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In vitro evaluation of sun protection factor of *Vasconcellea pubescens* fruit extract

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Abstract. *Vasconcellea pubescens* contains flavonoid compounds that can protect the skin from sunlight due to its antioxidant activity. The purpose of this study to investigates the in vitro sun protection factor (SPF) of *Vasconcellea pubescens* fruit extract. *Vasconcellea pubescens* is a promising candidate for use in cosmetic and pharmaceutical formulations. The experiment was done by extracting the *Vasconcellea pubescens* fruit with maseration method, and then the extracts were divided into five different concentration C1=150 ppm, C2=200 ppm, C3=250 ppm, C4=300 ppm, and C5=350 ppm. The sun protection factors were analyzed by UV-VIS spectrophotometry to determine the SPF value. The result of in vitro study gained SPF value for all five formulas were high enough C1=27.169; C2=32.466; C3=38.571; C4=40.948; and C5=44.449. This proved activity of plant showed its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sun protector that used now a day in the industry.

Keywords: *Vasconcellea pubescens*, sun protector value (SPF), in vitro

1. Introduction

Every year, more than one million people are diagnosed of skin cancer and about ten thousand people die because of malignant melanoma. Most skin cancers occurs on the areas which are most frequently exposed to the sun, such as face, neck, hand, and foot. The ultraviolet radiation (UVR) is classified into three types by its wavelengths such as UV-A, UV-B and UV-C. The dimensions of their wavelength are roughly 400-320 nm for UV-A, 320- 290 nm for UV-B and 209-200 nm for UV-C [1]. Various studies show the great influence of solar radiation on skin. UV-A and UV-B are mainly responsible for skin hazards such as sunburn, cutaneous degeneration, cell skin cancer. The ultraviolet radiation (UVR) is responsible to form a complex process associated with morphological and chemical reactions. DNA is an important macro molecule which absorbs UVR and causes mutate, in the future can result malignant trans-formation of the cell skin cancer [2].

The effective protection against UVR is available as preparations for topical use containing solar filters, known as sunscreens. The efficacy of such products is dependent on their capacity to absorb radiant energy. The effectiveness of a sunscreen is measured as a function of their sun protection factor (SPF). Thus, the necessity to provide high SPF and screening efficiency against both ultraviolet A and ultraviolet B wavelengths is evident [3].The higher the SPF, the more protection a sunscreen offers against sunburn. There is now an increasing body of evidence that the use of sunscreen is not entirely safe for sunscreen protection. Natural products are therefore important sources for research in new active compounds [4].. This offers the possibility of discovering new biological mechanisms to obtain new active molecules and to study their structure function relationships in order to develop more active drugs and to avoid unwanted side effects. Natural substances have been recently considered as potential sunscreen resources because of their absorption in the UV region and their antioxidant activity [5]. The various herbal formulation and chemicals are available to block various range of UV rays which prevent all types of skin from various damages [6].

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The carica fruit or mountain papaya (*Vasconcellea pubescens*) is a plant that grows in the Dieng Plateau. Carica fruit contains antioxidant compounds and sources of flavonoids that can counteract free radicals [7]. Flavonoids compounds are widely distributed in the plant kingdom and possess a biological action, especially antimicrobial, antioxidant and photo protective activities [8]. The demand for active flavonoid rich extracts has become an important component for the discovery of new molecules active to human photo-protection. That is due to its structural similarity to chemical filters which makes it susceptible to radiation absorption in the ultraviolet region [9]. Plant extracts rich in flavonoids are capable of absorbing ultraviolet, usually two maximum peaks of ultraviolet absorption in the UV-B and UV-A regions, what results in the possibility for the use of these extracts in the development of sunscreen formulations [2]. Based on the potential of carica fruit (*Vasconcellea pubescens*) this research will be conducted to investigates the in vitro sun protection factor (SPF) of *Vasconcellea pubescens* fruit extract which is a promising candidate for use in cosmetic and pharmaceutical formulations.

2. Matodology

The sample used was carica fruit (*Vasconcellea pubescens*) was taken from Dieng Sub-district, Wonosobo Regency, Central Java. Equipments was used UV-Vis Genesys spectrophotometer.

2.1. Methods

2.2.1 *Preparation and Extraction Stage of Carica Fruit Samples*

10 kg of carica fruit were cleaned and cut into small pieces, then the samples were dried using 40°C oven approximately 5x 24 hours to form dry simplicia. The extraction of carica fruit was solved using the maceration method using 70% ethanol solvent at room temperature (25°C). Stirring was done for 15 minutes using a stirring bar. The maseration was done for 3x24 hours. After that, screening, remaseration and evaporation was done to get the viscous extract.

2.2.2 *In Vitro* Test (SPF value)

The determination of the effectiveness of sunscreen was done by using UV-Vis spectrophotometer instrument. The extracts were divided into five different concentration C1=150 ppm, C2=200ppm, C3=250 ppm, C4=300 ppm, and C5=350 ppm. The sun protection factors were analyzed by UV-VIS spectrophotometry to determine the SPF value. Each concentration was read at a wavelength of 290-320 nm with a distance of 5 nm intervals. Aquadest was used as blanco. The absorbance value was used to calculate the SPF value [10].

$$SPF=CFx\sum_{290}^{320} EE\,(\lambda) \times I\,(\lambda) \times \text{absorbance}\,(\lambda) \qquad (1)$$

CF = Correction factor (10)
EE = Erythmogenic effect of radiation with wavelength λ
I = Solar Ray Simulation Spectrum
Abs = spectrophotometric absorbance values at wavelength λ

3. Result and Discussion

The SPF value of each carica fruit extracts concentration result of this study can be shown in Table 1.

Table 1. The SPF value of five different fruit extracts concentration

Concentration	SPF 1	SPF 2	SPF 3	TOTAL SPF	Protection
150 ppm	27.195	27.165	27.145	27.169 ± 0.025171	Medium
200 ppm	32.476	32.446	32.476	32.466 ± 0.017324	High
250 ppm	38.718	38.488	38.508	38.571 ± 0.127436	High
300 ppm	40.938	40.948	40.958	40.948 ± 0.010002	High
350 ppm	44.459	44.449	44.439	44.449 ± 0.010002	High

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. The effectiveness of sunscreen can be determined by in vitro test which was used UV spectrophotometric method that produced absorbance value. Absorbance value showed the value of light protection factor (SPF)[11]. To be effective in preventing sunburn and other skin damages, a sunscreen product should have a wide range of absorbance between 290 and 400 nm. The in-vitro SPF is useful for screening test during the product development [5]. The UV radiation was

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measured by UV spectrophotometer. The measurement was done by diluting the skin lotion into concentration 4000 ppm. The solution was read at the wavelength of 290-320 nm which was further calculated by following equation [10] :

$$SPF=CFx\sum_{290}^{320} E\,E\,(\lambda) \times I\,(\lambda) \times \text{absorbance}\,(\lambda) \quad (1)$$

CF = Correction factor (10)
EE = Erythmogenic effect of radiation with wavelength λ
I = Solar Ray Simulation Spectrum
Abs = Spectrophotometric absorbance values at wavelength λ

Each sample's absorbance was measured to evaluate the SPF value of sample containing a wide variety of chemicals that have specific absorbance in some parts of the UV spectrum. Due to containing a wide range of natural compounds, plant extracts usually cover full range of UV wavelengths. One approach to protecting the body from the harmful effects of UV irradiation is to use active photoprotectives. The phenolics may be beneficial in preventing UV-induced oxygen free radical generation and lipid peroxidation, *i.e.* events involved in pathological states such as photo aging and skin cancer [12]. Antioxidant activity is important in UV protection. High concentration of flavonoids such as rutin in plants may be used to prevent UV-induced oxygen free radical generation, too [13].

The carica fruit or mountain papaya (*Vasconcellea pubescens*) is a plant that grows in the Dieng Plateau. The morphology character, antioxidant capacity, and protein banding analysis on *Vasconcellea pubescens* have been studied by Laily [14], but specific research about the active compound of *Vasconcellea pubescens* to be drug raw material and its conservation have not been studied yet. Novalina states that carica leaf extract (*Vasconcellea pubescens*) contains flavonoids, alkaloids, tannins and phenols [15]. According to research from Lumbessy [7] carica fruit contains antioxidant compounds and sources of flavonoids that can counteract free radicals. The fruit of its plant contains flavonoid which is closely associated with antioxidant activity [16]. The phenolics exhibit wide variety of benevecial biological activities, including antiviral, antibacterial, immune stimulating, antioxidants, sunprotector, etc [17].

We made five different concentration of carica fruit extract to determine the SPF value of carica fruit extract and to know the relation between concentration and the SPF value. The concentration used are 150 ppm, 200 ppm, 250 ppm, 300 ppm and 350 ppm. And the result obtain shows that the higher concentration of carica fruit extract, lead to the higher SPF value too. It happens because the higher the level of flavonoid which contained in the extract of medicinal plants the more light absorbed by the active molecules at certain wavelengths so that the absorbance value is getting higher [18]. The higher absorbance value the higher SPF value. High concentration of flavonoids used to prevent UV-induced oxygen free radical generation. High chemical content in herbs include flavonoids and phenolics lead the extract of this plant have photoprotection potential because of their ability to absorb UV light. Flavonoids and phenolics also have the ability as antioxidants and also as anti-inflammatory and immunomodulatory. The role of flavonoids and phenolics in plants is also to protect plants from sun UV radiation [5].
SPF value can be divide into four different categories those are not sunscreen category, low, medium and high. The range less than 2 means it is not sunscreen category, the range between 2 until 11 means it has minimum protection, the range between 12 until 30 means it has medium protection, and the range more than or equal to 30 means it has high protection [19]. The result shows that carica fruit extract in 150 ppm has medium protection, meanwhile carica fruit extract in 200, 250, 300, and 350 ppm have high protection. This SPF value is good enough and proves that carica fruit extracts have the effectiveness as sun protector that can be derived from the existing flavonoid content. Further research is needed regarding its effectiveness when it has been formulated, because it can show different SPF values due to several aspects affecting the determination of SPF values, for example, the use of different solvents in which the sunscreen are dissolved; the combination and concentration of the ingredient; the nature of emulsiaon, etc [20].

Conclusion

The result obtained were showed the ability of extract to absorb UV radiation and proved UV protection ability. Further, isolated fruit extract of *Vasconcellea pubescens* have the major antioxidant is also stable when exposed to UVB irradiation. The result of in vitro study gained SPF value for all five formulas were high enough C1=27.169; C2=32.466; C3=38.571; C4=40.948; and C5=44.449. This proved activity of plant showed its importance and prophylactic utility in anti-solar formulation. This will be a better, cheaper and safe alternative to harmful chemical sunscreens that used now a day in the industry. Besides its antisolar activity and effects, making it a useful sun care as well as skin care product.

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