Antioxidant Activity, Sun Protection Factor (SPF) and Total Phenolic and Flavonoid Contents from Purified Extract of Stelechocarpus buharol (Bl.) Hook F. & Th. Leaves and its Classification with Chemometrics

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Abstract

Stelechocarpus buharol (BI.) Hook F. & Th leaves were contains flavonoids and have antioxidant activity. This study determined the antioxidant activity, Sun Protection Factor (SPF) value, total phenolic and flavonoid content of ethanol extract (EESL) and purified extract of S. buharol leaves (PESL). EESL was obtained by maceration method and PESL by using n-hexane to remove the non-polar compound then obtain the purified ethanol extract. EESL and PESL were determined the antioxidant activity by using 1,2-diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and β-carotene bleaching assay (BCB) and classified by using chemometrics of principal component analysis (PCA). The results showed antioxidant activity with DPPH were IC_{50} value of vitamin C 4.59±0.046 µg/ml, EESL 9.53±0.062 µg/ml and PESL 5.95±0.048 µg/ml, with FRAP method were IC_{50} values of vitamin C 6.66±0.150 µg/ml, EESL 16.13±0.156 µg/ml and PESL 24.53±0.114 µg/ml and with BCB method were IC_{50} values of quercetin 236.67±8.808 µg/ml, EESL 134.25±4.478 µg/ml and PESL 116.82±9.982 µg/ml. The SPF value of PESL was 5.70. Total phenolic content of EESL was 5.68 ± 0.042% and PESL was 6.11 ± 0.020%, while the total flavonoid content of EESL 5.62 ± 0.006% and PESL were 6.67 ±0.017%. Based on the results of the study it can be concluded that the results of the measurement of antioxidant activity in EESL and PESL provide good antioxidant activity. The SPF of PSEL at a concentration of 200 ppm of 5.70. The total phenolic and total flavonoid contents correlated with DPPH, FRAP, and BCB. PCA classified EESL and PESL using the variables of antioxidant activities and phenolic-flavonoid contents.

Keywords: *Stelechocarpus buharol leaves, Antioxidant activity, DPPH, BCB, FRAP, SPF, Total phenolic content, Total flavonoid content.*

1. INRODUCTION

The process of skin aging is happening faster in someone, especially in Indonesia, with a tropical climate. The most common cause of premature skin aging is due to exposure to free radicals in the form of ultraviolet (UV) which is referred to as photo aging [1]. UV rays can induce free radicals that will cause cellular aging and also cause skin damage to components of the epidermis, dermis and skin appendages tissue. Along with the development of science and process technology, it can be prevent [2]. One way to prevent premature aging naturally and safely is to use natural ingredients that contain flavonoids. Studies show phenolic compounds such as flavonoids have antioxidant activity free radical scavengers [3].

Antioxidants are molecules that can work on the skin to reduce the effects of

Reactive Oxygen Species (ROS). Research on natural antioxidants and free radicals (free radicals) is currently growing along with the increasing number of plants that can potentially protect the human body from the dangers of free radicals [4]. Natural material which is known to have antioxidant activity to counteract free radicals is the leaves of *S. burahol*, that is one of the fruit plants of the Annonaceae family which has medicinal properties.

This plant cannot bear fruit throughout the year, so that the alternative use of this plant part is its leaves [5]. This extract's total flavonoid contents by spectrophotometry method are 9.3±0.46% (w/w) [6]. The antihyperuricemia potential in ethanol extract of *S. burahol* leaves 60.86-78.33% and n-hexane extract 78.23-88.52% from were almost the same as allopurinol 50.82-91.16% in the xanthine oxidase inhibition test in vivo using mice [7].

Ethanolic fraction of *S. burahol* leaves has a high antioxidant activity with DPPH with IC_{50} value of 6.43 µg / mL [8]. It contains terpenoid and flavonoid compounds in n-hexane extract of 11.543 ± 0.889% and 9.535± 0.331%, which have potential as antihyperuricemia [9]. Kaempferol from *S. burahol* leaves as the most active isolate inhibits xanthine oxidase with IC_{50} of 0.27 µg/ml [10].

As an effort to search for natural antioxidants, in this study to determine the antioxidant activity, the value of Sun Protection Factor (SPF), and the determination of total phenolic and flavonoid contents from ethanol extract of *S. buharol* leaves (EESL) and purified extract of *S. buharol* leaves (PESL) with the methods of 2.2 diphenyl-1-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Beta Carotene Bleaching Assay (BCB) and its correlations with PCA chemometrics.

2. MATERIAL AND METHODS

2.1. Sample Preparation

S. buharol leaves are taken from Patikraja District, Central Java, Indonesia. The leaves collected are sorted then washed with running water and dried. The dry leaves were blended and sifted. The powder of dry leaves were macerated by using 70% ethanol with ratio of 1: 10 for 24 hours and re-macerated with ratio 1: 4. The extract were evaporated by using rotary evaporator at 50 ^{0}C to obtain thick extract (EESL). The extract was fractionated by using hexane to remove the non-polar compound then obtain the purified ethanol extract (PESL). Both of EESL and PESL determined the antioxidant activity, SPF value, total fenol content and total flavonoid content.

2.2. Determination of Antioxidant Activity

Antioxidant activity testing was carried out using three different methods. Antioxidant activity using 2.2 diphenyl-1-picrylhydrazy (DPPH), Ferric Reducing Antioxidant Power (FRAP) and Beta Carotene Bleaching Assay (BCB):

2.2.1. Antioxidant Activity using 2,2 Diphenyl-1-picrylhydrazy (DPPH) Method

In summary, EESL, PESL or vitamin C from various concentrations taken 1 ml was added to 2 ml DPPH then vortexed and incubated at room temperature for 30 minutes in a dark place. Then measured at a wavelength of 516 nm using a UV-Vis spectrophotometer. Negative control measurements were taken at the same wavelength consisting of 2 ml DPPH 40 ppm and 1 ml methanol p.a [11]. The inhibition was calculated based on the percentage of color fading of the DPPH solution to turn yellowish by the sample against the control (only DPPH solution). IC_{50} values are represented based on the sample concentration needed to reduce 50% DPPH which value is obtained from a linear regression graph. Replication is done 3 times.

2.2.2. Antioxidant Activity using Ferric Reducing Antioxidant Power (FRAP) Method

A sample solution of 1000 ppm was taken 1 ml each and 1 ml of phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferrycide mixture was incubated at 50˚C for 20 minutes. After incubation, 1 ml of tri chloro acetic acid (TCA) was added and homogenized for 10 minutes, then centrifuged at 3000 rpm for 10 minutes. The solution was taken as much as 1 ml, then added with 1 ml of distilled water and 0.5 ml of FeCl3 0.1% and read the absorption at a wavelength of 720 nm with a UV-Vis spectrophotometer. Calibration curves were made using ascorbic acid solution concentrations of 60, 70, 80, 90, 100 ppm. Replication is done 3 times.

2.2.3. Antioxidant Activity using Beta Carotene Bleaching Assay (BCB)

A sample solution of each concentration was taken 0.2 ml. The mixture was then added to 2.0 ml β-carotene-linoleic emulsion. The mixture is then incubated in a dark place at 50 ºC for 120 minutes, each time the 30 minute intervals are read for absorption. Uptake is read at a wavelength of 354 nm in a UV-Vis spectrophotometer. As a blank, 2.0 ml of a linoleic acid emulsion solution (such as solution b without β-carotene) was added to it in 0.2 ml of the sample with the same concentration as the concentration of the sample being absorbed. Replication is done 3 times.

2.3. Determination SPF Value

Determination of SPF values in vitro by the UV-Vis spectrophotometry method. A sample of 1 gram was dissolved in a 100 ml measuring flask and diluted with ethanol to the limit mark, then ultrasonified for 5 minutes, then filtered after this discarded the first 10 ml, then taken 5 ml then put into a 50 ml measuring flask and then diluted with ethanol until the boundary mark, then taken back 5 ml and then put in a 25 ml measuring flask, then diluted with ethanol until the limit mark. Furthermore absorbance was measured in the wavelength range of 290-320 nm at 5 nm intervals and ethanol was used as negative control. Then the SPF value is calculated by the Mansur equation [12].

2.4. Total Phenolic Contents

Determination of total phenolic contents was carried out by the Folin-Ciocalteu method with the use of gallic acid as a standard solution of series concentrations of 10, 20, 40, 60 and 80 µg / ml. Then take each 0.5 ml of a standard solution and the sample is added with the Folin-Ciocalteu reagent as much as 2 ml and 4 ml of sodium carbonate and allowed to stand for 30 minutes and read the absence at the maximum wavelength. Replicated 3 times.

2.5. Total Flavonoid Contents

Determination of total flavonoid contents using a standard solution of quercetin concentration series 60, 70, 80, 90 and 100 ppm. Each standard solution and sample taken as much as 1 ml and then added with 1 ml of 10% $AlCl_3$ and 8 ml of 5% acetic acid. Leave for 30 minutes, then absorbance is measured at the maximum wavelength. Replication is done 3 times.

3. RESULT AND DISCUSSION

Antioxidant activity was analysed using three methods are DPPH radical scavenging activity, iron reduction (FRAP), and inhibition of beta carotene degradation (BCB). The inhibitory concentration value to reduce the 50% DPPH radical (IC_{50}) was determined by plotting the linear regression curve between the DPPH radical reduction activity (%) with the sample concentration ratio. The results showed that PESL provided higher antioxidant activity with IC_{50} values 5.95±0.048 µg/ml and EESL 9.53± 0.063 µg/ml. However, when compared with a comparison of vitamin C 4.59±0.046 µg/ml, it was shown that PESL had lower antioxidant activity (Table 1).

This is because vitamin C is a pure compound that can quickly reduce DPPH radicals when compared to sample solutions [13].

The iron reduction test (FRAP) is based on the reduction reaction in an acidic atmosphere to the yellow Fe^{3+} complex compound (potassium hexacyanoferate) into a bluish green Fe^{2+} complex compound by antioxidants in an acidic atmosphere [14]. The more concentrated green that is formed indicates the formation of Fe^{2+} ions is increasing and causes an increase in the absorbance value which can indicate the antioxidant potential of the samples tested [15]. The results showed that EESL with IC_{50} values 16.13±0.156 µg/ml, where the FRAP values gave significantly better antioxidant activity compared to PESL 24.53±0.114 µg/ml. However, when compared with vitamin C 6.66±0.150 µg/ml it was shown that IC_{50} EESL values were lower because vitamin C was classified as a very active antioxidant with IC_{50} values below 10. The results of this study showed that flavonoids or phenolics in the sample of EESL and PESL contribute to the mechanism of metal ion chelation and reduce the possibility of hydroxyl radicals originating from superoxide radical anions [16]. A compound can be said to be a very strong antioxidant if it has an IC_{50} value of less than 50 µg/ml, strong if the IC_{50} value is 50-100 µg/ml, moderate if the IC_{50} value is 100-150 µg/ml, it is weak if the IC_{50} value> 150 µg/ml [17]. Measurement of antioxidant activity by the BCB method is based on the mechanism of inhibiting the rate of beta carotene degradation during the oxidation process that occurs when linoleic acid turns into hydroperoxide. The antioxidant activity of the sample is determined by observing the rate of degradation of the sample solution with control for 120 minutes at a time interval of 30 minutes. The degradation rate of β-carotene increases with time, but the rate of degradation of β-carotene can be inhibited by the addition of EESL and PESL. According to Hassimotto [18], the antioxidant power of the method β-carotene bleaching is classified into three levels: strong antioxidant (> 70%), intermediate (40-70%) and weak (<40%). The results showed that PESL provided higher antioxidant activity with IC_{50} values 116.82±9.982 µg/ml and EESL 134.25±4.478 µg/ml (Fig.1). However, when compared with a comparison of quercetin 236.67±8.808 µg/ml, it was shown that EESL and PESL had higher antioxidant activity.

Determination of antioxidant activity in each sample based on different methods shows different values of antioxidant activity. The difference in results between DPPH, FRAP and BCB tests is due to differences in the compounds responsible for testing the samples with all three methods. In testing with DPPH method, the responsible compound has a mechanism in the capture of free radicals by breaking the chain of radical reactions by giving or donating hydrogen radicals quickly [19]. In testing with the FRAP and BCB methods the compounds responsible are classified as secondary antioxidants where they have a mechanism in stabilizing hydro peroxidase to become free radicals. Compounds that can chew the metal are also included in this group of antioxidants. Secondary antioxidants work through the mechanism of binding of metal ion, capture oxygen, convert hydrogen peroxide into non-radical species and by deactivating singlet oxygen [20].

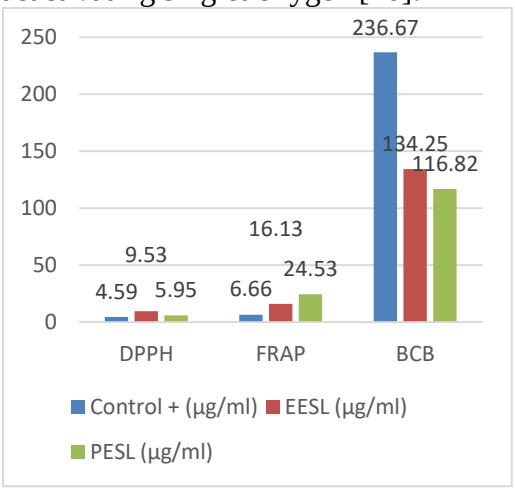

***Figure 1**. Result IC_{50} value of EESL and PESL using DPPH, FRAP and BCB methods*

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3.1. Determination of SPF Value

Determination of SPF quantitative analysis using UV-Vis spectrophotometry and the SPF value was calculated by the method developed by Mansur. The absorption value was taken in the wavelength range of 290-320 nm with intervals of 5 nm.

The results obtained in this study, the SPF value of PESL at a concentration of 200 ppm is 5.70, it is classified as medium SPF. The distribution of SPF values is classified as follows, SPF 2-4 is minimal, SPF 4-6 is moderate, SPF is 6-8 extra, SPF is maximum 8-15 and SPF>15 is ultra. The higher the concentration used, the SPF value obtained will be better (Tab.2).

***Table 2.** Result of SPF Value of PESL*

	Abs	A*EE	Abs	A*EE	Abs	A*EE
290	0.645	0.096	0.645	0.096	0.646	0.096
295	0.610	0.498	0.611	0.499	0.627	0.512
300	0.578	1.661	0.578	1.661	0.593	1.704
305	0.561	1.838	0.561	1.838	0.574	1.881
310	0.549	1.023	0.548	1.021	0.560	1.043
315	0.535	0.448	0.534	0.448	0.544	0.456
320	0.514	0.092	0.513	0.092	0.527	0.094
SPF		5.659		5.657		5.790

3.2. Total Phenolic and Flavonoid Contents

Determination of total phenolic content of EESL and PESL was carried out using the colorimetric method with follin-ciocalteu reagents and the results were expressed as % w/b equivalent gallac acid (% w/w EAG). The results of the study showed total phenolic content of EESL is 5.69 ± 0.042% and PESL is 6.11 ± 0.020% (Fig. 2). The total flavonoid content is based on the colorimetric method with $AlCl_3$ compared to the quercetin standard and the results are expressed as % b/b equivalent to quercetin. The results obtained by data on the total flavonoid content of EESL is 5.63 ± 0.006% and PESL is 6.67 ± 0.017%. Both of the total flavonoid content are less than ethanol extract of *S. burahol* 9.3±0.46% (w/w) from the reports of Diniatik [4].

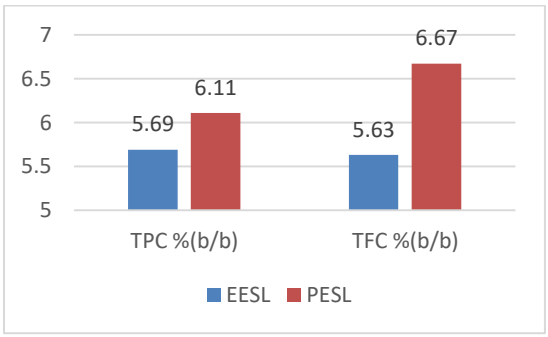

***Figure 2.** Result of total phenolic and total flavonoid contents of EESL and PESL*

3.3. PCA Analysis

Determination of total phenolic content of EESL and PCA is a technique for constructing new variables which are linier combinations of original variables [(21)]. Figure 3 shows the PCA plot scores from EESL and PESL, as shown in Table 4.

PCA plot scores are used for classification between samples and represent sample projections expressed by the first principle component (PC1) and the second principle component (PC2). Observed samples that have adjacent score plot values have the same physical chemical properties. Based on the plot score, the sample can be classified into three groups, as grouped in Figure 4. PESL1, PESL2 and PESL3 are in one group which means the three have similar physical and chemical properties.

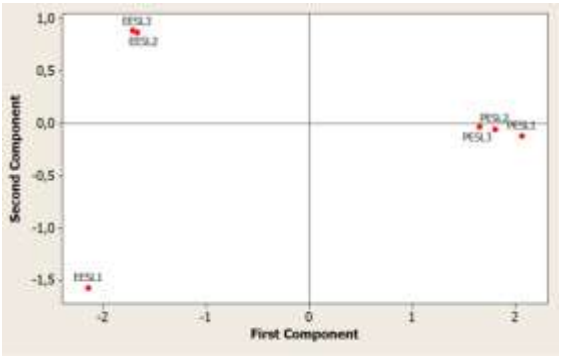

Figure 3. *PCA plot scores of EESL and PESL.*

To evaluate correlations between variables, loading plots can be used (Figure 5). The loading plot shows how strongly each variable affects principle components. Angles between vectors show how these variables correlate with each other. If two vectors are close to each other that form a narrow angle then it shows a positive correlation between the two variables. If the variables form an angle close to 90° the two do not correlate and if between variable vectors scatter and form an angle close to 180°, it shows that both are negatively correlated [22].

Figure 4 shows that shows TPC and TFC positively correlated with FRAP, do not correlated with BCB dan negatively correlated with DPPH.

4. CONCLUSION

Based on the results of the study it can be concluded that the measurement of antioxidants by DPPH, FRAP and BCB methods on EESL and PESL provides good antioxidant activity. SPF value of PESL at a concentration of 200 ppm is 5.70 as moderate, and the total phenolic and flavonoid content of PESL is greater than EESL.

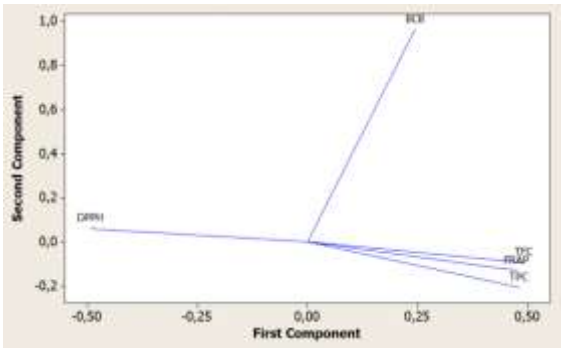

Figure 4. *PCA loading plot of EESL and PESL.*

Total phenolic and flavonoid contents are positively correlated with FRAP, do not correlated with BCB dan negatively correlated with DPPH. PCA succeeded in classifying EESL and PESL using antioxidant activity variables and total phenolic and flavonoid contents.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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