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Research Article

POTENCY OF PURIFICATION EXTRACT FROM BELIMBING WULUH (*AVERRHOA BILIMBI*) AS ANTIOXIDANT AND ANTI-TYROSINASE

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ABSTRACT

***Purpose**: The purpose was to investigate purified extract of belimbing wuluh (PEBW) leaves as Indonesian plant for natural whitening agent.*

***Method**: The purified extract of belimbing wuluh leaves used as a test Indonesian material. To investigate total phenolic content using folin-ciochalteu reagent, total flavonoids using AlCl3 reagent. The absorbance of belimbing wuluh were obtained on range 290-320 nm for SPF, 290-315 nm for percent erithema (%TE) 320-370 nm for percent pigmentation (%TP). Test for antioxidant activity with DPPH. Tyrosinase inhibitor activity using mushroom tyrosinase assay.*

***Results**: Purified extract of belimbing wuluh leaves have a total phenolic content of 42.22±0.44 mgGAE/g and total flavonoid content of 41.38±1.31 mgRE/g. SPF values at a concentration of 300 ppm were obtained 10.64±0.66 with %TE 38.79±6.01% and %TP 9.28±0.79%. The IC50 value of antioxidant activity is 77.09±8.21 µg/mL greater than rutin 5.92 µg/ml. The IC50 values of Tyrosinase inhibitor activity is 224.16±34.55 µg/mL greater than kojic acid 16.68 µg/ml.*

***Conclusion**: Purified extract of belimbing wuluh leaves have potential to be developed as sunscreen cosmetics and whitening agents.*

***KEYWORDS:** Belimbing wuluh, Purified extract, Sunscreens, Antoxidant, Tyrosinase inhibitor.*

INTRODUCTION

Tropical plants are known to be associated with many medicinal properties. In developed countries began to switch to medicinal ingredients [1]. In this study we chose the tropical plants commonly grown in Indonesia, namely *Averrhoa bilimbi*, known as Belimbing wuluh. Belimbing wuluh is widely used in traditional herbs for skin health. Methanol extract of belimbing wuluh fruit was investigated for total phenolic content [2]. Phytochemical content of fruit extracts contains flavonoids [3]. Total phenolic exploration and total flavonoids from various parts of the plant will produce different amounts [4].

Ultraviolet (UV) can be a major factor in skin problems such as aging, erythema, hyperpigmentation, wrinkles, dermatitis and skin cancer [5]. Natural chemicals such as polyphenols as flavonoids, are more effective than synthetic chemicals that are due to long-term effects that are useful especially against damage to the skin produced by free radicals along with UV-blocking. Skin requires several means of protection to produce long-term benefits and avoid chronic conditions such as cancer. Therefore the use of natural ingredients can be an alternative to sunscreen products. Skin whitening products for cosmetic purposes can be used on lighter skin appearance. Whitening agents act at various levels of melanin production in the skin. Many of them are known as competitive inhibitors of tyrosinase, a key enzyme in melanogenesis [6].

To our knowledge, there have been no reports for purified extracts on belimbing wuluh and test for total phenolic content, total flavonoid content and sun protection factors. The main objective of this study was to determine the total phenolic content, total flavonoid content using folin-ciocalteu reagent and colorimetric test, respectively and determination of sun protection factor (SPF) sunscreen with ultraviolet spectrophotometry [7] and antioxidant tests and inhibition of the tyrosinase enzyme. The purpose was to investigate purified extract of belimbing wuluh (PEBW) leaves as Indonesian plant for natural whitening agent.

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EXPERIMENTAL

Material and chemicals:

Materials:

Plant Material: Fresh of *Belimbing Wuluh* leaves were determination by a botanist at laboratory of pharmaceutical biology Sekolah Tinggi Ilmu Farmasi "Yayasan Pharmasi Semarang" (Stifar).

Chemical reagents: Ethanol, Methanol, Folin–Ciocalteu, Natrium Carbonate, aluminium chloride, Natrium Acetate, Gallic acid and Rutin were supplied by Sigma–Aldrich (St. Louis, MO, USA), DPPH kojic acid, tyrosinase from mushroom as enzym, L-tyrosine as a substrate. All chemicals and reagents used in the study were of analytical grade.

Extraction of belimbing wuluh:

The 200 g dried and powdered BW leaves were remaserated with 96% ethanol for 3 days at room temperature. Liquid extract was evaporated at 60°C, 100 RPM, to viscous extract.

Purification extract of belimbing wuluh (PEBW):

The viscous extract is added to the water and fractionated with a solvent starting from a non-polar solvent to a polar solvent. The solvents used are n-hexane, ethyl acetate and water itself. Water extract of BW, added solvent n-hexane placed in a separating funnel shaken, allowed to stand, and separated. Purification is then carried out sequentially with ethyl acetate. A layer of water is collected and evaporated. The resulting purified extract was used as a sample of this study [8].

Determination of Total Phenolic Content:

Analysis of total phenolic content using the Folin-Ciocalteu method whose absorbance measured at a wavelength of 750 nm [9]. About 100 mg PEBW added with aquadest in 10 ml flash. Added 0.5 ml extract (1 mg/ml) PEBW solution with 0,4 mL Folin-Ciocalteu reagent, incubated for 4-8 minutes. Moreover, added with 4.0 mL 7 % Natrium Carbonate. Then, added with distilled water, incubated 2 hours (as operating time) at room temperature. The last measured in 750 nm using a UV-Vis spectrophotometer (Shimadzu UV-1280, Japan). A calibration curve using a standard solution of Gallic acid *(mg GAE/g).* Gallic acid is made with a concentration variation of 20-140 μg/mL and the same reagent is added.

Determination of Total Flavonoid Content:

Analysis of total flavonoid content using $AlCl_3$ reagent according to [10, 11] with modification. PEBW with concentration 1.000 μg / mL in methanol. As much as 0.5 mL PEBW added with 1,5 mL methanol, 0,1 mL 10 % $AlCl_3$, 0,1 mL Natrium acetate 1 M and added 2,8 mL distillated water. Then, incubated for 30 minutes (as operating time) in room temperature and measured in 415 nm using a UV- Vis spectrophotometer (Shimadzu UV-1280, Japan). *Rutin was used as a standard for calibration curve and the results were expressed as rutin equivalents (*mg *RE/g). Rutin is made concentration variation of 100 – 400* μg/mL and the same reagent is added.

In Vitro Assesment of Sunscreens Activity:

Determination of Sun Protection Factor (SPF):

Determination of SPF in vitro using spectrophotometer UV [12]. Each 100, 200 and 300 ppm extract of PEBW diluted with aquadest. The absorbance of PEBW solution were obtained on range 290-320 nm for SPF, 290-315 nm for percent erithema, 320-370 nm for percent pigmentation. The absorbance data were obtained with interval 5 nm [13]. SPF values can be calculated using the eq 1.

$$SPF(spectrophotometric) = \text{CF } x \sum_{290}^{320} EE\ (\lambda)\ x\ I\ (\lambda)\ x\ Abs\ (\lambda) \quad \text{.....(1)} \ [13]$$

Determination of transmission erythma (%TE) and transmission pigmentation (%TP):

The extracts were prepared same as in the determination of SPF, then measured uptake with UV-Vis spectrophotometer in wavelengths of 292 nm - 372 nm. Absorbance (A), the transmission (T) is calculated by the formula, A = - log T. Then, Transmission of erythm (Te) calculated by the formula, Te = T x Fe. Where, (Fe) is flux of erythema whose value at certain wavelengths can be seen on[14]. Value of flux erythm which is continued by the sunscreen (Ee) is calculated by the formula, Ee = Σ (T x Fe). Erythema transmission (%TE) can be calculated using the eq 2.

$$\text{\% Erythema transmission} = \frac{Ee}{\sum Fe} = \frac{\sum (T \times Fe)}{\sum Fe} \quad \text{.....(2)} \ [14]$$

With same concentrations of SPF and erythm, than absorbance measured on Wavelengh 292-372 nm. Calculated transmission of pigmentation (TP) follow the formula Tp= T x Fp, Where Fp is flux of pigmentation with wavelength certain. Have a lot of flux pigmentation follow the formula, Ep = Σ (T x Fp). While the pigmentation transmission % (%TP) is calculated by the eq 3.

$$\text{\% transmission pigmentation} = \frac{Ep}{\sum Fp} = \frac{\sum (T \times Fp)}{\sum Fp} \quad \text{.....(3)} \ [14]$$

Determination of antioxidant activity:

The antioxidant activity of PEBW was evaluated using 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according [15]. PEBW 10 mg diluted with methanol in 10 ml flask. From this PEBW solution a series of concentrations was made from 100 μg/mL to 5 μg/ mL. One mL of this solution added with 1 mL DPPH 0.4 mM and methanol until 5 mL in volumetric flask. The contents were mixed and incubated for 30 minutes as aperating time same as. PEBW series solution measured at a maximum wavelength of 517 nm as λmax with Vis spectrophotometer [16]. Rutin standard antioxidant are used as a comparison made with concentration 2 μg/mL to 10 μg/ mL.

IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals [17].

Determination of enzyme inhibition activity:

In the PEBW test for inhibition of tyrosinase activity used Lai method [18] with modification, 500 µL of PEBW with various concentrations mixed in 1000 µL of phosphate buffer solvent pH 6.8 was added with 500 µL L-tyrosine (2.5 µM) in a phosphate buffer pH 6.8. The solution mixture was incubated in a dark place for 10 minutes. Then, 500 µL of mushroom tyrosinase solution (25 KU) was added to the mixture, which was then incubated for 30 minutes at room temperature. Absorbance was measured with spectrophotometer at 480 nm. The concentration of kojic acid was made at a concentration of 10-20 µg/mL and treated with the same sample. For blanks, complete analytical procedures are followed, including all chemicals and solvents, but no samples are addedInhibitory effects of the PEBW were expressed as the inhibitor concentration causing a 50 % loss of enzyme activity (IC50) [19].

RESULT

Total Phenolic Content:

PEBW as a sample and gallic acid as a standard were reacted with Folin-Ciocalteu reagent which produced a yellow color which showed that containing phenol, then added with Na2CO3 solution would produce a blue color which was a molybdenum-tungsten complex. Phenolic compounds from PEBW react with the Folin-Ciocalteu reagent in an alkaline atmosphere to allow dissociation of protons in phenolic compounds into phenolic ions because of the addition of Na2CO3 solution to the sample. The maximum wavelength obtained is 718 nm with an operating time of 120 minutes. The equation of the standard gallic acid curve is obtained (y = 0.0054 x + 0.0325, R = 0.99784). The standard gallic acid curve can be seen in fig 1. Total phenolic content of PEBW can be seen at table 1.

Total Flavonoid Content:

PEBW samples and rutin standards were dissolved in methanol and reacted with AlCl3 reagent to form a routine complex-AlCl3, a wavelength shift was shown by the solution which produced a more yellow color. Sodium acetate is added to maintain the wavelength in the visible region. The maximum wavelength obtained is 413 nm with an operating time of 30 minutes. The standard curve equation obtained is routine (y = 0.0026 x + 0.018, R = 0.99755). The standard rutin curve can be seen in fig 2. Total flavonoid content of PEBW can be seen at figure 1.

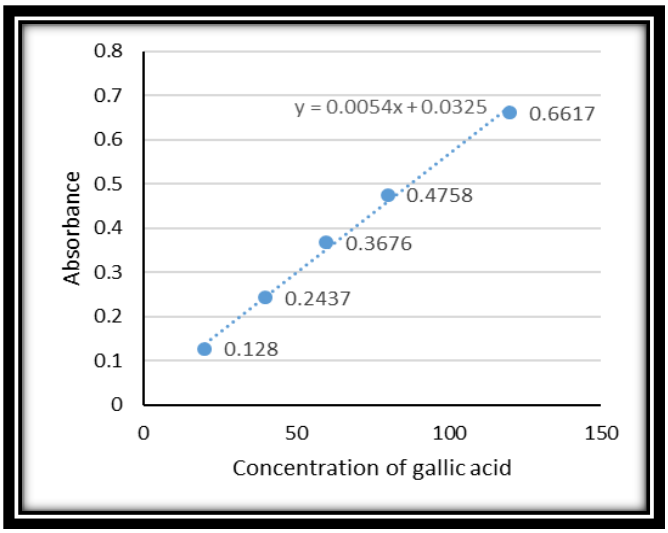

Fig. 1: Standard gallic acid curve

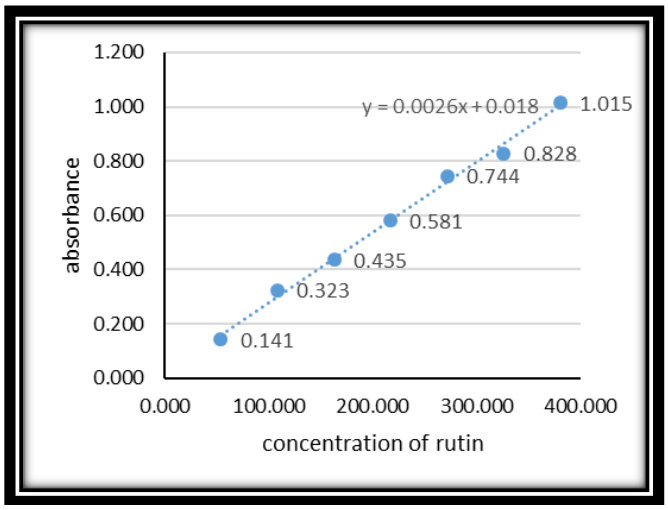

Fig. 2: Standard Rutin Curve

Table No. 1: Quantitative Result of PEBW

Quantitative	PEBW
Total phenolic content	42,22±0,43 mgGAE/g of sample.
Total flavonoid content	41,38±1,30 mgRE/g of sample

In Vitro Assesment of Sunscreens Activity:

Determination of Sun Protection Factor (SPF):

The SPF in vitro was determined by the spectrophotometric method developed by Mansur [13] using the UVB region, considered to be the region of greatest incidence during the day in which people are exposed for longer [12]. Percent of TE (transmission erythema) and TP (transmission pigmentation) sunscreen can be determined using spectrophotometry which is measuring the intensity of light transmitted by the active substance of the sunscreen in the wave range erythema and pigmentation. TE and TP can be calculated by measuring absorption at each wavelength, TE at a wavelength of 292.5 - 337.5 and TP 322.5 - 372.5 with intervals of 5 nm. From the absorption value obtained calculated the absorption value and the percent transmittance value with the formula A = - log T. The value of erythema transmission is calculated by multiplying the transmission value by Erythema (Fe) effectiveness factor. The pigmentation transmission value is calculated by multiplying the T value by the pigmentation effectiveness factor (Fp). Next value percent of erythema and pigmentation transmission is calculated by equation (2) and (3). The sunscreens activity can be seen in table 2.

Table No. 2: Sunscreens Activity of PEBW

sunscreens activity	PEBW concentration		
	100 µg/mL	**200 µg/mL**	**300 µg/mL**
SPF value	3.98±0.16	7.26±0.22	10.64±0.66
% Erythema transmission	190.66±8.03	88.36±3.43	38.79±6.01
% Pigmentation transmission	25.25±0.52	14.61±1.41	9.28±0.79

Determination of antioxidant activity:

The principle of this antioxidant activity test method is measurement Quantitative antioxidant activity is by doing DPPH radical capture measurements by a compound have antioxidant activity using UV-Vis spectrophotometry so that the value of the activity will be known free radical reduction expressed with IC50 value (Inhibitory Concentration). The IC 50 value is defined as the concentration of the test compound which can reduce free radicals by 50%. The smaller the IC50 value, the higher the activity of free radicals. The working principle of this measurement is the presence of free radicals stable is DPPH which is mixed with antioxidant compounds that have the ability to donate hydrogen, so that free radicals can be muted [19, 20]. The IC_{50} value of antioxidant activity of PEBW greater than rutin as standard can be seen at table 3.

Determination of tyrosinase activity:

PEBW has tyrosinase enzyme inhibitory activity. This is reflected in the increase in the concentration of PEBW has increased the percentage of inhibition of the enzyme tyrosinase or IC 50 is getting smaller. Tyrosinase inhibitor activity of PEBW can be seen from IC_{50} values greater than kojic acid as standard.

Table No. 3: Antioxidant and Inhibition of Tyrosinase Activity PEBW

Activity	IC50 PEBW (µg/mL)	IC50 standard (µg/mL)
Antioxidant	77.09±8.21	5.92
Inhibition of tyrosinase	224,16±34.55	16.68

DISCUSSION

Liquid extract obtained from belimbing wuluh extraction process with use organic solvents or water often contains compounds which is often detrimental to stability and reduce the levels of active compounds inside extract so it must be removed (such as dyes (pigments), carbohydrates, waxes, resin). Purification goal namely to remove compounds disturbing but still maintaining active compound. Purified extract the results obtained in this study are positive contains phenolic and flavonoids. This matter indicates that the purification process is not remove the active compound in the extract [8, 11].

From table 2 it can be seen that SPF value of PEBW is in the maximum potential at the lowest concentration (100 ppm) to ultra-potential at high concentrations (300 ppm) [21]. When associated with total phenolic and total flavonoid data, table 2 shows that the sunscreen active ingredients found on the leaves of belimbing wuluh are suspected of phenolic and flavonoid compounds and other components that have a conjugated double bond that can absorb UV light in the 290-320 nm range [4]. From the calculation data of erythema and pigmentation % transmission which can be seen in table 2 it can be seen that PEBW which tested has protective effect to the radiation of sunlight, especially UV-A and UV-B. This is seen in the % erythema value and the % pigmentation value at concentrations of 100 to 300 ppm decreases. Only one PEBW concentration of 300 ppm can be drawn in the standard suntan category. Some of the mechanisms of action of flavonoids in photo protection include Inhibits UV mediated induction of ornithine decarboxylase activity, down-regulates COX-2 expression in macrophages, Inhibits UV mediated induction of ROS and Protects skin's antioxidant systems (glutathione peroxidase, glutathione reductase, catalase and superoxide dismutase activities), prevention of UVC radiation-induced liposome peroxidation SPF [5].

Antioxidant activity is mainly exhibited by phenolic and flavonoid components from PEBW. The way this compound works to reduce skin melanin is by inhibiting tyrosinase activity

[22]. The color of the skin depends on the pigment known as melanin in the dermis of the skin. Skin pigmentation occurs mainly due to increased melanogensis by melanocytes stimulated by UV light exposure [6]. Melanocytes produce melanin in the skin as a mixture of two eumelanin pigments and phaeomelanin [23]. Melanogenesis is achieved by a series of oxidative reactions controlled by various enzymes. Tyrosinase is the main Tyrosinase is the main catalyst for this phenomenon.

CONCLUSION

PEBW leaves have potential to be developed as sunscreen cosmetics and whitening agents.

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REFERENCES:

1. AG. Patil, SP. Koli and DA. Patil. Pharmcognostical standardization and HPTLC fingerprint of Averrhoa bilimbi (L.) fruits. J Pharm Res **2013**;6(1):145–150.
2. M. Hasanuzzaman, MR. Ali, M. Hossain, S. Kuri and MS. Islam. Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of Averrhoa bilimbi (fruits). Int Curr Pharm J **2013**;2(4):92–96.
3. Ashok Kumar. A Review on Phytochemical Constituents and Biological Assays of Averrhoa Bilimbi. Int J Pharm Pharm Sci Res **2013**;3(4)136–139.
4. Yinjie Jin, Yanbing Zhang and Ke Yuan. The study on antioxidant activity and content of total flavonoidsand total phenolic in different parts of Abelmoschus esculentus L. IEEE Int Sympos on IT in Med and Edu **2011**;169–172.
5. M. Donglikar, S. Deore, S. Deore and S. Deore. Sunscreens: A review. Pharmacogn J **2016**;8(3):171–179.
6. N. Smit, J. Vicanova and S. Pavel. The hunt for natural skin whitening agents. Int J Mol Sci **2009**;10(12)5326-49.
7. A. Hosu, VM. Cristea and C. Cimpoiu. Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines using artificial neural networks. Food Chem **2014**;150:113–118.
8. A. Trejo-González et al. A purified extract from prickly pear cactus (Opuntia fuliginosa) controls experimentally induced diabetes in rats. J Ethnopharmacol **1996**.
9. R. Suharsanti, N. Sugihartini, E. Lukitaningsih and RR. Rahardhian. Effect of Different Solvent on Total Phenolic, Total Flavonoid, and Sun Protection Factor of Belimbing Wuluh (Averrhoa Bilimbi). J Glob Pharm Tech **2019**; 11(01S):154–162.
10. CC. Chang, MH. Yang, HM. Wen and JC. Chern. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. **2002**;10.
11. A. Nugroho, A. Malik and S. Pramono. Total phenolic and flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentale L.). **2012**;20.
12. EA. Dutra, DAG. da C. Oliveira, ERM. Kedor-Hackmann and MIRM. Santoro. Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry. Rev Bras Ciências Farm **2004**.
13. JS. Mansur, MNR. Breder, MCA. Mansur and RD. Azulay. Determination of Sun Protection Factor by Spectrofotometry. An Bras Dermatol **1986**.
14. MS. Balsam and E. Sagarin. 1913-1986, Eds., Cosmetics : Science and technology / Edited by M. S. Balsam and Edward Sagarin. New York: Wiley-Interscience, **1972**.
15. MRRR. Ririn Suharsanti, Nining Sugihartini, Endang Lukitaningsih. Potency of Belimbing Wuluh (Averrhoa Bilimbi) as Antioxidat and Tyrosinase Inhibitor for Skin Whitening Product. J Pharm Res **2019**;8(4):151–154.
16. N. Aktar, H. Khan, S. Ashraf, I. Mohammad and A. Ali. Skin depigmentation activity of Crocus sativus extract cream. Trop. J Pharm Res **2014**;13(11):1803.
17. F. Pourmorad, S. Hosseinimehr and N. Shahabimajd. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotech **2002**;5(11):1143–1145.
18. J. Lai, C. Lin and T. Chiang. Tyrosinase Inhibitory Activity and Thermostability of the Flavonoid Complex from Sophora japonica L (Fabaceae). Trop J Pharm Res **2014**; 13(2):243.
19. C. Namngam and P. Pinsirodom. Antioxidant properties, selected enzyme inhibition capacities, and a cosmetic cream formulation of Thai mango seed kernel extracts. Trop J Pharm Res **2017**;16(1):9.
20. SB. Kedare and RP. Singh. Genesis and development of DPPH method of antioxidant assay. J Food Sci Tech **2011**; 48(4):412–22.
21. JB. Wilkinson and RJ. Moore. Harry's Cosmeticology (7th edition). New York: Chemical Publishing Company, **1982**.
22. S. Momtaz et al. Tyrosinase inhibition by extracts and constituents of Sideroxylon inerme L. stem bark, used in South Africa for skin lightening. J Ethnopharmacol **2008**.
23. SM. De Leeuw et al. Melanin content of cultured human melanocytes and UV-induced cytotoxicity. J Photochem Photobiol B **2001**;61(3):106-13.

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