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Sunscreen Activity of Fraction n-hexane, Chloroform, and Ethyl Acetate of Ethanol 96% Flamboyant Leaf (*Delonix regia*. Raf) Extract

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ABSTRACT

The sunscreen test of fraction n-hexane, chloroform, and ethyl acetate of ethanol 96% flamboyant leaf (*Delonix regia*. Raf) extract had been performed. This research begins the extraction of a flamboyant leaf using 96% ethanol. Extraction used the maceration method. The extract is fractionation with some solvents such as n-hexane, chloroform, and ethyl acetate. Each fraction was identified secondary metabolite and sunscreen test involving SPF, %Te, and %Tp measurement using UV-Vis Spectrophotometer. The result of phytochemical screening exhibited flavonoids, alkaloids, saponins, tannins, and phenolics in ethyl acetate fraction. n-hexane and chloroform fraction don't show saponins and flavonoid content. The sunscreen test shows that chloroform fraction has good protection power toward UV light with SPF, %Te, and %Tp value is 54.27±0.462, 7.46±0.473, and 12.83±0.047 in 250 mg/L, respectively.
Keywords: Flamboyant leaf (*Delonix regia*. Raf); Sunscreen; Maceration

INTRODUCTION

Sunscreens that are organic and inorganic agent have been able to protect skin from UV light. Protection ability showed in the mechanism of action of active compounds in a sunscreen agent. In general, the mechanism of action of organic compounds absorbs high energy of UV in the π bond of benzene ring which is conjugated carbonyl group and it released the low energy of UV (Ngurah *et al*., 2014). Another thing, the sunscreen from the inorganic compound is scattering and reflecting UV light so it can't transmit to the skin (Manaia *et al*., 2013).

The active compound of sunscreen agents is generally sourced from synthetic materials. Several synthetic materials of an organic compound have been used as sunscreen agents such as benzophenone derivates while inorganic compounds can be found from titanium oxide and zinc oxide. The use of synthetic material for sunscreen agents shows not good for applications on the skin (Warnida and Nurhasnawati, 2017). Because it generates photo irritation, photosensitivity, and allergic reactions on the skin (Saewan and Jimtaisong, 2013), we need a solution to the use of a safe sunscreen which is found from a natural substance.

Flamboyant (*Delonix regia*. Raf) is a plant that belongs to the Fabaceae family, bright red flowers, and oval-shaped leaves (Suhane *et al*., 2016). The phytochemical screening of flamboyant leaf shows that it contains flavonoids, alkaloids, tannins, and phenolics (Tjitda and Nitbani, 2019). The phenolic and flavonoid total content in the methanol extract of the flamboyant leaf had been measured by Gizawy *et al* (2018) and they are 5.5 g GAE/100 g DE and 53.3 g CE/100 g DE, respectively. The presence of phenolic and flavonoid compounds in flamboyant makes great potential to develop as an active compound for sunscreen agents.

Saewan and Jimtaisong (2013) report aromatic compound conjugated carbonyl group that was flavonoid compound has photoprotective power. The use of flamboyant leaves as sunscreens has been reported by Tjitda (2018). This research exhibit alcoholic extract which methanol extract has extra protection with an SPF value of 6.97 in concentration 600 mg/L. The effectiveness of the sunscreen of flamboyant leaf showed from %Te and %Tp. They are 6.15 and 22.45, respectively.

Based on previous data research, this research aims to find out sunscreen potential from n-hexane, chloroform, and ethyl acetate fraction of 96% ethanol extract of the flamboyant leaf. The best activity from these fractions should be a reference to the isolation active compound which is the sunscreen activity responsibility.

METHODOLOGY

Materials

The material used in this study is n-hexane (Merck), chloroform (Merck), ethyl acetate (Merck), 96% and 70% ethanol, sulfuric acid (Merck), ferric chloride (Merck), sodium hydroxide (Merck), acetic acid (Merck), Mayer reagents, hydrochloride acid (Merck), and aquades.

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The sample used in this study was flamboyant leaf (Delonix regia. Raf).

Instrumentation

The instrumentation in this study is laboratory glassware, 60 mesh sieve, blender, analytical mass balance (EW-220-3NM), rotary evaporator (Eyela N-100), Waterbath (Memmert), and UV-Vis Spectrophotometer (Shimadzu Type UV- 1700).

Methods

Preparation and extraction of flamboyant leaf (*Delonix regia*. Raf)

The flamboyant leaves were washed, dried, destroyed, and sieved with a 60 mesh cycle. A total of 400 g of flamboyant leaf powder is dissolved in 1200 mL 96% ethanol and hashed up in 5 days when it is stirring in every day. On the 5th day, the extract is filtered to produce filtrate. The remaining pulp was re-maceration by adding 400 mL of 96% ethanol solution for two days. All macerates were filtered and collected in one container. The filtrate was evaporated at 60 °C temperature using a rotary evaporator until it produced concentrated extract. The yield of extract was calculated by the following equation:

$$Rendement = \frac{weight\ of concetrated\ extract\ (g)}{weight\ of\ initial\ extract\ (g)} \times 100\%$$

Fractionation 96% ethanol extract of flamboyant leaf (*Delonix regia*. Raf)

A total of 30 g of 96% ethanol extract of flamboyant leaves was put into a separating funnel and dissolved in a 100 mL methanol: water (2: 3) solvent mixture. This solution is partitioned with several solvents. The first partition is to add 100 mL of n-hexane solution to the separating funnel and vibrate and leave it into two layers. The n-hexane layer is separated and collected in a container. Next, mix the methanol: water solution again with n-hexane until the n-hexane layer is clear. The second partition adds 100 mL of chloroform to the residual layer until the chloroform layer appears clear. The third partition, ethyl acetate as a solvent is used to partition the residue layer. Each fraction collected evaporates using a rotary evaporator and becomes a concentrated fraction.

Phytochemical screening

Secondary metabolite content of n-hexane, chloroform, and ethyl acetate is worked by phytochemical screening. The confirmation of secondary metabolite in each fraction was performed by colour test to determine flavonoid, alkaloids, saponins, phenolic, and tannins. The phytochemical screening procedure was worked by following Gafur and Bialangi (2013).

Sunscreen test

Determination of the sun protection factor (SPF)

Determination of SPF to n-hexane, chloroform, and ethyl acetate fraction was conducted by UV-Vis Spectrophotometer. Each fraction weighing 0.1 g which dissolved in 100 mL 96% ethanol produced stock solution (1000 mg/L). The stock solution was similarly dilute in solvent to generate the concentration of fractions that are 50, 100, 150, 200, and 250 mg/L. The absorbance of each concentration fraction measured every 5 nm throughout a wavelength of 290 nm. Then, the absorbance value is used to find out the area under cover (AUC) by followed equation bellow:

$$AUC = \frac{Aa + Ab}{2} \times (dP_{a-b})$$

AUC values obtained is then determining SPF value by followed equation bellow:

$$log\ SPF = \frac{AUC}{\lambda n - \lambda 1} \times 2$$

Determination of %Te and %Tp value

Each of the fractions had n-hexane, chloroform, and ethyl acetate measured the value of %Te by measuring absorbance at wavelengths of 292.5-317.5 nm while the value of %Tp by measuring absorbance at wavelengths of 322.5-372.5 nm. The absorbance value obtained is then converted to the value of %Transmittance using the equation:

$$\%T = antilog(2 - A)$$

Then, %T value used to determine %Te and %Tp by followed equation below:

$$Ee = \Sigma T \times Fe$$

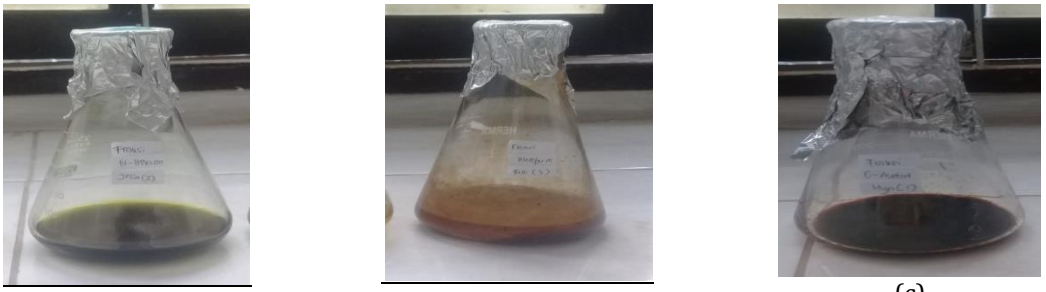
$$\%\ erythema\ \ transmission = \frac{Ee}{\Sigma Fe}$$

$$Ep = \Sigma T \times Fp$$

$$\%\ pigmentation\ transmission = \frac{Ep}{\Sigma Fp}$$

Where: Ee : the amount of erythema flux passed by the extract at wavelengths of 292.5-317.5 nm; Fe : erythema flux; T : transmission value; Ep : the amount of pigmentation flux carried by the extract at a wavelength of 322.5-372.5 nm; Fp : pigmentation flux.

The measurement of SPF, %Te, and %Tp value is worked in the triplicate experiment and examined by statistical analysis to show mean ± standard deviation (SD).



(a) (b) (c)

Figure 2. The results of fractionation using a) n-hexane, b) chloroform, and c) ethyl acetate

Table I. Percent yield of fractionation of 96% ethanol extract of flamboyant leaves (*Delonix regia*. Raf)

Fraction	Extract Weight (g)	Fraction weight (g)	% Yield (b/b)	Extract color	Form
n-hexane	15	2,26	15,06	Blackish green	Pasta
Chloroform	15	5,75	38,35	Brownish red	Pasta
Ethyl acetate	15	2,41	16,06	Black	Pasta

RESULT AND DISCUSSION

Preparation and extraction of flamboyant leaf (*Delonix regia*. Raf)

Figure 1. Extract ethanol 96% of flamboyant leaves (*Delonix regia*. Raf)

At this step, fresh flamboyant leaves were prepared and dried without directly exposed to sunlight. It produces dried leaves. The dried leaves obtained are continually extracted with 96% ethanol in five days. Extraction uses the maceration method. The maceration is a cold extraction method which it is proper extraction for the unstable active compound. Flavonoid and phenolic are the groups of secondary metabolites that straightforward degraded in thermal conditions.

The usage of alcohol as solvent extraction is due to able to penetrate the membrane cell of the plant. Therefore, the secondary metabolite is simply the extraction (Tiwari *et al*., 2011). Alcohol is a polar compound that has a worthy appeal to the polar secondary metabolite (Abubakar and Haque, 2020). This treatment is according to the like dissolve like principle.

The maceration of flamboyant leaves in five days, which is a fresh solvent used in two days, it worked. This process aims to increase effective extraction. Further, the active compound can be optimal to captivate. Subsequently, the evaporation of extract solution is executed at 60 °C to remove the solvent. This treatment is to have concentrated extract in blackish green with a rendement of 12.50%.

Fractionation ethanol 96% extract of flamboyant leaf (*Delonix regia*. Raf)

The concentrated extract of the flamboyant leaf using 96% ethanol was subsequently partitioned using various solvents through the liquid-liquid extraction method. The solvent in the fractionation process of concentrated extracts of the flamboyant leaf was n-hexane, chloroform, and ethyl acetate. Partitioning using n-hexane solvent is expected to bind several non-polar components such as lipids and coumarin (Cowan, 1999). Partitioning products using n-hexane produces a black-green paste with a yield of 15.06%. The remaining fraction is then partitioned with chloroform to produce a brownish-red product with a yield of 38.35%. The remaining fraction was partitioned again with ethyl acetate to dissolve the semi-polar compound and the product was obtained in the form of a black paste with a yield of 16.06%. The physical appearance of the product from each fraction is shown in Figure 2 and the yield data is presented in Table I.

Table II. Phytochemical screening of n-hexane, chloroform, and ethyl acetate fractions from ethanol extract 96% flamboyant leaves (*Delonix regia*. Raf)

Secondary metabolites	Testing method	Sampel		
		n-hexane	Chloroform	Ethyl acetate
Flavonoid	0.1 g of fraction and NaOH	-	+	+
Alkaloid	Mayer's reagent	+	+	+
Saponin	Hot water and Suffle	-	-	+
Tannin	$FeCl_3$ 1%	+	+	+
Phenolic	$FeCl_3$ reagent	+	+	+

notice: (+) : the positive result of secondary metabolites; (-) : the negative result of secondary metabolites

Table III. Sunscreen activity of n-hexane, chloroform, and ethyl acetate fraction as expressed by SPF value

Fraction	SPF value				
	Concentration (mg/L)				
	50	100	150	200	250
n-hexane	1.28±0.203	1.75±0.700	4.08±0.177	5.83±0.076	11.80±0.347
Chloroform	1.90±0.830	3.44±0.040	6.65±0.241	16.47±0.364	54.27±0.462
Ethyl acetate	1.63±0.446	3.07±0.001	3.91±0.522	12.33±0.149	40.76±0.837

The preliminary phytochemical screening conducts on every fraction by the colour test. Screening tests to reveal secondary metabolites contained in each fraction are performed. The result illustrated secondary metabolites of each fraction in the following Table II. Based on Table II, the n-hexane fraction shows the negative result on the flavonoids and the saponin components. This is supported by the absence of light brown and foam-forming for flavonoid and saponin indicated, respectively. The n-hexane solvent that is an alkane compound with six aliphatic carbon chains has a hydrophobic character, so it is unable to form hydrogen bonds with flavonoids or saponins. As a result, they are insoluble in n-hexane solvents.

On the other hand, the chloroform fraction does not contain saponins. Saponin is integrated triterpenoid compounds with glucose molecules and presence in aglycones form. Therefore, this compound is easily interacted with by polar solvents. The presence of secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and phenolics contained in the ethyl acetate fraction. This metabolite constitutes is also present in another article (Esan *et al*., 2020). The semi-polar character of the ethyl acetate compound allows several secondary metabolites to dissolve well.

Sunscreen test

Sunscreen activity tests of n-hexane, chloroform, and ethyl acetate fraction from 96% ethanol extract of flamboyant leaves were carried out. The use of 96% ethanol as solvent extraction was based on the results of previous research by Tjitda and Nitbani (2019). It shows that extraction using alcohol has potential as a sunscreen. Sunscreen activity assay conducts with the Spectrophotometry method. The ability to protect UV light at each fraction is determined by measuring the absorbance at wavelengths from 290 to 400 nm (Donglikar and Deore, 2016).

The absorbance value obtained at each measurement will determine both the Area Under Cover (AUC) value and the AUC value. They are used to calculate the SPF value. SPF values indicate the ability of sunscreen agents against UV rays (Siregar *et al*., 2019). The higher SPF value of a sunscreen agent shows that the compound can absorb UV rays well. The chromophore group in flavonoid and phenolic will absorb UV rays cause vibrational and rotational movement. It prompts to be electron excitation. Subsequently, the electron moved to the ground state and accompanied by a low level of energy emission as harmless long wave IR. Conversely, high energy emission transmitted by sunscreen compounds is to have an awful impact on the skin (Wu *et al*., 2016)

The results of determining the SPF value of the product in the n-hexane, chloroform, and ethyl acetate fractions from 96% ethanol crude extract of flamboyant leaves give in Table III. Also, Figure 3 provides the SPF value comparison of each fraction. Based on Figure 3 shows a similar pattern where the increase in extract concentration goes along with an enhancement for the ability of UV absorption from 50 mg/L to 200 mg/L. In contrast, the absorption UV light of every fraction climbs

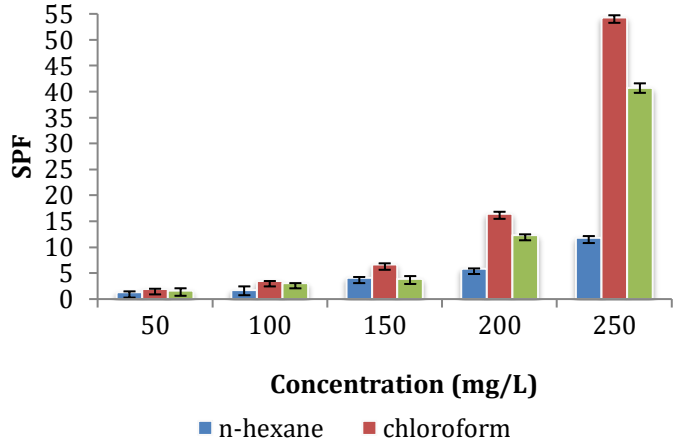


Figure 3. Graph of SPF values of n-hexane, chloroform, and ethyl acetate fractions of 96% ethanol extract of flamboyant leaves (*Delonix regia*. Raf)

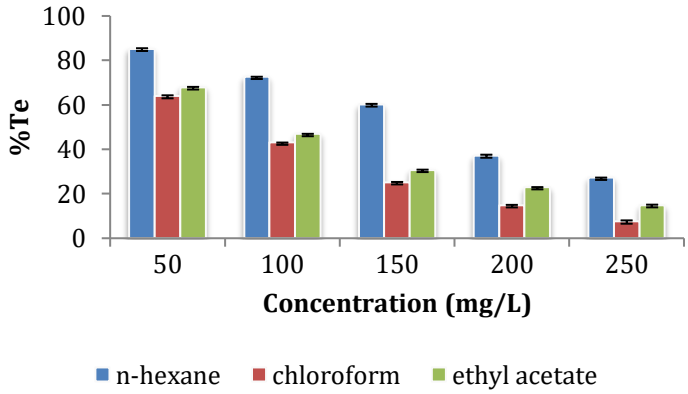


Figure 4. Graph of %Te value of n-hexane, chloroform, and ethyl acetate fractions of 96% ethanol extract of flamboyant leaves (*Delonix regia*. Raf)

dramatically between 200 mg/L and 250 mg/L. The enhancement is due to the growth in the number of substance molecules per liter of the solution. The best absorption ability of UV light is indicated by the highest SPF value of chloroform fraction among the fraction of n-hexane and ethyl acetate. At a concentration of 250 mg/L, the product from the chloroform fraction showed the highest SPF value of 54.27±0.462. This fact gives information about chloroform fraction that has the potential to protect the skin from UV rays.

The ability of protection against UV rays is affected by existing active compounds. The presence of flavonoid compounds contributes to sunscreen activity (Saewan and Jimtaisong, 2013). Structurally, flavonoids have two aromatic rings connected with heterocyclic rings. The carbonyl group in the flavonoid structure provides electrons π to be excited when it exposed to UV light. The excited electron will return to the ground state via emitting energy with high wavelengths (Manaia *et al*., 2013).

Flavonoids in the protection of UV rays also can undergo antioxidant mechanisms. The appearance of a hydroxyl substituent that serves as an electron-releasing group can prevent the formation of radical oxygen species (ROS) and radical nitrogen species (RNS). This mechanism occurs within a process of electron delocalization from the -OH group to the carbonyl group as an electron-acceptor group (Bustamante *et al*., 2020) so it can prevent cell membrane damage.

The effectiveness of sunscreen protection products can also analyze by determining the value of %Te and %Tp. %Te is a value that shows the ability of a sunscreen product to absorb UVB rays in the wavelength range of 292.5-317.5 nm. When the compound can absorb UVB rays, as well as show the low %T, UVB rays cannot reach surface skin. Thus, the phenomenon of erythema does not

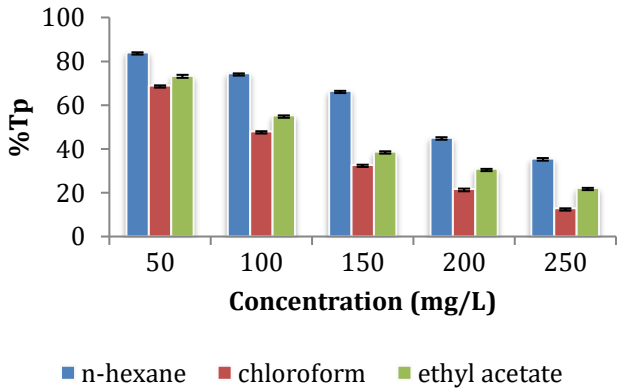


Figure 5. Graph of %Tp values of n-hexane, chloroform, and ethyl acetate fractions of ethanol 96% extract of flamboyant leaves (*Delonix regia*. Raf)

Table IV. Sunscreen activity of n-hexane, chloroform, and ethyl acetate fraction as expressed by %Te value

Fraction	%Te value				
	Concentration (mg/L)				
	50	100	150	200	250
n-hexane	85.24±0.232	72.64±0.010	60.23±0.218	37.22±0.352	27.23±0.045
Chloroform	63.95±0.341	43.02±0.015	25.21±0.101	14.86±0.137	7.54±0.473
Ethyl acetate	67.90±0.178	46.90±0.070	30.77±0.077	22.99±0.010	14.88±0.215

Table V. Sunscreen activity of n-hexane, chloroform, and ethyl acetate fraction as expressed by %Tp value

Fraction	%Te value				
	Concentration (mg/L)				
	50	100	150	200	250
n-hexane	84.05±0.035	74.46±0.011	66.49±0.007	45.18±0.151	35.62±0.206
Chloroform	68.97±0.022	48.03±0.027	32.74±0.037	21.65±0.230	12.83±0.047
Ethyl acetate	73.44±0.371	55.20±0.094	38.84±0.068	30.86±0.018	22.19±0.020

happen. The %Te value in the n-hexane, chloroform, and ethyl acetate fractions are in the following Table IV. The data in Figure 4 shows that the n-hexane, chloroform, and ethyl acetate fraction show a sufficient protective ability against UVB. It can be seen that increasing the concentration of products in each fraction can decrease the %Te value. The highest concentration provides more active compounds that contribute to UVB protection. The presence of secondary metabolites in the form of flavonoids and phenolic compounds is considered to have a part in the protection of UVB rays. This statement is confirmed by the data that chloroform fraction containing flavonoids and phenolics gives the lowest %Te value at a concentration of 250 mg/L of 7,456±0.473.

UVA is one part of UV light radiation that has a long wavelength and low energy. When exposing UVA to the skin for a prolonged time, it happened, it will cause a serious problem that is pigmentation occurrence. In this work, all fractions are measuring the percent transmission by pigmentation assay to show the UVA protection capability (Table V). The measurement of five concentration series worked by using absorbance analysis. The absorbance value converted to a percent transmission value. The graph in Figure 5 illustrated that the n-hexane, chloroform, and ethyl acetate fraction have absorbed UVA rays well. Figure 5 also shows a similar trend graph as well as %erythema assay. The growing concentration reduces %Tp value. The chloroform fraction had the lowest %Tp value at each concentration tested. This fact gives information about the chloroform fraction from flamboyant leaves can absorb UVA rays better than the n-hexane and ethyl acetate fractions. Based data analysis reveals chloroform fraction that has better UVA and UVB absorption. The SPF value of the chloroform fraction is high among fractions. The presence of flavonoid and phenolic in the phytochemical screening of

chloroform fraction is a presumption that they play a role in sunscreen activity.

CONCLUSION

The sunscreen activity test for n-hexane, chloroform, and ethyl acetate fractions from 96% ethanol extract of flamboyant leaves has the ability as a UV protection agent (sunscreen). The best ability as a sunscreen agent is shown by the chloroform fraction with SPF, %Te, and %Tp values at a concentration of 250 mg/L of 54.27±0.462, 7.46±0.473, and 12.83±0.047, respectively.

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