



## RESEARCH ARTICLE

## ***In Vitro* Assessment of Total Phenolic, Total Flavonoid and Sunscreen Activities of Crude Ethanolic Extract of Belimbing wuluh (*Averrhoa bilimbi*) Fruits and Leaves**

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### **Abstract**

*Averrhoa bilimbi*, namely belimbing wuluh in Indonesia. It's the fruits used to food in vegetable dishes. Belimbing wuluh's leaves and fruits are traditional herb in Indonesian. This study aim to determined total phenolic content, total phenolic content and sunscreen activity from ethanolic extract of Belimbing Wuluh fruits and leaves. Total phenolic content of fruits ethanolic extract is  $19.8022 \pm 1.6696$  mg GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g. Both have significant differences after tested t test ( $\text{sig} < 0.05$ ). Total flavonoid content of fruits ethanolic extract  $24.7458 \pm 0.3291$  mg RE/g more than leaves ethanolic extract  $13.3126 \pm 0.5695$  mg RE/g. Both have significant differences after tested t test ( $\text{sig} < 0.05$ ). Sun Protecting Factor of leaves ethanolic extract better than fruits ethanolic extract. Only one leaves ethanolic extract concentration of 300 ppm can be drawn in the total block valuation category with ultra protection. The conclusion is leaves ethanolic extract has good potential as an active sunscreen ingredient although it has a total phenolic content and a lower total of flavonoids than fruits ethanolic extract of belimbing wuluh. These data indicate that the sunscreen active ingredients found on the leaves of belimbing wuluh are suspected of phenolic compounds and other components that have a conjugated double bond that can absorb UV light in the 290-320 nm range.

**Keywords:** *Averrhoa bilimbi, Total Phenolic Content, Total Flavonoid Content, Sunscreen Activity.*

### **Introduction**

Exposure of Ultraviolet (UV) chronic in the skin can is key factor of skin problem like as ageing, erythema, hypopigmentation, hyperpigmentation, cracks, burns, immune suppression, wrinkles, dermatitis, and most complicated skin cancers[1]. Natural chemical like polyphenols as flavonoids, are more effective over synthetic chemicals which is due to their long term useful effects mainly against free radical generated skin damages along with UV-rays blocking.

Photoradiation mediated skin damages require multiple protection means to produce long term benefits and avoidance of chronic conditions like cancers. Hence following natural chemicals use can be ideal in

sunscreen products. Tropical fruits are well known to be related with many medicinal properties. Synchronously, many people in developed countries have started to turn to alternative or complementary cures, including medicinal herbs [2]. In this study we chose of tropical fruits commonly grown in Indonesia, viz. *Averrhoa bilimbi*, a native cultivar known as Belimbing Wuluh. Belimbing Wuluh is one of the natural constituents that are extensively used in traditional herbs for skin health.

Methanolic extract of Belimbing Wuluh fruits was investigate the total phenolic content [3]. Phytochemical constituents the fruits extract contain flavonoids [4]. Total phenolic

exploration and total flavonoids from different parts of plants will produce different quantities [5]. The total phenolic content showed a good correlation with antioxidant activity but not the total flavonoid and tannin content [6]. To our knowledge, no reports are available to compare extract ethanolic of leaves and fruits on the total phenolic content, total flavonoid content and sun protection factor. The main objective of this research to determine total phenolic content, total flavonoid content using Folin-Ciocalteu reagent and colorimetric assay, respectively and determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry methods [7, 8]. To extract the sample using conventional solvent is ethanol.

## Materials and Method

### Materials

#### Plant Material

Fresh of *Belimbing Wuluh* leaves and fruits were determined by a botanist at laboratory of pharmaceutical biology Sekolah Tinggi Ilmu Farmasi “Yayasan Pharmasi Semarang” (Stifar).

#### Chemical and Reagents

Ethanol, Methanol, Folin-Ciocalteu, Sodium Carbonate, aluminium chloride, Sodium Acetate, Gallic acid and Rutin were supplied by Sigma–Aldrich (St. Louis, MO, USA). All chemicals and reagents used in the study were of analytical grade.

### Methods

#### Extraction of Belimbing Wuluh

A number of 3 kg each leaves and fruits of belimbing *Wuluh* were dried and powdered. 300 g of powder was macerated by 96% ethanol for 24 h. Consequently, residues were macerated by same solvent three times. Accumulated extract and evaporated at 60°C, 100 RPM, to give viscous extract.

#### Determination of Total Phenolic Content (TPC)

Modifications have been made to the analysis of the total phenolic determination of previous content [8, 9]. 0.5 ml both sample fruits and leaves extract (1 mg/ml) belimbing wuluh added with 0, 4 mL Folin-Ciocalteu reagent, incubated for 4-8 minutes. Moreover, added with 4.0 mL 7 % Sodium Carbonate. Then, added with distilled water, incubated 2 h 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).

#### Determination of Total Flavonoid Content (TFC)

TFC was determined according Chang method [10], validated by Nugroho [9] with modification. 0,5 ml both sample extract of belimbing wuluh fruits and leaves Added with 1,5 mL methanol, 0,1 mL 10 %  $AlCl_3$ , 0,1 mL Sodium acetate 1 M and added 2,5 mL distilled water. Then, incubated for 30 minutes 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).

#### In Vitro Assessment of Sunscreens Activity

##### Determination of Sun Protection Factor (SPF)

Determination of sun protection factor (SPF) of sunscreens by ultraviolet spectrophotometry using Dutra method [7]. Each 100, 200 and 300 ppm extract of belimbing wuluh fruits and leaves diluted with aquadest. The absorbance of belimbing wuluh extract in solution were obtained on range 290-320 nm for SPF, 290-315 nm for percent erythema, 320-370 nm for percent pigmentation. The absorbance data were obtained with interval 5 nm and triplet measured, followed by Mansur equation [11].

$$SPF(\text{spectrophotometric}) = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

EE (λ) erythemal effect spectrum; I (λ) solar intensity spectrum; Abs (λ) absorbance of sample; CF correction factor (= 10). Value of EE (λ) x I (λ) are constant, showed on Table 1.

**Table 1: The Normalized product function used in the calculation of sun protection factor (SPF)**

Wavelength (λ nm)	EE x I (Normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

**Determination of Erythma and Pigmentation Transmission and Transmission**

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,  $Te = T \times Fe$ . Where, (Fe) is flux of erythema whose value at certain wavelengths can be seen on[12]. Value of flux erythm which is continued by the sunscreen (Ee) is calculated by the formula,  $Ee = \sum (T \times Fe)$ . While the erythm transmission% is calculated by the formula:

$$\% \text{ transmission erythm} = \frac{Ee}{\sum Fe} = \frac{\sum(T \times Fe)}{\sum Fe}$$

With same concentrations of SPF and erythm, than absorbance measured on Wavelength 292-372 nm. Calculated transmission of pigmentation (TP) follow the formula  $Tp = T \times Fp$ , Where Fp is flux of

pigmentation with wavelength certain. Have a lot of flux pigmentation follow the formula,  $Ep = \sum (T \times Fp)$ . While the pigmentation transmission % is calculated by the formula:

$$\% \text{ transmission pigmentation} = \frac{Ep}{\sum Fp} = \frac{\sum(T \times Fp)}{\sum Fp}$$

The flux of erythm and pigmentation at certain wavelengths can be seen in Table 2.

**Table 2: The flux erythema and pigmentation on sun screen**

Wavelength (λ nm)	Flux of erythema	Flux of Pigmentation
290-295	0,1105	-
295-300	0,6720	-
300-305	1,0000	-
305-310	0,2008	-
310-315	0,1364	-
315-320	0,1125	-
320-325	-	0,1079
325-330	-	0,1020
330-335	-	0,0936
335-340	-	0,0798
340-345	-	0,0669
345-350	-	0,0570
350-355	-	0,0488
355-360	-	0,0456
360-365	-	0,0356
365-370	-	0,0310
370-375	-	0,0260
Total	2,2322	2,9264



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The sunscreen activity category is then assessed by percent of erythema and pigmentation as seen in Table 3.

Table 3: Table of sunscreens category

Category	Range of transmission UV (%)	
	Erythema	Pigmentation
Sunblock	<1	3-40
Extra Protection	1-6	42-86
Standard Suntan	6-12	45-86
Fast tanning	10-18	45-86

## Results

### Total Phenolic Content

The total phenolic content of the crude ethanolic extract of belimbing wuluh fruits and leaves was estimated by Folin-Ciocalteu reagent and expressed in gallic acid equivalents (GAE). Fruits and leaves ethanolic extract as samples and gallic acid as standard were reacted with Folin-Ciocalteu reagent producing a yellow color indicating that it contained phenol, after which it was added with  $\text{Na}_2\text{CO}_3$  solution resulting in a blue color which is a molybdenum-tungsten complex.

The phenolic compound reacts with the Folin-Ciocalteu reagent only in an alkaline atmosphere to allow proton dissociation in the phenolic compound into phenolic ions, thus adding  $\text{Na}_2\text{CO}_3$  solution to the sample. The stable absorbance of the mixture of gallic acid with the folin-ciocalteu reagent occurred in 120 minutes. The maximum wavelength produced will be used to obtain an absorbance value that gives the highest measurement sensitivity.

Maximum wavelength obtained is 718 nm. The equation of the standard curve gallic acid obtained ( $y = 0.0054x + 0.0325$ ,  $R = 0.99784$ ). Total phenolic content of fruits ethanolic extract is  $19.8022 \pm 1.6696$  mg GAE/g more than leaves ethanolic extract  $6.5886 \pm 0.0590$  mg GAE/g. Both have significant differences after *t test* tested ( $sig < 0.05$ ).

### Total Flavonoid Content

The total flavonoid content of crude ethanolic extract was determined via aluminum chloride colorimetric method. Fruits and

leaves ethanolic extract samples and rutin standard are reacted with  $\text{AlCl}_3$  reagent which can form a rutin complex- $\text{AlCl}_3$ , so that a visible wavelength shift is indicated by the solution yielding a more yellow color. The addition of sodium acetate to maintain the wavelength in the visible region (visible).The

stable absorbance of the mixture of rutin with the sodium acetate and aluminum chloride reagent occurred in the 30 minute. Maximum wavelength obtained is 413 nm. The equation of the standard curve rutin obtained ( $y = 0.0026x + 0.018$ ,  $R = 0.99755$ ) and expressed as rutin equivalent per gram of plant extract. Total flavonoids content of fruits ethanolic extract is  $24,7458 \pm 0,3291$  mg GAE/g more than leaves ethanolic extract  $13,3126 \pm 0,5695$  mg GAE/g. Both have significant differences after *t test* tested ( $sig < 0.05$ ).

### In Vitro Sunscreen Activity

#### Sun Protecting Factor (SPF)

The SPF in vitro was determined by the spectrophotometric method developed by Mansur (1986) using the UVB region, considered to be the region of greatest incidence during the day in which people are exposed for longer (Dutra et.al.2004).

In figure 1, it can be seen that SPF value of fruits ethanolic extract belimbing wuluh is in the minimum potential range at low concentration (100 ppm) up to maximum potential at high concentration (300 ppm). Meanwhile, belimbing wuluh leaves have the maximum potential at the lowest

concentration (100 ppm) to ultra protection at high concentrations (300 ppm).

If matched with total phenolic and total flavonoid data, from the figure shows that leaves ethanolic extract has good potential as an active sunscreen ingredient although it has a lower total phenolic and total of

flavonoids content than fruits ethanolic extract of belimbing wuluh. These data indicate that the sunscreen active ingredients found on the leaves of belimbing wuluh are suspected of phenolic compounds and other components that have a conjugated double bond that can absorb UV light in the 290-320 nm range.

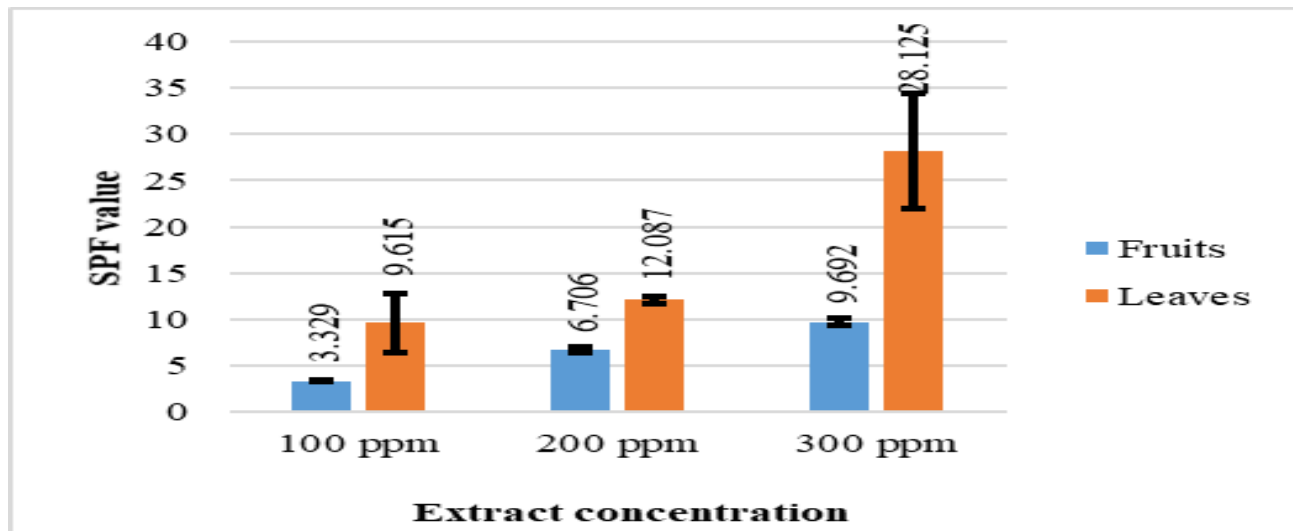


Figure 1: SPF value of ethanolic extract belimbing wuluh fruits and leaves

### Percent (%) Transmission of Erythema and Pigmentation

Exposure to ultraviolet light at wavelengths of 290-320 nm triggers an inflammatory reaction and causes skin color to become red or erythema. Stages of erythema are divided into three phases, namely skin redness, skin shrinkage, and epidermal cell release. From the calculation data of erythema and pigmentation % transmission which can be seen in figure 2 it can be seen that each fruits and leaves of belimbing wuluh 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 but the effect is still weak to be used as a single sunscreen. Only one leaves concentration of 300 ppm can be drawn in the total block valuation category. In general all the extracts are active as sunscreen, this is it caused by the presence of secondary metabolite compounds. Secondary metabolite compounds that can act as a sunscreen include flavonoids and phenolic.

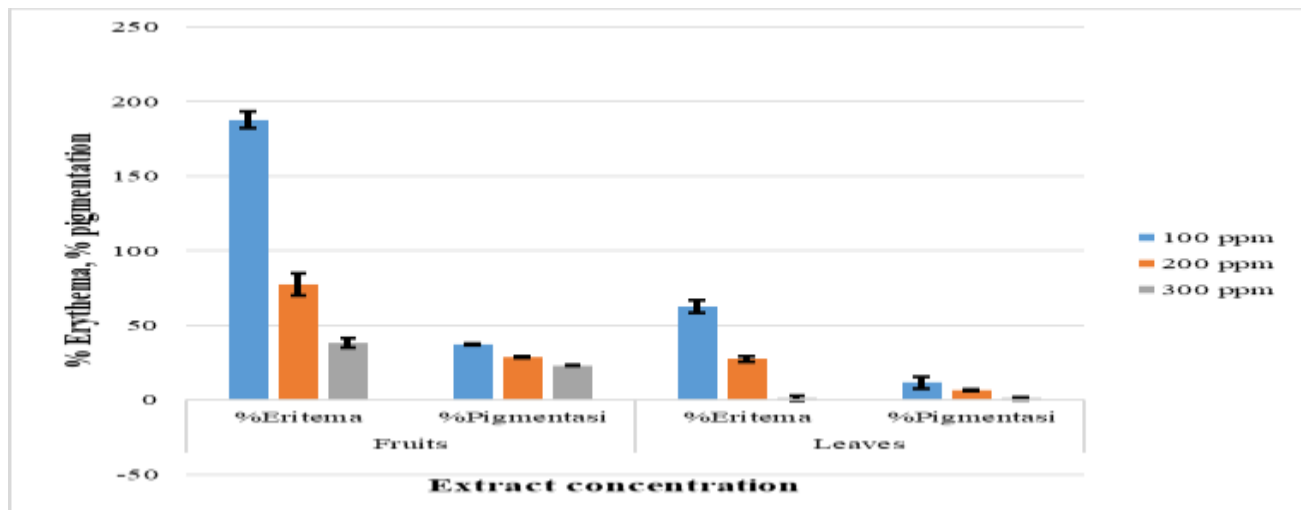


Figure 2: Percent (%) erythema and pigmentation of ethanolic extract of belimbing wuluh fruits and leaves

## Conclusion

From this study that concluded belimbing wuluh (*Averrhoa bilimbi*) leaves are better than fruits source of total phenolic content, total flavonoid content, and sunscreen activity. Hence, further studies are suggested to be conducted for elucidating the extended uses of sun protection factor to topical dosage form.

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