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Research Article

The Antiradical Activity of Insoluble Water Suji (*Pleomele angustifolia* N.E. Brown) Leaf Extract and Its Application as Natural Colorant in Bread product

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ABSTRACT

Currently, there are some synthetic colouring agents present in food products. The synthetic colouring agents are supposed to give deleterious effect to human health; therefore, natural coloring agents derived from plant is continuously explored to replace the synthetic ones. Suji (*Pleomele angustifolia*) leaf is one of the potential plant to be used as natural coloring agents. The aim of this study is to explore the natural colour potency and antiradical activity of insoluble water extract of suji leaf from three different regions. Furthermore, green components obtained from suji leaf extract (SLE) was used for coloring food product (bread product), and bread added with extract was subjected to preference test, namely color and taste preferences by trained panelist. The result showed that region and maturity level affect the intensity of green components obtained from SLE. The highest level of green components (2.57 %) among three regions evaluated was observed from Jumantono. The water insoluble extract of suji leaf can also make coloring effects toward bread and exhibits low radical scavenging against 2,2-diphenyl-1-picrylhidrazyl. The preference test against color and taste of bread added with synthetic coloring agents and mixed with SLE showed that the coloured bread is less preferred than that without coloring agent.

Keywords: suji leaf; coloring agents; 2,2-diphenyl-1-picrylhidrazyl; preference test.

1. Introduction

The use of food additives in food products including the food colorants is an emerging issue, especially in health aspect. The appearance of the food, especially its color is important factor affecting the attractiveness of consumers to consume. Hence, the food colorants become so important in the production of food. Food colorants can be classified as synthetic and natural. Synthetic colorants have been widely used in the food industry due to its effectiveness (Amalia *et al*., 2013). However, the application of synthetic colorants for the food or beverages can cause toxic and carcinogenic effects. As a consequence, some food scientists try to use natural colorants in order to hinder the negative effects of synthetic colorants (Chengaiah *et al*., 2010).

Natural coloring agents are derived from naturally occurring sources such as plants, animal and minerals. The current preference for naturally derived colorants is due to their healthfulness and excellent performance

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(Hernandez-Ceruelos *et al*., 2002; Chengaiah *et al*., 2010). Some plants which have been successfully used as natural colorants and its biological activities are turmeric (yellow in color) having antioxidant activity (Kalpravidh *et al*., 2010) and anti-inflammatory activity (Skrzypezac-Jankun *et al*., 2000), *Caesalpinia sappan* bark produced red color and had hepatoprotective activity (Srilakshmi *et al*., 2010), roselle extract in a model system of a drink (Duangmal *et al*., 2008), and Black Rice Bran in Yogurt (Nontasan *et al*., 2010). From literature review, there are more 20 plants used as natural colorants (Chengaiah *et al*., 2010), including suji (*Pleomele angustifolia* N.E. Brown) leaf.

In traditional medicine, suji leaf has been used in cough and lung disease (Roosita *et al*., 2008). Suji leaf (Figure 1) also contains chlorophyll at level of 1 % (Prangdimurti *et al*., 2006). The use of chlorophyll has been correlated with benefit effects to human health; as a consequence, some food producers can still advertise many chlorophyll-containing products, mostly in dietary supplements and juices. The advertisement may be supported by the chemical similarity between chlorophyll and the prosthetic group (heme) of hemoglobin which are responsible for protecting the human body from several diseases (Tumolo and Lanfer-Marquez, 2012).

Fig 1. The suji leave (*Pleomelleangustifolia* (Roxb.) N.E.Br.

Chlorophyll is natural compound derived from leaf and is suspected to posses antioxidant activity due to the conjugated double bond present in chlorophyll. Therefore in this study, we evaluate the antioxidant activity of suji leaf extracted using 2,2-diphenyl-1-pycrilhydrazil (DPPH) radical scavenging test. Furthermore, the extract obtained was also used as natural colorant in food model (bread products), and bread added with suji leaf extract was tested by panelist.

2. Materials and Methods

Suji leaf was obtained from three different regions in Central java, namely Jumantoto, Tawangmangu and Mateseh. Botanical identification was performed in the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. 2,2-diphenyl-1-pycrilhydrazil (DPPH) radical was obtained from Sigma (Alldrich, USA). The other chemicals and solvents used were of analytical grade, unless if they are specified.

2.1. Extraction of Suji leaf

An approximately of each 100 g of leaf from three different region was added with 800 mL of water, blended for 2 min using commercial blender and filtered. The residue was then blended using 800 mL of water, and the filtrate obtained was pooled with the first filtrate. The filtrate was boiled until the coagulant (insoluble water suji leaf extract) present in the top of water. The coagulant was taken and dried on conventional oven at 70 oC (for 12 hour). The extract obtained was further subjected to several tests including antiradical activity.

2.2. Determination of color intensity of Suji leaf extract

The color intensity of suji leaf extract (SLE) obtained during extract preparation was measured as follows: A- 200.0 mg of SLE was accurately weighed using calibrated analytical balance, added with 10 mL methanol and stand for 5 minute, shaken vigorously for 2 x 5 minute, and centrifuged for 10 minute. The filtrated was scanned using UV-vis spectrophotometer at λ 200 – 700 nm using methanol as blank. The λ_{max} of SLE was determined.

2.3. Antioxidant activity of Suji leaf extract

The evaluation of the antioxidant activity of suji leaf extract was carried out using 2,2-diphenyl-1-pycrilhydrazil (DPPH) radical scavenging test according to Kikuzaki *et al*. (2003). A-200.0 mg of SLE was accurately weighed, added with 10 mL methanol and stand for 5 minute, shaken vigorously 2 x 5 minute, and centrifuged for 10 minute. The filtrate was taken and added with 5 mL methanol. Take the solution with certain concentration by pipetting 0 mL, 0.5 mL, 1mL, 1.5 mL and 2 mL and added with metanol until 2 mL. The test solutions was added with 2 mL methanolic-DPPH having absorbance of 1.300 at λ 515. The mixture was shaken vigorously using vortex for 1 minute, and allowed to stand for 30 min at room temperature in a dark room. The blank during measurement is extract dissolved in methanol. After that, the absorbance of solution was measured using spectrophotometer (Genesys 10) at 515 nm. The radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging activity}\,(\%) = \frac{\text{Ac} - \text{As}}{\text{Ac}}\ \text{x}\ 100$$

where Ac is absorbance of control (DPPH free radical without the addition of test solution), As is sample absorbance (absorbance of DPPH free radical after the addition of test solutions).

2.4. Preparation of bread

Bread is used as food model to be added with SLE as natural coloring agent. The preparation of bread is as follows: A-0.5 kg of egg was added with 0.5 kg sucrose and salt. The mixture was vigorously shaken, added with 0.5 kg flour, sodium bicarbonate and vanilla. The mixture was allowed to stand up for 30 minute. A-50 mL of bread dough was added with 0.3 g of SLE and or synrhetic coloring agent. The bread dough was further taken for steaming for 10 minute. The bread formed was evaluated for its antiradical activity against 2,2-diphenyl-1-pycrilhydrazil (DPPH).

2.5. Preference test

Bread with and without addition of SLE and or synthetic coloring agent was further taken for preference test. The bread was given to 20 panelists to be evaluated for its colour and taste. The scoring for evaluation form is as follows:

Score 1 : very dislike
Score 2 : dislike
Score 3 : neutral
Score 4 : like
Score 5 : very like

The level of preference for each group (bread without coloring agent, bread with SLE of 2 g/4 kg and 4 g/kg flour, and bread added with fresh juice of suji extract) was scored, and the score obtained was analyzed using analysis of variance (ANOVA).

3. Results and Discussion

3.1. Characteristic of suji leaf

Suji leaf was obtained from three different regions, namely Jumantono, Matesih and Tawangmangu (all are in Central java Province) having bit different characteristics. Table 1 showed stomata index and land characteristic analysis. The aim of stomata index is to investigate leaf development in relation to light intensity. According to Reid *et al*. (2003), the climate change and CO_2 concentration affect the index and density of stomata. The highest stomata index was observed in SLE coming from Tawangmangu; however, this difference is not statistically significant ($P > 0.05$).

Table 1. The characteristics of suji leaf used during the study.

The origin of plant	Stomata Index	The level of nitrogen total (%)	Level of Mg (ppm)	Level of Fe (ppm)	Level of Mn (ppm)
Jumantono	0.224 ± 0.3 (a)	0.01	219.95	5.23	1126.5
Tawangmangu	0.225 ± 0.7 (a)	0.05	553.25	1.60	275.5
Mateseh	0.215 ± 0.3 (a)	0.06	497.48	2.02	360.23

The different letter indicated the different result

3.2. Extraction of Suji leaf

The suji leaf was extracted with water with the aid of heating. The main component exploited during extraction is chlorophyll. Basically, chlorophyll is more soluble in organic solvent such as chloroform and hexane; however, due to safety reason for being used in food product, the water was chosen as extracting solvent. The chlorophyll is insoluble in water, therefore, the coagulant (insoluble SLE) appears in the top of water during extraction is removed and used for further study (Figure 2). The coagulant was taken and dried on conventional oven at 70°C (for 12 hour).

Figure 2. The coagulant (insoluble suji leaf extract) appears in the top of water during suji leave extraction with water as extracting solvent. It contains chlorophyll.

Table 2. The levels of suji leaf extract from three different regions (Tawangmangu, Mateseh and Jumantoro) and with different maturity

Region / Maturity	Tawangmangu	Mateseh	Jumantono
Matture	2.37 (b)*	2.57 (b)	2.40 (b)
Immature	1.47(a)*	1.13 (a)	1.30 (a)

*the different letter indicated the significant difference at level of P 0.05.

Table 2 showed the concentration of SLE from three regions. The highest level of extract was observed in mature SLE from Tawangmangu. The mature leaf contains more chlorophyll than immature one. The development of chlorophyll in mature leaf occurs more completely than that in immature one.

3.3. UV-vis spectra of Suji leaf extract

The suji leaf extracts (SLEs) were subjected to UV-vis spectra measurement. All extracts revealed the similar UV-vis spectra profile. Thi UV-vis spectra of SLE correspond to UV-vis spectra of chlorophyll. The maximum wavelength is observed at 410 nm and 660 nm, respectively as shown in Figure 3. The extract also contains several peaks with low intensity, indicating the presence of other components in that extract. This

component can occur from degradation products of chlorophyll during extraction.

Table 3. The absorbance average of suji leaf extract in methanol (0.02 gram/10 mL) at 410 nm.

Region / Maturity	Mateseh	Tawangmangu	Jumantono
Immature	0.403 (a)	0.488 (a)	0.769 (b)
Mature	0.780 (b)	0.826 (b,c)	0.995 (c)

*the different letter indicated the significant difference at level of P 0.05.

The mature extract showed the higher intensity than the immature one, especially at wavelength of 410 nm. This indicated that the mature extract yield more green color component than that in immature one. Table 3 showed the absorbance average of suji leaf extract in methanol at wavelength of 410 nm with same concentration.

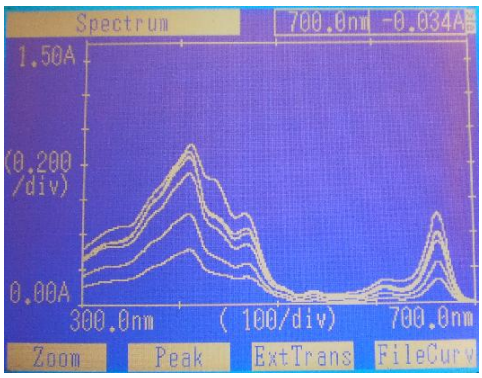

Fig3. The UV-vis spectra of insoluble water suji leaf extract in methanol.

3.4. The antiradical activity of Suji leaf extract and that in bread

The antiradical activity of SLE and bread added with SLE was evaluated using synthetic radical 2,2-diphenyl-1-picrylhidrazyl (DPPH). The method is based on the reduction of methanolic-DPPH solution because of the presence of antioxidant substances present in SLE having hydrogen donating groups to form non radical DPPH-H form (*Paixao* et al., 2007).

Table 4. The IC_{50} values of suji leaf extract from three different regions (Tawangmangu, Mateseh and Jumantoro) and with different maturity.

Region / Maturity	Nilai IC_{50}		
	Mateseh	Tawangmangu	Jumantono
Immature	1.61%	1.65%	1.41%
Mature	1.15%	1.19%	1.25%

The parameter used to evaluate radical scavenging activity of SLE is IC_{50} value, defined as the concentration of SLE required for 50% scavenging of DPPH radical in certain time period. The smaller IC_{50} value, the higher radical scavenging activity (Maisuthisaku *et al.*, 2007). The IC_{50} values of SLE were shown in Table 4. All extracts yielded high IC_{50} indicating the weak radical scavenging, especially if compared with IC_{50} of vitamin C (2.7 ppm) (Rohman *et al.*, 2010). As a consequence, the bread added with SLE has very weak antiradical capacity. This study indicated that SLE is not good component used for antiradical in food product.

3.5. Preference test

After being used as coloring agent in food product (bread), the preference test was taken. Bread with and without addition of natural coloring agents from SLE and from synthetic ones (tartrazine and *brilliant blue*) as well fresh juice of suji leaf was given to panelist for preference evaluation. Table 5 showed the result of preference test from 20 panelists.

The panelist did not know the composition of coloring agent during preference test. The preference test against color and taste of bread added with synthetic coloring agents and mixed with SLE showed that the colored bread is less preferred than that without coloring agent.

Table 5. The preference level for bread added with coloring agents.

Preference / Coloring agents	The color score*	The taste score*
Without coloring agent	3.45 ± 1.19 a.b	3.65 ±1.04 b.c
Suji leave extract (2 g/kg flour)	3.25 ± 0.97 a.b	3.1 ±0.85 a.b
Suji leave extract (2 g/kg flour)	2.7 ± 1.13 a	2.75 ± 0.85 a
Synthetic coloring agent with high intensity	3.25 ± 0.79 a.b	3.5 ± 0.69 a.b.c
Synthetic coloring agent with low intensity	3.75 ± 0.91 b	3.5 ± 1.10 a.b.c
Fresh juice of suji leave	3.6 ± 1.05 b	3.8 ± 1.01 b.c

*the different letter indicated the significant difference at level of P 0.05. The score is based on: Score 1= very dislike; Score 2 = dislike; Score 3 = neutral; Score 4= like; and Score 5= very like

4. Conclusion

Suji (*Pleomele angustifolia*) leaf from three different regions (Jumantono, Matesih and Tawangmangu) and with different maturity level has been exploited as natural coloring agents. The region and maturity level affect the intensity of green components (chlorophyll) obtained from suji leaf extract (SLE). The highest level of green components was observed in SLE from Jumantono. The water insoluble extract of suji leaf can also exhibits low radical scavenging toward 2,2-diphenyl-1-picrylhidrazyl. The preference test against color and taste of bread added with synthetic coloring agents and mixed with suji leaf extract showed that the coloured bread is less preferred than that without coloring agent.

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