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Estimation of Andrographolide and Antioxidant Activities in *Andrographis peniculata* Commercial Products by Color Parameters

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Abstract

Andrographis paniculata (Burm.f.) Wall.ex Nees is a standing out medicinal herb. It contains a large quantity of andrographolide and wildly used a traditional herb in many countries. Andrographolide is found in all part of the plant. However, those part are different in color, active compound content and antioxidant activity. Even though, there are several methods to determine andrographolide and antioxidant. They are chemical involved, complicated and time consuming. Therefore, the objective of this study was to develop estimation method of andrograhpholide content and antioxidant activities by CIE color parameters. The result showed that range of CIE color parameters of andrographolide sample were broad with range from light greyish yellow-green to greyish dark green. Antioxidant activity was barely correlated with colors. However, andrographolide content showed moderate correlation with colors. Dark green andrographis herb contain higher andrographolide than light greyish yellow-green one. Furthermore, andrographolide content could be numerical estimated by CIELab and CIELCh color parameters. The developed estimating method were easy, rapid and no chemical involved.

Keywords: Andrographis herb, Andrographolide, Color, CIELab, CIELch, Antioxidant activity, Estimation

Introduction

Andrographis paniculata (Burm.f.) Wall.ex Nees is a prominent medicinal herb. It contains a large quantity of andrographolide, a bitter diterpenoid lactone. This herb is commonly found in south and southeast Asia and some other countries including Cambodia, Caribbean islands, China, India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand and Vietnam. It has been widely used as traditional herb for the treatment of snake and bug bite, diabetes, dysentery, fever and malaria [1]. Therapeutic potentials of this herb are immunomodulatory, antibacterial and anti-inflammatory, laxative, depurative, prophylactic, hepatoprotective and cardiovascular effects [2]. Despite of immemorial usage of this herb, during the COVID-19 crisis, andrographis herb has become more standing out. The demand was sharply increased, causing the short product supply afterward. Apart of that, variation of andrographolide content and quality was also concerned. Treatment with low quality product could be leading to not only economic loss, but also disease progressing and treatment failure.

Although, andrographolide is found in all part of the plant, however, leaves showed the highest amount. While andrographolide in roots was almost 100 times lesser than that of in leaves [3]. Moreover, there was report that clearly showed that matured leaves contained higher amount of andrographolide than young one [4]. Antioxidant activities were also different in leaf, stem and fruit of the plant [5]. Hence, disparity of andrographolide content and antioxidant activity in products may be partly cause by using different part of the plant. Despite the fact that, there are several methods to determine andrographolide and antioxidant [6-9]. They are chemical involved, complicated and time consuming.

Andrographis leaf and stem are dark green, while flower is white corolla with rose-purple spots on the petals. Roots and seed are yellowish-brown [10]. However, color is a subjective apparent property of object. Since 1931, the International Commission on Illumination (Commission Internationale de l'Eclairage, CIE) has proposed first numeric CIE color system [11]. CIELab and CIELCh color systems were later proposed. For CIELab system, L* refers to lightness, where its value ranges from 0 (darkest) to 100 (lightest). The a* and b* chromatic coordinate values denote the opposite color of red/green and yellow/blue colors, respectively. The a* color axis ranges from positive value (green) to negative value (red). In the same manner, positive value on b* axis shows yellow which opposite to blue color (negative value). CIELCh represents for CIELab in cylindrical coordinates. L* is the same as CIELab. The chroma

(C*) and hue angle (h) are derived from a* and b*. The C* is relative saturation or vividness of color. Hue angle of 0°/360°, 90°, 180° and 270° represent red, yellow, green and blue, respectively [12]. There are several reports of study of relationship of color, antioxidant and active ingredient in herb, fruit and vegetable. A study of in 2020, showed that reddish and bluish-reddish of 57 fruits and vegetables contain high antioxidant [13]. Furthermore, a study of mulberry wine showed that total phenolics, total anthocyanins and cyanidin-3-O-rutinoside (a monomer anthocyanin) correlated with color parameters [14]. Comparable results also have been reported that color parameter in CIE system related with total anthocyanins, total flavonols and total phenolics of grape wines [15]. Lycopene and beta-carotene content in tomatoes were indicated by yellow to red colors [16]. To our knowledge, there are scantly report of color parameters and antioxidant and andrographolide content of andrographis herb. Moreover, to assist rapid quality checking of andrographis production, the objective of this study was to develop estimation method of andrograhpholide content and antioxidant activities by CIE color parameters.

Materials and methods

Chemical and reagents

Andrographolide standard, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-*s*-triazine (TPTZ), gallic acid were sodium acetate trihydrate were obtained from Sigma-Aldrich. Acetonitrile gradient grade for liquid chromatography and methanol (analytical grade) were purchased from Merck.

Andrographis samples

Dried and grounded 90 samples of andrographis were purchased from local market in Bangkok, Nakorn Pratom, Nontabururi and Suphanburi and from online market throughout Thailand. The samples were used without further preparation.

Analysis of color

The of CIE color space including L*, a*, b*, C* and h of andrographis powder was determined using a colorimeter (HunterLab, Ultrascan Pro, USA) with illuminant D_{65}, 10° observation, 25 mm illuminated port diameter and a 10 mm path length cell. The samples were measured in reflectance-specular excluded mode.

Andrographolide determination

The extraction was performed according to Wattananat *et al*. [17] with slightly modified. Two hundred mg of andrographis powder was extracted with 25 mL methanol. Then the mixture was sonicated for 15 min at room temperature. After filtrated, the sample extract was then assayed for andrographolide content by UHPLC equipped with PDA detector (Shimadzu, Japan). The extract was separated on C18 column (2.1×100 mm^2, 1.9 μm) by isocratic elution using 35 % acetonitrile with flow rate of 0.5 mL/min. Detection was monitored at 230 nm. The amount of andrographolide in sample were calculated by comparing with analytical curve of andrographolide standard and expressed as % by weight.

DPPH radical scavenging activity assay

DPPH radical scavenging activity was measured using the method as described elsewhere [18]. A hundred μL of sample extract from andrographolide determination experiment was reacted with 2.9 mL of 0.06 mmol/L DPPH reagent. After incubation for 60 min in the dark, the absorbance at 517 nm was measured. The DPPH radical scavenging activity of the sample was determined against standard curve of gallic acid and expressed as grams gallic acid equivalent per kilogram of sample (g GAE/kg).

FRAP assay

FRAP was analyzed as described previously with slight modifications [19]. A hundred μL of the extract was added to 3 mL freshly prepared FRAP reagent (1 part of 10 mmol/L 2,4,6-tri(2-pyridyl)-*s*-triazine, 1 part of 20 mmol/L ferric chloride and 10 parts of 300 mmol/L sodium acetate buffer pH 3.6). After incubation at 37 °C for 4 min the reaction was measure at 593 nm. The FRAP of the sample was calculated against standard curve of gallic acid and expressed as grams gallic acid equivalent per kilogram of sample (g GAE/kg).

Statistical analysis

All samples were analyzed in triplicate. Correlation analysis among color parameters, andrographolide content and antioxidant activity were performed based on Pearson's correlation test. Estimating equations were obtained from enter method of linear regression analysis. A *p*-value less than 0.05 was considered significant. All statistical tests were carried out using SPSS for Windows.

Results and discussion

The range of CIE color parameters were vast. The lightness of samples was range from fairy dark (35.17) to slightly light (64.58). While a* result showed both plus and minus value, indicated that some andrographis sample with plus a* value contained slightly red color. However, all studied sample were shift toward yellowish color by positive b* value. Average chroma (C*) value was 20.87 with ranged from 12.58 to 27.69. It was demonstrated that andrographis samples color were in grey zone and not vivid. Hue angle, h* result showed that samples were range from yellow (86.16) to green (113.28). Other than wide range of color parameters, andrographolide content and antioxidant activities were also broad as shown in **Table 1**.

Table 1 CIE color andrographolide and antioxidant activities of andrographis herb.

Parameter	Mean ± SD	Range in values
lightness, L*	50.57 ± 7.18	35.74 - 64.34
red/green value, a*	–3.93 ± 2.19	–9.02 - 1.43
yellow/blue value, b*	20.39 ± 2.83	11.97 - 27.28
chroma, C*	20.87 ± 2.88	12.58 - 27.69
hue angle, h* (°)	100.84 ± 5.96	86.16 - 113.28
andrographolide (%)	3.29 ± 2.46	0.19 - 11.67
DPPH (g GAE/kg)	1.03 ± 0.86	0.01 - 8.12
FRAP (g GAE/kg)	1.90 ± 1.62	0.25 - 12.60

According to a guide to use of correlation coefficient that publish elsewhere [20,21], CIE color parameters showed low to negligible correlation with antioxidant activities. Nevertheless, h* was negatively correlated with both DPPH and FRAP. Samples with lower h* or shift toward yellow may contain higher DPPH and FRAP.

Though, almost all CIE color parameters were significant correlated with andrographolide content, b* showed a weak correlation. While L*, a* and h showed moderate correlation with andrographolide content. Decreasing in L* and a* resulted in increasing in andrographolide content. However, lower in hue angle value, h* showed higher value of andrographolide as shown in **Table 2**. This was not surprising, due to foliage part with dark green color of andrographis contain high amount of andrographolide. However, correlation of andrographolide and a* which represent red/green value of sample was not as high as that of h*. This may due to h* were derived from a* and b* value. It accounted for both red/green and yellow/blue color axes. It was able to represent natural mixed color of plant better than a* that accounted for only single-color parameter.

Although andrographolide contains antioxidant property, however, the results showed the negative correlation between andrographolide and antioxidant activities. It was plausible that andrographis contained other high antioxidant compounds. Furthermore, the negative significant between h* and antioxidant activities results suggested that those other compounds may be increased when color of sample shifted toward yellow.

Table 2 Correlation coefficients among CIE color parameters, andrographolide content and antioxidant activities.

	L*	a*	b*	C	h*	andro-grapholide	DPPH
a*	0.534**						
b*	0.257**	–0.227**					
C*	0.167**	–0.354**	0.990**				
h*	–0.626**	–0.952**	–0.061	0.071			
andrograp holide	–0.630**	–0.556**	–0.129*	–0.038	0.631**		
DPPH	0.117	0.283**	0.047	0.019	–0.305**	–0.017	
FRAP	–0.137*	0.008	0.083	0.093	–0.031**	–0.318**	0.838**

Note: * and ** = correlation was significant at 0.05 and 0.01 levels, respectively.

The slight correlation results indicated that color parameters were not proper to predict antioxidant activity. However, these parameters showed adequate correlation to estimate andrographolide content. Andrographolide estimating equations from regression analysis were shown in **Table 3**. Based on the coefficient of determination (R^2) and root mean square error (RMSE) for the estimation, the equation with CIELCh was slightly better than the one with CIELab. Despite that, combination of both CIE systems resulted in better estimation power.

Table 3 Regression equations for estimation of andrographolide content.

Parameters	Equation	R^2	RMSE
CIELab	andrographolide = –0.136L* –0.413a* –0.105b* +10.666	0.546	25.516
CIELCh	andrographolide = –0.128L* –0.011C* +0.164h* –6.612	0.564	25.020
Both	andrographolide = –0.117L* +1.758a* –2.304b* +2.628C* +0.639h* –56.252	0.621	23.335

For discrimination of high and low andrographolide content according to array of colors. Studied sample were divided into 4 groups (A, B, C and D) base on ca. half value of 2 highest correlation value of color parameters (L* and h*). Ranges of L* and h* in each group were as below:

Group A: L* = 35.0 - 50.0 and h* = 100.0° - 115.0°
Group B: L* = 49.9 - 65.0 and h* = 100.0° - 115.0°
Group C: L* = 35.0 - 50.0 and h* = 85.0° - 99.9°
Group D: L* = 49.9 - 65.0 and h* = 85.0° - 99.9°

Andrographolide in group A were significantly higher than that of in the other groups, especially group D. Average andrographolide of group A was 1.72, 1.67 and 3.34 times higher than that of group B, C and D, respectively, as illustrated in **Figures 2**(**A**) and **2**(**B**).

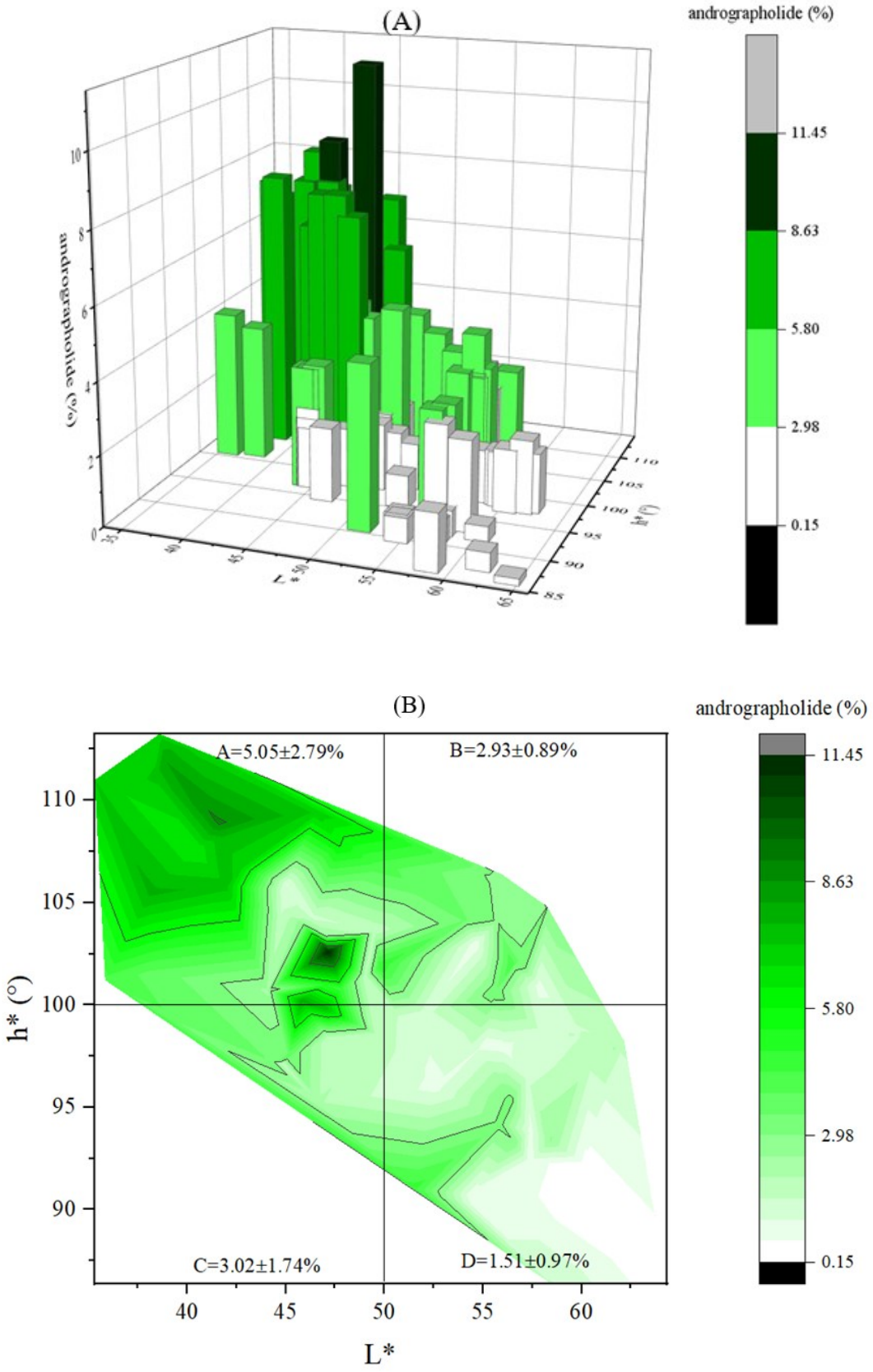

Figure 2 Relationship between color parameters (L* and h*) and andrographolide content.

This andrographolide estimation method required only color measurement. No extraction and separation were needed. Despite of several advantages, limitation of this method was distinguishing between authentic and counterfeit products, especially one with similar color.

Conclusions

Apparent color of andrographis samples were range from light greyish yellow-green to greyish dark green. Antioxidant activity was barely correlated with colors. However, andrographolide content showed moderate correlation with colors. Darker and greener andrographis herb was assumed that contain higher amount of andrographolide. In general, dark green andrographis herb contain andrographolide ca. 3 times higher than light greyish yellow-green one. Furthermore, CIELab and CIELCh color parameters could estimate numerical andrographolide content. This estimating method were easy, rapid and no chemical involved. This method could be used as rapid quality checking of andrographis production.

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