Indonesian Journal of Pharmaceutics
IdJP
ISSN : 2597-8748

Vol. 5, Issue 2, 2023 (422-431)
http://journal.unpad.ac.id/IdJP

The Sunscreen activities of ethanol, ethyl acetate, n-hexane, and water fractions from papaya (*Carica papaya* l.) leaf extract

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Submitted: 26/04/2023, Revised: 19/07/2024, Accepted: 20/12/2024, Published: 24/01/2025

ABSTRACT

The application of sunscreen products containing natural compounds is one of alternative popular ways to protect the skin from the harmful effects of sun exposure. The aim of this study was to evaluate the sunscreen activity of papaya leaf extract. The ethanol extract of papaya leaf was fractionated to obtain ethanol, n-hexane, ethyl acetate, and water extracts. The value of SPF 15, percent transmission of erythema (1% Te), and the percent transmission of pigmentation 40 (% Tp) of each extract were calculated *in vitro* by using spectrophotometric method. among the extract examined, the ethyl acetate extract was found to be the most effective to reach Sun Protection Factor (SPF) 15 (ultra-protection category) and 1% Te (sunblock category) at a minimum concentration of 90.08 ppm and 63.49 ppm. Meanwhile n-hexane extract was the most effective to reach 40% tp (sunblock category) at a minimum concentration of 30.68 ppm. The minimum concentration of papaya leaf extracts required to reach SPF 15, 1% Te and 40% Tp were far below the concentration of sunscreen compounds allowed in commercial cosmetic products. It can be concluded that papaya leaf extract have significant sunscreen property for use as cosmetic ingredient.

Keywords: *Carica papaya*, SPF, Transmission of erythema, Transmission of pigmentation

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https://doi.org/10.24198/idjp.v5i2.46557

1. Introduction

Excessive exposure to sunlight is considered to be the main cause of many types of skin cancer including melanoma, the most violent form of skin cancer (1). The prolonged exposure to direct sunlight also triggers the skin aging and other skin damage such as dry skin, wrinkles, over pigmentation, actinic keratosis marked by scaly and rough skin texture, freckles and abnormal skin discoloration (2). Despite the negative effects, exposure to sunlight cannot be eliminated. Lack of sunlight led to a deficiency of serotonin and vitamin D in the body and increase the risk for depression, osteoporosis, heart disease, cancers, infectious diseases, fatigue, and tiredness (3).

The application of sunscreen products was used to overcome these harmful effects of sunlight exposure. Ingredients in sunscreen products protect skin from UV light in two different ways. Inorganic compounds like titanium dioxide and zinc oxide, act as a physical barrier that reflects UV light whereas organic compounds provide chemical structures that absorb UV radiation (4). The sunscreen material derived from natural sources such as plant phenolic, flavonoids, and carotenoids gain its popularity as a natural sunscreen related to their capacity to absorb UV ray and minimizes the negative effect of sunlight on the skin (5). The sunscreen capacity of plant compounds is obtained from the structure of the chromophore that absorbs sunlight and transform it into thermal or fluorescent/phosphorescent energy. The protection activity of secondary plant metabolites against sunlight spans a wide spectrum range from UV irradiation to visible and infrared light (6).

Papaya plant including its leaf, seeds, ripe and unripe fruits, and juice are commonly used as a traditional medicine. The leaves of papaya are knowns to contain many active compounds such as papain, chymopapain, cystatin, tocopherol, ascorbic acid, flavonoids, cyanogenic glucosides and glycosylates. These compounds proved to have immunomodulatory and antioxidant effects, reduce lipid peroxidation, exhibit anti-tumor activity and prevent inappropriate proteolysis. The papain enzyme from papaya leaves is used topically to remove dead cells from skin's surface to help the skin rejuvenate. (7, 8). Many reports of *C. papaya* leaf application to improve skin health have been published but more specific studies to find out about the capacity of papaya leaves to protect skin from sunlight exposure are still lacking. Thus the aim of this study was to evaluate the potential role of papaya leaf extract as a sunscreen agent.

2. Method

Plant material: papaya leaf var. *calina* was collected from Indonesian Research Institute for Medicinal Spices (BALITRO) and authenticated at the Indonesian Institute of Sciences (LIPI), Botanical Gardens, Bogor. The papaya leaf was washed and dried in electric oven at a temperature of 50^0-60^0C and then grinded to produce fine powder material. The dried papaya leave powder stored in a vacuum flask at room temperature for further usage.

2.1. Reagents and instruments

Reagents and instruments used in this study were of analytical grade i.e. ethanol, methanol, n-hexane and ethyl acetate (Merck); *rotary evaporator* (IKA®) and UV-Visible spectrophotometer (Jasco V-730®).

2.2.2 Extraction and fractionation

The sample was macerated using 96% ethanol (1: 10 w/w) for 24 hours, filtered and dried in *rotary evaporator* to obtain condensed crude extract. The condensed crude extract was diluted with hot double distilled water (20:100 w/v) prior to fractionation. The liquid-liquid fractionation method based on the polarity of the solvent was applied with hexane, ethyl acetate and ethanol successively to fractionate the papaya leaf extract. Furthermore, the sunscreen activity of each fraction was determined using UV-VIS spectrophotometer.

2.3. Determination of sunscreen activity.

A series of concentrations for each extract were prepared in….(please mentioned the solvent applied) prior to the determination of sunscreen activity using a spectrophotometer as listed in table 1.

Table 1 Concentration of Papaya Leaf Extract Fractions

Sampel	Concentration (ppm)
Ethanol extract	25; 50; 75; 100
n-heksan extract	10; 25; 50; 75
ethyl acetate extract	25; 30; 35; 40
Water extract	25; 50; 75; 100

2.4. Determination of SPF 15, % TE AND % TP

Sunscreen activity of papaya leaf extracts were determined *in vitro* using the spectrophotometry method. The absorbance data to calculate SPF value was read at wavelength 290-320 nm every 5 nm interval meanwhile the percent transmission of erythema (% Te) and the percent transmission of pigmentation (% Tp) was read at wavelength 292,5 -372,5 every 5 nm interval. The value of SPF was calculated according to Mansur equation (9) as follows:

$$\mathrm{SPF} = \mathrm{CF}\, X \sum_{290}^{310} \mathrm{EE}(\lambda) X\, I\, (\lambda) X\, abs\, (\lambda)\, X\, fp \qquad (1)$$

Where:

CF = correction factor (10),

EE (λ) = erythemal effect of radiation at wavelength λ,

Abs (I) = spectrophotometric absorbance of sample at wavelength λ.

The SPF value obtained was extrapolated to set up the linear equation and the linear equation will be used to calculate the value of SPF 15.

Value of % Te and % Tp was calculated using the following equations:

A = -log T (Equation 1) (2)

Where:

A = Absorbance of sample

T = Transmition value

The T value obtained from equation 1 put into Balsam equation (10) to obtain (%) Te and (%) Tp.

$$(\%)\ Te = \frac{\Sigma Ee}{\Sigma Fe} = \frac{\Sigma(T \times Fe)}{\Sigma Fe} \quad (2) \qquad (3)$$

$$(\%)\ Tp = \frac{\Sigma Ep}{\Sigma Fp} = \frac{\Sigma(T \times Fp)}{\Sigma Fp} \quad (3) \qquad (4)$$

Where:

T = Transmission value

Fe = erythema flux at wavelength λ

Ee = The erythema flux passed by sunscreen

Fp = Pigmentation flux at wavelength λ

Ep = Pigmentation flux passed by sunscreen

The % Te and Tp value obtained from each concentration was extrapolated to set up the linear equation to calculate the 1% Te and 40% Tp.

3. Result and Discussion

The sunscreen activities of papaya leaf extracts were displayed in Figure 1. The effectiveness of papaya leaves extracts as sunscreen was evaluated from the concentration of extract required to reach SPF 15, 1% Te, and 40% Tp. The chart in Fig. 1 indicates a negative correlation between the concentration of extracts and its sunscreen capacity. The lower the concentration required to reach SPF 15, 1% Te and 40% Tp, the higher sunscreen capacity of papaya leaf extracts.

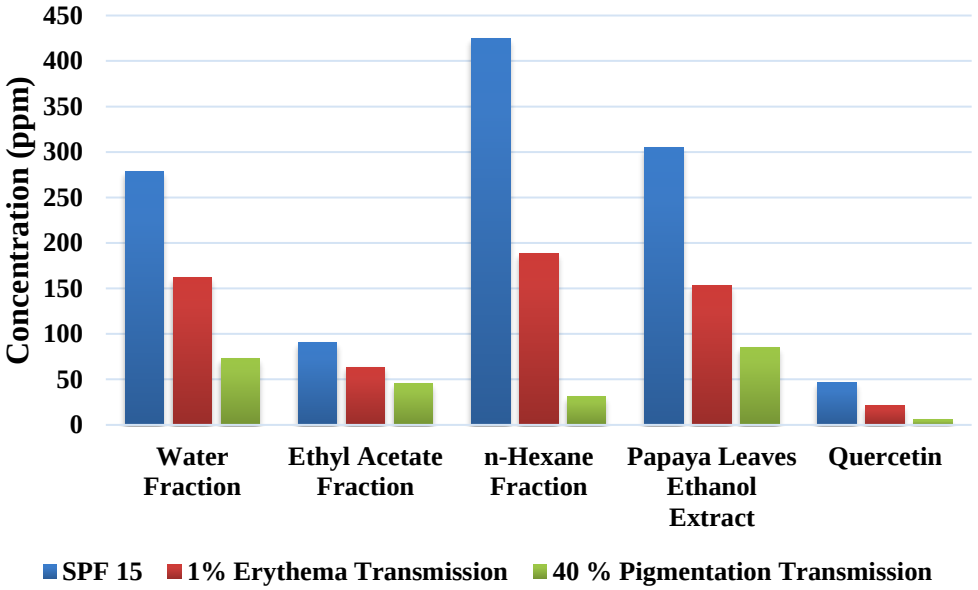

Figure 1: Sunscreen activities from different fractions of *Carica papaya* leaves extract

SPF is a measurement that shows how much the sunscreen will protect the skin from the burning radiation of UV rays. The SPF value according to European Commission (EC) Recommendation (11) categorized as follows: SPF 6-10 (low protection), SPF 15-25 (medium protection), SPF 30-50 (high protection), SPF 50+ value (very high protection). The SPF 15 evaluated in this study fall into medium protection category which block about 93% of UV B rays. The concentration of papaya leaf extracts required to reach SPF 15 are shown in table 2.

Table 1. Concentration of papaya leaf extracts at SPF 15

Sample	Concentration (ppm)
Water extract	278.29
Ethyl acetate extract	90.08
n-hexane extract	424.83
Ethanol extract	305.03
Quercetin (control)	46.33

Table 1. shows that the ethyl acetate extract has the most effective sunscreen activity with a minimum concentration to reach the SPF 15 occur at concentration of 90.08 ppm. This concentration is quite low compared to the compounds allowed in cosmetics products such as glyceryl PABA (3%), escalol 75A (5%), 2-ethylhexyl salicylate (5%), sunarome WMO (5%), digalloyl trioleate (3%), homomenthyl salicylate (8%), propylene glycol salicylate 4% and other natural compounds (12, 13).

The sunscreen capacity of papaya leaf ethyl acetate extract was supported by study of Marliani *et al.*, (14) and Sachin *et al.* (15). Previous data reveal it was required 50 µg / mL to 7 0 µg / mL papaya peel extract to reach SPF 2 (minimal protection category), and 14.2% papaya fruit extract to reach SPF 16.

The sunscreen activity of ethyl acetate extract could be associated with the present of the phenolic compounds (65.12 mg GAE/g) in its dry leave powder (16). Phenolic well known as main a compound that are responsible for the antioxidant activity as demonstrated by various natural ingredients (17, 18). Many researches also confirm the strong correlation between antioxidant activity and the sunscreen activity of phenol compounds (19, 20). For example, some Brazilian medicinal plants which contain phenolic compounds up to 3.77 to 57.14 mg GAE/g showing sunscreen activity ranging from SPF = 20.12 to SPF = 26.82 (21). Among the various phenolic compounds, the flavonoids are perhaps the most important group. The sunscreen activity of flavonoids against UV radiation were provided by the presence of double bonds structure and hydroxyl groups which act as strong UV absorbing agent (22). Flavonoids are suspected as a responsible compound for sunscreen activity also due to property of ethyl acetate as one of the best flavonoids' solvent (23). Other compounds possible for sunscreen activity in the ethyl acetate extract come from natural benzophenones group which is soluble in ethyl acetate (24). Benzophenones are a naturally occurring compounds in some flowering plants that block ultraviolet (UV) rays and widely used as sunscreen ingredients in cosmetic products.

Percent transmission of erythema (% Te) and transmission of pigmentation (% Tp) are the amount of sunlight transmitted to the skin surface after passing through the sunscreen which causes erythema and pigmentation. Erythema was a hypersensitivity reaction of the skin to UV radiation characterized by redness and inflammation symptoms, and the pigmentation was a darkening (tanning) of certain skin areas because of the formation of new melanin in the skin surface. The value of (%) Te and (%) Tp were used to determine the sunscreen category of a material i.e. % Te <1 and % Tp 3-40 is categorized as sunblock, Te% 1-6 and% Tp 42-86 is categorized as extra protection, % Te 6 -12 and % Tp 45-86 is categorized as suntan, and Te% 10-18 and% Tp 45-86 and is categorized as tanning. Table 2 and Table 3 show the concentrations of papaya leaf extracts required to reach 1 % Te and 40% Tp at the extra protection category.

Tabel 2. The concentration of papaya leave extracts at 1% Te

Sample	Concentration (ppm)
Water extract	161.91
Ethyl acetate extract	63.49
N-hexane extract	188.55
Ethanol extract	152.87

Quercetin (control)	20.88

Table 2 show that ethyl acetate extract was the most effective to prevent the incidence erythema less than one % at a concentration of 63,49 ppm. Similar to the evaluation of SPF 15, the compounds responsible for blocking sunlight and reducing the incidence of erythema in ethyl acetate extract are suspected to come from the phenolic group. Apart from phenolic, alkaloids are the possible compounds that act as a sunscreen because the alkaloids dissolve in ethyl acetate. Several studies have confirmed the positive correlation between plant alkaloids and sunscreen activity.

Tabel 3. Concentration of papaya leave extracts at 40% Tp

Sample	Concentration (ppm)
Water extract	72.57
Ethyl acetate extract	45.21
N-hexane extract	30.68
Ethanol extract	84.96
Quercetin (Control)	5.48

Different from the determination of SPF 15 and 1 % Te, table 3 shows that n-hexane extract was the most effective to reach 40% Tp. N-hexane extract reduce 40% pigmentation at the concentration of 30,68 ppm. The difference may occurs due to the different cause of erythema and pigmentation. Erythema was induced by exposure of skin to UV-B rays while pigmentation was induced by exposure to UV-A rays, therefore the determination of %Te was applied to show protection against UV-B rays and determination of % Tp was applied to show protection against UV-A rays.

The sunscreen compounds which inhibit transmission of pigmentation n-hexane extracts probably comes from terpenoids group especially carotenoids. The presence of carotenoids in n-hexane extract is associated with n-hexane property as a non-polar solvent suitable for extracting the terpenoid/carotenoid compounds from fruit and plant. Study of Ugo *et al.* (25) revealed the presence of beta carotene in papaya leaves extract up to 303.5 mg/100g. The carotenoids in plants act to protect the photosynthetic elements from damage by absorbing the excess of light energy. The application these compounds in skin are expected to possess similar effect to protect the skin from negative effects of excessive sunlight (26, 27). The sunscreen property of carotenoids was confirmed in a recent study of Darvin *et al.* (28). The study revealed that application beta carotene topically effectively neutralized free radicals produced on the skin surface after IR irradiation. Other study also confirmed the positive effects of combined oral/topical antioxidant treatment with lutein and zeaxanthin on human skin (29).

4. Conclusion

The results of this study confirmed that papaya leaf extracts have a significant sunscreen property. The ethyl acetate and n-hexane extracts proved to reduce the occurrence of erythema and pigmentation at low concentrations of 63.49 ppm and 30.68 ppm respectively. A combination of two extracts has the potential for use as an active ingredient in sunscreen cosmetic. Further study should be conduct to elucidate the compounds responsible for sunscreen activity and how to utilize as a safe cosmetic ingredient.

5. Acknowledgements

The authors thank to Faculty of Sciences and Mathematic and Natural Sciences, Universitas Pakuan, Indonesia for funding this study.

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