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Photoprotective and Inhibitory Activity of Tyrosinase in Extract and Fractions of *Terminalia catappa* L.

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Abstract

BACKGROUND: Chronic exposure to ultraviolet (UV) radiation causes various skin damages. One of the most significant risks to skin occurrence is cancer and photoaging. Recent strategies for photoprotection have included incorporating natural sunscreens and antityrosinase.

AIM: This study aimed to determine the sun protection factor (SPF) and inhibitory activity of tyrosinase of the extract and fractions of leaves of *Terminalia catappa* Linn.

METHOD: The dried leaves were macerated with 96% ethanol and fractionated using n-hexane, ethyl acetate, and water. The extract and fractions were screened for their phytochemical profile, and tyrosinase inhibitory activity was evaluated and expressed as IC_{50}. The photoprotective activity of extract and fractions were measured by a UV spectrophotometric.

RESULTS: Among the tested samples, the ethyl acetate fraction showed ultraprotection on erythema transmission rate (%TE), sunblock on pigmentation transmission (%TP), and minimum protection on SPF. Ethyl acetate fraction showed the highest activity to inhibit tyrosinase (IC50 was 50.54±2.37 µg/mL). The phytochemical analysis of ethyl acetate fraction revealed the presence of phenolic and flavonoid compounds.

CONCLUSION: This study's findings revealed a higher tyrosinase inhibitor and sun protection capacity of ethyl acetate fraction of leaves of T. catappa and suitable to develop as a cosmetic agent.

Introductions

Becoming the largest organ of the human body, skin consists of 16% of body mass. Skin covers and protects everything inside the body. Normal human skin is organized into two primary layers, epidermis and dermis. The epidermis, of ectodermal origin, is the outer layer and direct contact with the environment. At the same time, the dermis is located inside and made up of epithelial, mesenchymal, glandular, and neurovascular components. Epidermis, with its physical characteristics, plays an important role in protecting the body from harmful chemicals such as infections, chemical agents, sharp objects, and ultraviolet (UV) radiation [1], [2], [3].

Prolonged exposure to UV radiation of human skin may cause several damages. These damages include skin cancer, sunburn, oxidative stress, and photoaging. The degree of damage depends on the amount and duration of exposure and the form and type of UV radiation [4]. Based on its wavelength, UV radiation is divided into three main segments which are UV C (100–290 nm), UV B (290–320 nm), and UV A (320–400 nm) [5].

The main important thing of UV radiation is leading to the formation of deoxyribonucleic acid (DNA) and changing between adjacent pyrimidine bases. Implication on unwanted protein production or broken cell or organ. These damaged DNA molecules can stimulate repair mechanisms known to cause inflammatory responses within the skin and increase the production of radicals. Free radical, singlet oxygen, and reactive oxygen species (ROS) can, in turn, cause other ROS, and in combination, attack cellular components such as cell membranes, cellular proteins, lipids, and nuclear and mitochondrial DNA. Many chromophores groups (chemicals capable of absorbing UV radiation and generating damaging ROS) exist within the skin by neutralizing the singlet racial oxygen [6].

Physiologically, antioxidant mechanism comes in the skin, including chemical and enzymatic

antioxidants. Water-soluble antioxidants (Vitamin C, glutathione, lipoic acid, and uric acid) or lipid-soluble antioxidants (Vitamin E, ubiquinone, and carotenoids) are usually coming from diet and nutrition. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione transferase. Both chemical and enzymatic antioxidants protect the skin from damage by ROS and free radicals. However, with age and environmental stress, or chronic exposure can deplete the natural antioxidants, resulting in more vulnerable skin damage.[7], [8].

There has been an increasing interest in using antioxidants in sunscreens to provide supplemental photoprotective action activity. Antioxidants from natural sources may offer new possibilities for treating and preventing UV-mediated diseases. Therefore, natural products are essential sources for research in new active compounds to discover new biological mechanisms, obtain new active molecules, and study their structure-function relationships to develop more active drugs and avoid unwanted side effects [9], [10].

Wen *et al*., 2011, reported that water extract of *Terminalia catappa* protected skin from photodamage by inhibiting the mitogen-activated protein kinase (MAPK)/activator protein-1 (AP-1)/ matrix metalloproteinase (MMP) pathway [11]. The present study indicated that *T. catappa* exhibits potent free radical scavenging and antioxidant activity due to polyphenol and flavonoid content. The leaves extract of *T. catappa* protects the skin damage from radical H_2O_2 by inhibiting the protein expression of MMP, AP-1, MAPK, and cyclooxygenase-2 (COX-2) [12]. However, the activity and related mechanisms of *T. catappa* against UV oxidative stress-induced skin damaging are unclear. Therefore, this study investigated the effects of *T. catappa* ethanol extract and fractions to protect skin from UV radiation through the sun protection factor (SPF) value and their capability to inhibit tyrosinase.

Materials and Methods

Materials

Tyrosinase from the mushroom in lyophilized powder was obtained from Sigma Chemical Co. (St. Louis, USA). All chemicals and reagents used in the experiment were analytical grade and purchased from Sigma-Aldrich (St. Louis, USA).

Sample collection and identification

The leaves of *T. catappa* were collected from Makassar, South Sulawesi, Indonesia. The collected leaves were air-dried at 37°C for 3 hours and grounded into a powder. Identification and authentication of the plant were made by the Indonesia Institute of Science Research Center for Biology, Bogor, Indonesia.

Extraction and fractionation

The powdered form of air-dried leaves of *T. catappa* was subjected to macerate extraction using 96% ethanol. After complete extraction, the solvent was evaporated using a rotary evaporator (Büchi, German). Further, the ethanol extract (3.0 g) in water (50 mL) was extracted with two different solvents, which are hexane and ethyl acetate, using the liquid-liquid extraction (LLE) method, respectively. All the fractions were then dried in Freeze Dryer Lyophilizer (Büchi L200, German). The lyophilized powders were transferred into a vial and stored in a desiccator for subsequent analysis.

Phytochemical screening

Flavonoids

A total of 2.0 g of extract and fractions were dissolved in 2 mL of methanol, then Mg powdered, and five drops of HCl concentrated were added. The presence of flavonoids is indicated by the formation of red or orange colors [13].

Phenolic

The extract was added with 5% $FeCl_3$; a dark blue or black color is formed, which indicates a phenolic compound [14].

Antityrosinase activity

Antityrosinase activity was determined with the previous method describes by Wu *et al*., 2019 [15]. First, the extract and fractions were dissolved in dimethyl sulfoxide (DMSO) and diluted to seven different concentrations. The final concentration in water of the extract and fractions was 7.81; 15.63; 31.25; 62.50; 125.00; 250.00; and 500.00 μg/mL. Subsequently, 30 μL of the extract and fractions was mixed with 100 μL sodium phosphate buffer (0.05 mM), and 100 μL of 100 mg/L L-tyrosine and 20 μL of tyrosinase solution (350 units/mL) were next added. This reaction solution was homogeneously mixed and incubated at room temperature. The final absorbance was recorded at 490 nm using a microplate reader after 20 min of incubation. The concentration of extract and fractions at which 50% of the original tyrosinase activity is inhibited (IC_{50}) was determined for extract and fractions. The antityrosinase activity of the extract and fractions leaves of *T. catappa* is expressed as a percentage of tyrosinase inhibition as follows:

$$\text{Tyrosinase inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

In vitro sunscreen activity

Preparation of solution

The ethanol extract was dissolved in ethanol to a final concentration of 50.0; 75.0; 100.0; 125.0; and 150.0 μg/mL. Hexane fraction was dissolved in ethanol to a final concentration of 60.0; 120.0; 180.0; 240.0; and 300.0 μg/mL; ethyl acetate fraction was dissolved in ethanol to a final concentration 32.0; 48.0; 64.0; 80.0; and 96.0 μg/mL; while water fraction was dissolved in ethanol to a final concentration of 50.0; 75.0; 100.0; 125.0; and 150.0 μg/mL.

Determination of percent transmission of erythema (TE)

The TE value was determined using a UV spectrophotometer (Shimadzu UV-1800) by measuring the transmittance of several concentrations of the sample solution at the wavelength causing erythema of 292.5–372.5 nm at 5 nm intervals with ethanol as the blank. The TE value was calculated by:

$$TE = \frac{\Sigma erythema\, transmission\,(TxFe)}{\Sigma flux\, erythema\,(Fe)} \times 100$$

Determination of percent transmission of pigmentation (TP)

The TP value was determined using a UV spectrophotometer (Shimadzu UV-1800) by measuring the transmittance of several concentrations of the sample solution at the wavelength causing erythema of 292.5–372.5 nm at 5 nm intervals with ethanol as the blank. The TP value was calculated by:

$$TP = \frac{\Sigma\, pigmentation\, transmission\,(TxFp)}{\Sigma flux\, pigmentation\,(Fp)} \times 100$$

Determination of SPF

The SPF model used in this study was according to the methodology described by Costa *et al.* (2015). The sample absorbances were measured in wavelength range 292.5–372.5 nm, with 5 nm increments, and three determinations were made at each point [16]. The SPF value is calculated using the formula:

$$\text{Area Under Curve } (\text{AUC}) = \frac{Aa + Ab}{2} \times (\text{dP}_{a-b})$$

$$\text{Log SPF} = \frac{AUC}{\lambda n - \lambda 1} \times \text{DF}$$

Where:

A_a = Absorbance in wavelength a nm
A_b = Absorbance in wavelength b nm
dP_{a-b} = Difference in wavelengths a and b
λn= The largest wavelength (with A ≥0.05)
λ_1 = Smallest wavelength (290 nm)
DF= Dilution factor.

Data analysis

The analyses were performed in triplicate, and the results expressed as mean ± standard deviation (SD).

Results

The yields for the dried extract and fractions in each solvent are presented in Table 1. The results showed that the percentage of ethanol extract yield was 28.08%. After fractionation, the fraction showed a pattern for percent yield, as follows: Water >h-hexane >ethyl acetate. These results indicated that the leaves of *T. catappa* primarily contain polar compounds.

Table 1: The yields of leaves of *T. catappa* extracts and fractions prepared using different extraction solvents

Sample	Yield (%)
Ethanol extract	28.08
Hexane fraction	11.30
Ethyl acetate fraction	4.66
Water fraction	15.97

Phytochemical screening was carried out on extract and fractions of leaves of *T. catappa* using a color reaction or precipitation. From the results of the tests conducted, it was stated that the extract and all fractions contained flavonoid and phenolic compounds, except hexane only phenolic (Table 2).

Table 2: Phytochemical screening of extract and fractions of leaves of *T. catappa*

Sample	Parameters	
	Flavonoid	Phenolic
Ethanol extract	+	+
Hexane fraction	-	+
Ethyl acetate fraction	+	+
Water fraction	+	+

The effectiveness of the leaves of T. catappa as sunscreen is based on the percentage of TE and TP, and also SPF value. The concentrations of extract and fractions were used in this study, which were 32.0 till 300 μg/mL. The selection of concentrations is based on optimization results as well to see the effect of increased fraction concentration on sunscreen activity. The present value of %TE, as well as the %TP, shows that both extract and fractions an increase in concentration indicates a decrease in value of leaves of *T. catappa* (Figures 1 and 2). This illustrates that all samples can absorb UV light and reduce the side effect of exposure received by the skin, but ethyl acetate fraction is more active than others.

Table 3 shows the protection categories of extract and fractions of leaves of *T. catappa.* Ethanol

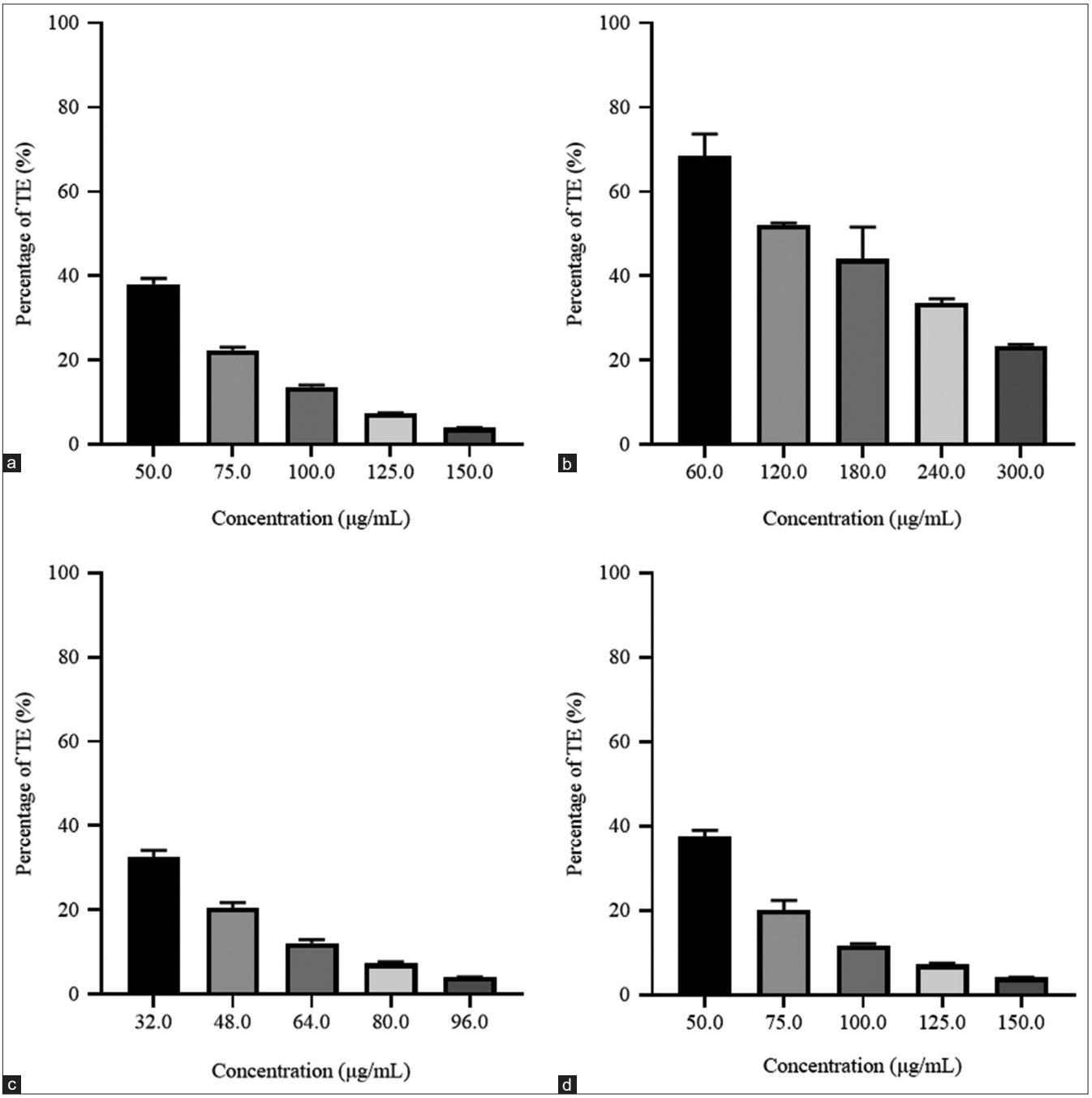

Figure 1: Percentage transmission of erythema of leaves of T. catappa: Ethanol extract (a); hexane fraction (b); ethyl acetate fraction (c); water fraction (d). Data are expressed as the means ± SD of three independent experiments

extract at the concentration 100.0 till 150.00 µg/mL has minimum category; hexane fraction at the concentration 60.0 till 300 µg/mL no showing activity; ethyl acetate fraction at the concentration 64.9 till 96.0 µg/mL has minimum category; while water fraction at the concentration 100.0–150.0 µg/mL has a minimum category. From this result, extract and fractions have a minimum category at a specific concentration, except hexane fraction.

Inhibition on tyrosinase was evaluated to measure the ability of extract and fractions to interrupt this enzyme. The result showed that both extract and fractions could inhibit tyrosinase activity in a dose-dependent manner at a concentration of 7.81–500.00 µg/mL (Figure 3). The most pronounced inhibition was observed in the ethyl acetate fraction. This can be seen from the lowest IC_{50} value compared to the others (Table 4).

Discussion

The aim of SPF measurement is quantitative of the effectiveness of a sunscreen. To effectively prevent

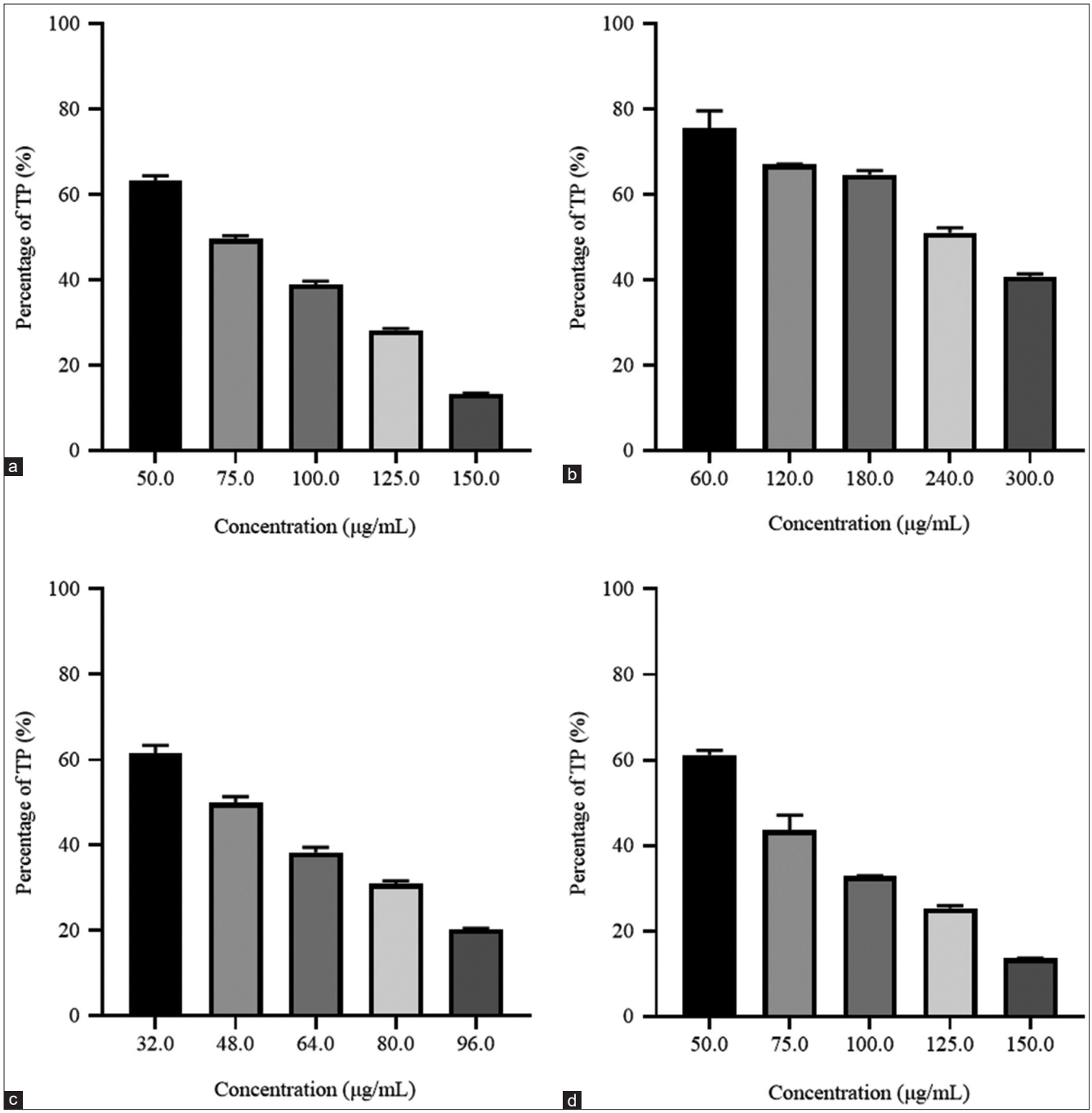

Figure 2: Percentage transmission of pigmentation of leaves of Terminalia catappa: Ethanol extract (a); hexane fraction (b); ethyl acetate fraction (c); water fraction (d). Data are expressed as the means ± SD of three independent experiments

sunburn and other skin damage, a sunscreen product should have a wide range of absorbance between 290 and 400 nm [17]. In this research, the ethanol extract and its fractions of leaves of *T. catappa* were evaluated by UV spectrophotometry applying a mathematical equation.

The leaves of *T. catappa* were extracted with 96% ethanol using the maceration method. The extraction process steps are size reduction, extraction, filtration, concentration, and drying [18]. The solvent depends on the type of bioactive compounds that will be extracted. In general, polar solvents such as water are used to extract polar compounds, non-polar solvents such as hexane are used to extract non-polar compounds. In contrast, semi-polar like ethanol can extract both polar and non-polar compounds. LLE method was used to divide the active compounds based on their polarity. Non-polar compounds will accumulate in hexane fraction, slightly polar compounds will accumulate in ethyl acetate fraction, while polar compounds will accumulate in water fraction (residue).

Phytochemical screening was carried out on extract and fractions of leaves of T. catappa using a color reaction or precipitation. It was stated that the extract and all fractions contained flavonoid and phenolic compounds, except hexane only phenolic. The

Table 3: Protection categories based on SPF values of extract and fractions of leaves of *T. catappa*

Sample	Concentration (μg/mL)	SPF value	Protection category
Ethanol extract	50.0	1.44	-
	75.0	1.76	-
	100.0	2.09	Minimal protection
	125.0	2.74	Minimal protection
	150.0	3.68	Minimal protection
Hexane fraction	60.0	1.16	-
	120.0	1.32	-
	180.0	1.37	-
	240.0	1.54	-
	300.0	1.77	-
Ethyl acetate fraction	32.0	1.51	-
	48.0	1.80	-
	64.0	2.23	Minimal protection
	80.0	2.71	Minimal protection
	96.0	3.51	Minimal protection
Water extract	50.0	1.44	-
	75.0	1.84	-
	100.0	2.27	Minimal protection
	125.0	2.76	Minimal protection
	150.0	3.61	Minimal protection

high amounts of phenolic influence sunscreen activity. The phenolic compound in the extract has conjugated bonds in the benzene core. When exposed to UV light, resonance occurs through electronic transfer. The standard conjugation system for phenolic compounds and chemicals usually contained in sunscreens gives these compounds the potential to be photoprotective. Flavonoid is the largest group of phenolic compounds. Flavonoid also has potential as sunscreens because of chromophore groups' presence, which generally gives plants a yellow color. The conjugated aromatic system becomes the identity of flavonoid, which provides a powerful ability to absorb light of UV A and UV B [19].

Table 4: The IC_{50} values of the extract and fractions of leaves of *T. catappa* on the tyrosinase activity

Sample	IC_{50} (μg/mL)
Ethanol extract	93.01 ± 2.28
n-hexane fraction	275.93 ± 67.74
Ethyl acetate fraction	50.54 ± 2.37
Water fraction	123.12 ± 2.01

The percentage of erythema transmission describes the amount of sunlight transmitted after being exposed to sunlight, causing skin pigmentation. Based on this, the lower the percentage value of erythema and pigmentation transmission means that the sunscreen's potential to protect the skin is better than a bigger concentration. However, SPF is a universal indicator that explains the effectiveness of a product or substance that is a UV protector. The higher the SPF value of a product or active sunscreen, the more effective it is to protect the skin from the destructive effects [20], [21]. UV B light induces sunburn (burning skin), at high and long exposure induces skin cancer formation. Even though UV A amount reaches to earth is 10% more than UV B, more erythema production is caused by UV B [22]. Substances can provide a protective effect as a sunscreen if its SPF value is >2 [23]. On 2009, European Commission (EC) classified SPF value as the following: Low protection (SPF 6–10), protection (SPF 15-25), high protection (SPF 30-50), and extremely high protection (SPF >50) [24]. In fact, the higher the SPF value desired, the higher the amount of active substance is needed to be added. A substance can provide a protective effect as a sunscreen if its SPF value is >2 [23].

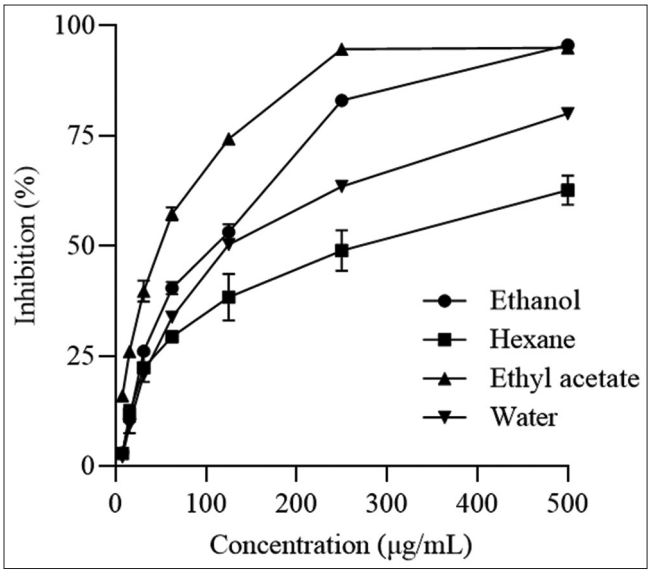

Figure 3: The inhibition effects Terminalia catappa extract and fractions on tyrosinase activity. Data are expressed as the means ± SD of three independent experiments

The actual skin color is affected by many substances, but the most important substance is melanin. Melanin causes the skin color of darker human skin and produces within the melanocytes cell. Melanin synthesis was catalytic by the enzyme tyrosinase. Tyrosinase catalyzes L-tyrosine to L-3.4-dihydroxyphenylalanine (DOPA) and subsequently oxidized become dopachrome. [25]. Therefore, tyrosinase inhibitors can be used to avoid skin pigmentation both in medicine and in cosmetics. The most pronounced inhibition was observed in the ethyl acetate fraction. The highest activity can be inferred from the presence of phenolic and flavonoid compounds. Much literature describes that these compounds are responsible mainly for the inhibition of tyrosinase activity [26], [27].

T. catappa and its constituents have shown many beneficial pharmacological potentials. Free radical, 1,1-diphenyl-2-picrylhydrazyl, can be scavenged by methanol extract of *T. catappa* able to scavenge [28]. The free radicals are the main risk of skin damage. Presenting antioxidants can stimulate the skin to repair and build itself naturally. Strong antioxidants protect humans from free radicals or oxidative stress. Several studies have indicated that strong antioxidants from natural ingredients are able to resist oxidative damage to the skin because of free radicals [29], [30].

Conclusion

This study showed that the ethyl acetate of leaves of *T. catappa* exhibited higher SPF activities

compared to the ethanol, hexane, and water fraction. The SPF activity of ethyl acetate fraction could be attributed to their relatively content phenolic and flavonoid. These fractions can, therefore, be proposed as new potential sources of natural additives for cosmetics.

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