ANTIOXIDANT AND ANTITYROSINASE ACTIVITIES OF FLAVONOID FROM *BLUMEA BALSAMIFERA* (L.) DC. LEAVES EXTRACT

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ASTRACT

The aim of this present research is to evaluate antioxidant and antityrosinase potentials of flavonoid from *Blumea balsamifera* of ethanol leaf extract. The content of flavonoids in the examined plant extracts was determined through reaction with aluminum cloric ($AlCl_3$), Antioxidant activity was measured by using two different free radical scavenging methods, 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), whereas flavonoid contents were evaluated by using Folin-Ciocalteau, and aluminum nitrate methods respectively. Tyrosinase inhibition was evaluated using commercial enzyme and dihydroxyphenylalanine (DOPA) as substrate. The results showed that the content flavonoid of leaf extract 23.34 ± 0.67, The extract showed strong antioxidant activity with IC_{50} of 0.021 µg/ml and tyrosinase inhibition activity received with IC_{50} of 398.16 µg/ml.

Keywords: *Blumea balsamifera,* flavonoid, Antioxidant, antityrosinase.

INTRODUCTION

Blumea balsamifera is a medicinal plant that grows in Southeast Asia, belongs to the family Asteraceae. The leaves are also used as a tea, and as a cure for certain disorders such as rheumatism and hypertension. Its leaves have attracted attention as a part of the plant with various physiological activities, including plasmin-inhibitory, antifungal, and liver-protective effects (Norikura et al., 2008). The dominant components in the oil from leaves were borneol (33.22%), caryophyllene (8.24%), ledol (7.12%), tetracyclo [6,3,2,0,(2.5).0(1,8) tridecan-9-ol, 4,4-dimethyl (5.18%), phytol(4.63%), caryophyllene oxide(4.07%), guaiol (3.44%), thujopsene-13 (4.42%), dimethoxydurene (3.59%) and γ-eudesmol (3.18%) (Bhuiyan et al., 2009). Several studies on the chemical constituents of *B. balsamifera* DC have been reported and a number of flavanoids, have been isolated from this plant (Ruangrungsi et al., 1981; Barua and Sharma, 1992; Fazilatun et al., 2001; Fazilatun et al., 2004; Ali et al., 2005). In addition, hydroxylated flavonoids are good target compounds for tyrosinase inhibitors because they share structural similarities with the natural substrate for tyrosinase (Jeong et al., 2009).

Variations in skin color are outstanding to different levels of a pigment in the skin called melanin. Melanin is synthesized by special cells named melanosomes found in melanocytes with the action of an enzyme, tyrosinase (Hasan and Eltahir, 2010). The melanocytes distribute the melanin to the skin that gives the dark tint color to the skin (Kumar and Gupta, 2012). Melanin plays an important role in protection of UV-induced dermal irritation. However, overproduction of melanin causes an esthetic problem as well as dermatological issue (Lin et al., 2008). Most skin lightening products are targeted to inhibit tyrosinase because it is the first step in the pigment formation and can therefore block all pigment

producing pathways (Kumar and Gupta, 2013). Therefore, this study aimed to examine the flavanoids from *B. balsamifera* DC leaves with antioxidant activities and the inhibition of mushroom tyrosinase.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained as pure commercial products and used without further purification. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), aluminum nitrate, 2,2-diphenyl-1- picrylhydrazyl radical (DPPH•), Folin-Ciocalteu phenol reagent, catechin, mushroom tyrosinase and L-3,4-dihydroxyphenylalanine (L-DOPA) were from Sigma Chemical Co (St. Louis, USA).

Preparation of Extract

B. balsamifera DC leaves were only used that has grown in the medicinal garden of Institute of Tropical Biology- Vietnamese Academy Science and Technology. Leaves was washed, dried at 50^{o}C and grinded to dust. 10g of powder was soaked in 600 mL of ethanolic at temperature 80^{o}C for 90 minutes. After filtration, the ethanol were evaporated to dryness under vacuum at 60°C. These extracts were used for the further biological study including flavonoid content and anti-oxidative, anti-oxidant, anti-tyrosinase and anti-melanin deposition activity.

Determination of total flavonoid content

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Quettier et al., 2000). The sample contained 1 ml of ethanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% $AlCl_3$ solution dissolved in ethanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of catechin and a dilution series of catechin of concentration 45, 90, 180, 360 and 450 µg/ml was prepared and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (µg/ml) on the calibration line; then, the content of flavonoids in the extracts was expressed in terms of catechin equivalent.

Determination of *in vitro* antioxidant activities:

The stock solution of extracts were prepared in ethanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain a concentrations of 0.005, 0.01, 0.015, 0.02 and 0.025 mg/ml. Diluted solutions (1 ml each) were mixed with 1 ml of methanol solution of DPPH in concentration of 1 mg/ml. After 30 min incubation in darkness at room temperature (23^{o}C), the absorbance was recorded at 517 nm. Control sample contains all the reagents except the extract. Percentage inhibition was calculated using equation 1, whilst IC_{50} values were estimated from the percentage inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3). The percentage of antioxidant activity was calculated follows:

% inhibition = $(A_{control} - A_{sample})/A_{control} * 100$.

Where “A” stands for Absorbance.

Tyrosinase Inhibitive Activity

Tyrosinase-inhibition activity of the extract of *B. balsamifera* DC leaves was performed by using L-DOPA as a substrate according to Kubo et al. (2000) with slight modification. The extract was first dissolved in DMSO at 1.0 mg/ml and then diluted to different concentration using DMSO. Each sample (100 µl) was diluted with 1800 µl of 0.1 M sodium phosphate (pH 6.8) and 1000 µl of L-DOPA solution (with 0.1 M sodium phosphate, pH 6.8). Then, 100 µl of mushroom tyrosinase solution (138 units) was added in the reaction. The dopachrome formation was measured using UV-Vis spectrophotometer at 475 nm, for 6 min. The percentage of tyrosinase-inhibition activity was calculated follows:

% Tyrosinase inhibition = [A- (B-C)]/A * 100

Where, A = absorbance of control treatment; without test sample, B = absorbance of test sample treatment; with tyrosinase, C = absorbance of test sample blank treatment; without tyrosinase.

The extent of inhibition by the addition of samples is expressed as the percentage necessary for 50% inhibition (IC_{50}).

RESULTS AND DISCUSSION

Total Flavonoids

The concentration of flavonoids in leaf extract of *B. balsamifera* DC.was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of catechin equivalent (the standard curve equation: y = 0,0037x – 0,0345, R^2 = 0.9979), mg/g of extract (Table 2). The total flavonoid content in the examined leaf extract was 23.34 ± 0.67 mg/g of extract. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation (Min and Chun-Zhao, 2005).

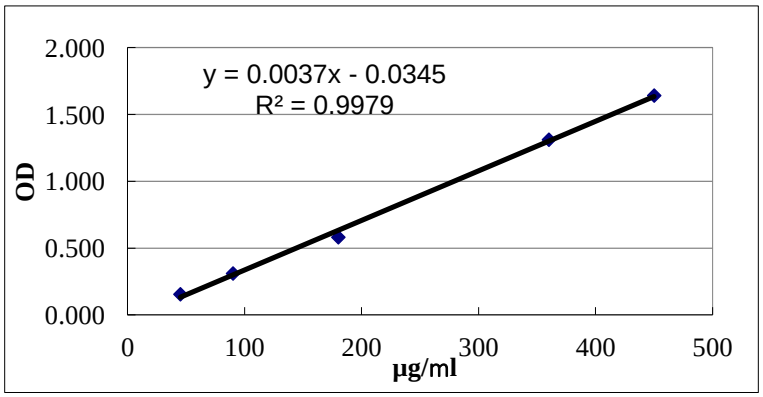

Figure 1. Showing the Calibration Plot for the determination of Flavonoids.

Table 1. Concentration Verses Absorbance of catechin

S.No.	Concentration (µg/ml)	absorbance
1	45	0.155
2	90	0.310
3	180	0.581
4	360	1.311
5	450	1.641

Table 2. Total Flavonoid content in extract espressed in mg/g equivalent to Catechin of extract.

S.No.	absorbance	Conc.of Extract	Total Flavonoid content (mg/g) equiv. to catechin
1	0.933	1 mg/ml	23.0029
2	1.028	1 mg/ml	24.1141
3	0.929	1 mg/ml	22.9077
Mean ±SD			23.34 ± 0.67

Antioxidant activities of *B. balsamifera* DC leaf extract

The antioxidant activity of the ethanol crude extract was determined using a ethanol solution of DPPH solution, as DPPH is very stable free radical. There is no certainty of any side reaction like metal ion chelation and enzyme inhibition as found in *in vitro* generated free radicals such as hydroxyl radical and superoxide anion. A deep purple colour of freshly prepared DPPH fades when antioxidant molecules quench DPPH free radicals (i.e. by providing hydrogen atom or by electron donation, conceivably via a free radical attack on th DPPH molecule) and convert them into colorless/ bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band (Amarowicz, et al., 2003). The ethanol leaf extract of *B. balsamifera* DC have shown significant activity comparable to the activity of Ascorbic acid as standard, as shown in Table. 3 and Figure 2. The result showed that antioxidant activity of the extract was higher ascorbic acid with correspongding contents were 0.021 (mg/ml) and 0.029 (mg/mg).

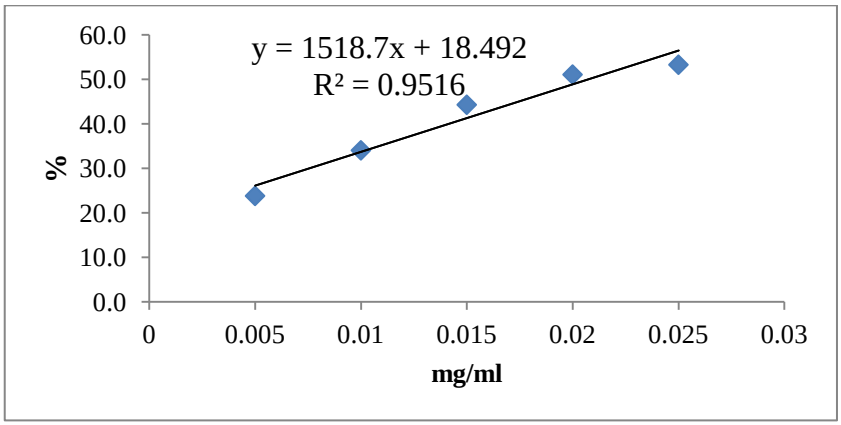

Figure 2. Showing the % Inhibition of ethanol extract of *B. balsamifera* DC using DPPH assay.

Table 3. Evaluation for antioxidant capacity of ethanol extract of *B. balsamifera* DC (DPPH Assay).

Concentration (mg/ml) and % inhibition						
Conc.	0.005	0.01	0.015	0.02	0.025	IC50 (mg/ml)
Ascorbic acid	26.92	33.31	41.89	53.28	62.39	0.029
extract	23.79	34.03	44.26	51.04	53.25	0.021

Anti-tyrosinase properties of *B. balsamifera* DC leaf extracts

Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Therefore, these agents have good

commercial potential in both food processing and cosmetic industries. Kojic acid was used as a positive control, as it has been reported to display the highest tyrosinase inhibition activity. At 398.16 µg/ml the tyrosinase inhibition of extracts is 50%, which indicate that the extracts have weak activity compared to kojic acid. The inhibition of tyrosinase ability might depend on the hydroxyl groups of the flavonoid compounds of the *B. balsamifera* extracts that could form a hydrogen bond to active site of the enzyme, leading to a lower enzymatic activity. Some tyrosinase inhibitors act through hydroxyl groups that bind to the active site on tyrosinase, resulting in steric hindrance or change conformation. Flavonoid compounds from leaf extract of *B. balsamifera* DC proved to be effective inhibitors of tyrosinase activity as reported before (Baek et al., 2008). The tyrosinase inhibitory activity of the isolated flavonoids might be due to chelating with copper in the active center of tyrosinase. The binding ability with copper in mushroom tyrosinase enzyme of the isolated compounds was investigated by adding Cu^{2+} and by incubation with the enzyme (Saewan et al., 2011).

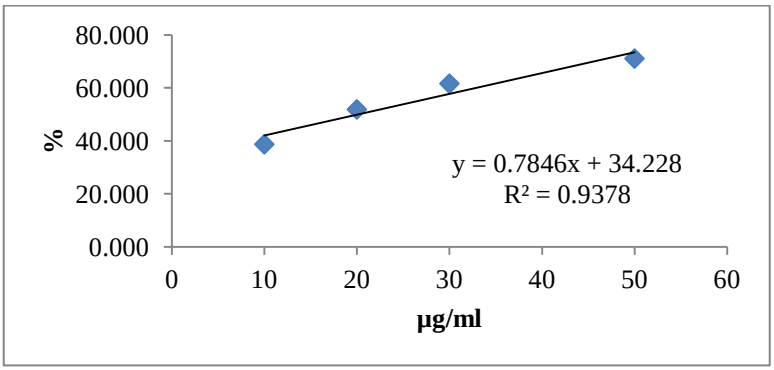

Figure 3. Showing the % Inhibition tyrosinase of ethanol extract of *B. balsamifera* DC

Table 4. Evaluation for anti-tyrosinase capacity of ethanol extract of *B. balsamifera* DC.

Concentration (µg/ml) and % inhibition					
Conc.	10	20	30	50	IC_{50} (µg/ml)
Kojic acid	38.70	51.86	61.61	71.05	20.1
Conc.	100	200	300	500	IC_{50} (µg/ml)
extract	38.25	42.80	48.10	54.92	398.16

CONCLUSION

The total flavonoid content of the ethanol extract of *B. balsamifera* was 23.34 ± 0.67 (mg/g). The ethanol leaf extract of *B. balsamifera* showed powerful antioxidant activities as compared to the standard ascorbic acid but antityrosinase activities was less than kojic acid. Hence the results of this study on ethanol leaf extract of *B. balsamifera* shows that it can be used as easily accessible source of antioxidant and to survey inhibition synthesis melianin activity on cells in order to apply into reality.

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