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DETERMINATION OF THE TOTAL PHENOLIC CONTENT FROM THE BULBS OF CRINIUM LATIFOLIUM (L.) BY FOLIN-CIOCALTEU METHOD

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Abstract

Crinum latifolium is a widely used traditional herb in Viet Nam. Phytochemical study of *C. latifolium (L.)* bulbs revealed the presence of triterpenoid, alkaloid, polyphenol, saponin, and polyuronid. Polyphenols are phytochemicals, compounds found abundantly in natural plant food sources that have antioxidant properties. The aim of this present study was to develop and validate an analytical method to quantitate the content of total polyphenols (TP) in an extract isolated from *C. latifolium (L.)* bulbs using UV/Vis spectrophotometric method. The optimum conditions such as analysis time, wavelength and ratio of reagents were examined and identified. Under these conditions, the analytical procedure validation proved the method to be linear, specific, precise, accurate, and reproducible. The total phenolic content of ethanolic extract was 475,58 mg GAE/100 g dry weight.

Key words: *Crinum latifolium (L.),* Total phenolic content, Folin-Ciocalteu.

1. Introduction

Crinum latifolium is a widely used traditional herb in Viet Nam virtually because of its antitumor activity. In addition, *C. latifolium (L.)* has a number of other valuable biological effects such as antioxidant, anti-inflammatory, detoxification, tissue regeneration, hormone balancing, enhancing cell-mediated immunity and being an effective T-lymphocyte activator [1,8]. So far, most studies have been conducted using leaves extract, but little is known about the phytochemicals of *Crinum latifolium (L.)* bulbs extract.

Phenolics are a class of chemical compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon group. There are two groups of phenolics - simple phenols and polyphenols. Polyphenols whose antioxidant activity have the ability to reduce or prevent damage caused by free radicals promote positive effects on human health [5]. Therefore, in this present study, we tried to determine the total phenolic content of powdered *Crinum latifolium L.* bulbs extract.

2. Materials and Methods

2.1 Materials and chemicals

The bulbs of C. latifolium (L.). were collected in Binh Dinh province, Viet Nam in 2016 Gallic acid (99% purity) was used as the reference standard (China).

2.2 Instrumentation

The absorbance of all samples was measured using a Thermo Scientific Evolution 300 UV/Vis Spectrophotometer with VisionPro software.

2.3 Procedures

2.3.1 Phytochemical screening

Dried powder of the bulbs was extracted with different solvents of increasing polarities: ethyl ether, ethanol, and water. Determination of compound groups in each extract is carried out using specific chemical reactions.

2.3.2 Total phenolic content determination

The total phenolics content in extracts was determined by the Folin-Ciocalteu (FC) reagent [2, 6, 7].

Extraction of plant materials

2.0 g of the powdered bulbs was extracted with 70 ml of 70% ethanol on an ultrasonic bath at 50 ^{0}C for 60 min. The extract was let to cool down to room temperature, filtered and transfered to a volumetric flask, then the flask was filled up to 100 ml using ethanol 70%.

Standard solution

Gallic acid was used as a standard and the total phenolics were expressed as mg/100 g of gallic acid equivalents (GAE). Standard solutions of gallic acid are of following concentrations:10, 25, 50, 75, 100, and 150 μg/ml.

Determination of the optimum conditions including analysis time, wavelength, and ratio of reagents.

The method was validated according to the guidance on analytical procedures and methods validation. The standard used was gallic acid. Specificity, linearity, accuracy and precision were tested.[3,4]

Results and Discussions

3.1. Phytochemical screening

Phytochemical study of *C. latifolium (L.)* revealed the presence of triterpenoid, alkaloid, polyphenol, saponin, carbohydrates and polyuronid.

3.2. Method optimization

The maximum absorption wavelength of the standard solutions and samples ranged from 750 nm to 765 nm. Absorbance of each solution was compared at three wavelengths of 750 nm, 760 nm and 765 nm. The 750 nm is the most stable wavelength. Using the wavelength of 750 nm, the next step was to determine the reaction kinetics with respect to the time period prior to the spectrophotometric measurement. The increase in absorbance of each solution relatively from 0 to 120 min was determined. These data showed that the Folin-Ciocalteu reaction was stable after 60 min. Ratio of reagents, and the volume of extract were determined using the Design wizard, the Box-Behken model in MODDE 5.0 software progress 15 experiment to record and predict optimal conditions.

Table 1. Levels of factorial

	Levels		
	-1	0	+1
Volume of extract	0.25 ml	0.5 ml	0.75 ml
Concentration of FC reagents	5%	10%	15%
Concentration of Na_2CO_3	7.5%	10%	12.5%

Results of ANOVA analysis show that prediction goodness Q2 = 0.882 > 0; the statistical criterion of fit goodness R2 = 0.991 > 0.9; p = 0 <0.05.

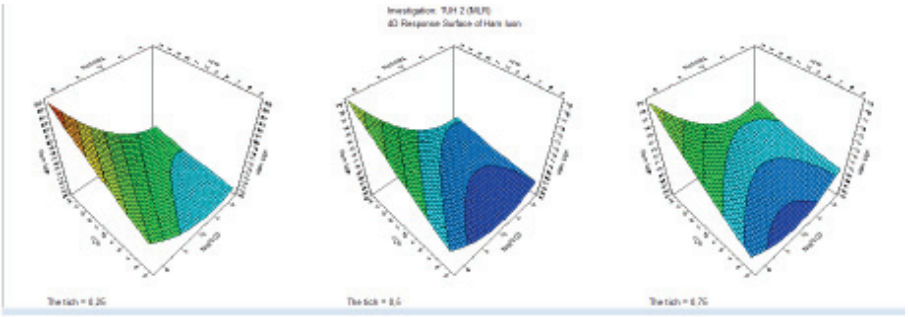

Figure 1. Three-dimensional response surface plot for total polyphenol content

The increase in the extract volume and the concentration of Na_2CO_3 led to the decrease in the total phenolic content. However, the concentration of Na_2CO_3 must be enough to maintain the alkaline medium.

The optimal conditions were 0.25 ml of the plant extract, 15% Folin-Ciocalteu reagent, and 7.5% Na_2CO_3.

From the aforementioned results, total phenolic content was determined using the Folin-Ciocalteu's reagent. In detail, 0.25 ml of the extract solution (0.25 ml) was added into a volumetric flask, then 5.0 ml of 15% Folin-Ciocalteu reagent were added and the flask was shaken thoroughly. After 5 min, 4 ml of 7.5% Na_2CO_3 was added, 70% ethanol was added to fill up to 10 ml, and the mixture was allowed to stand for 60 min. Absorbance was measured at 750 nm. The same procedure was repeated for standard solution of 75 μg/ ml of gallic acid.

The total polyphenol (TP) expressed as mg/100 g of gallic acid equivalents (GAE) of the extract from *C. latifolium (L.)* was calculated by Equation:

$$TP = \frac{A_x \times C_{control}}{A_{control}} \times \frac{100}{m \times 1000} \times \frac{100 \times 0.99}{(100 - h)\%}$$

3.3. Method Validation

Specificity (selectivity)

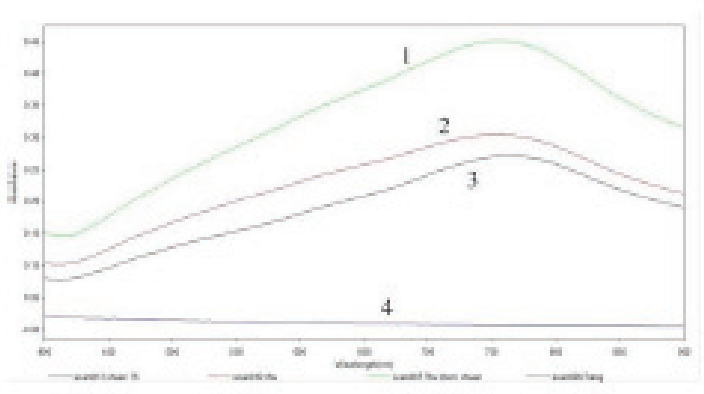

Figure 2. Absorption spectra (400 to 900 nm) of (1) sample spiked with gallic acid, (2) sample, (3) gallic acid standards, (4) blank sample.

Linearity of concentration

Seven sets of concentrations at 0.25, 0.65, 1.25, 1.875, 2.5, 3.75, and 5.0 g/ml of standard gallic acid solutions were made. Five sets of concentrations at 0.2; 0.5; 1.0; 1.5 and 2.0 mg/ml of extract solution were made.

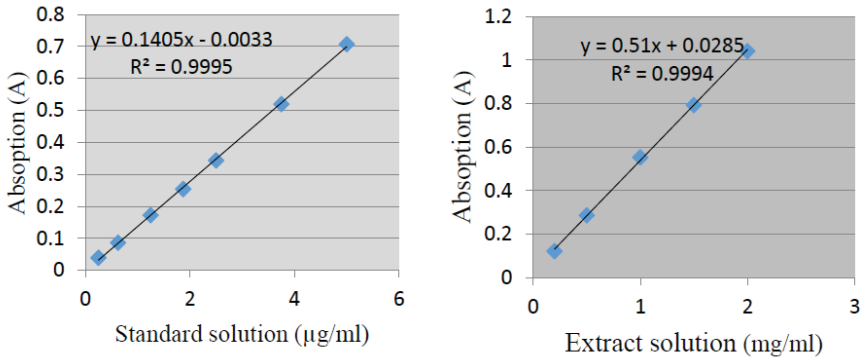

Figure 3. Linear line of gallic acid solution and extract solution

The results show that the absorption and the concentration of the standard gallic acid/ extract solution with correlation coefficient $R^2 = 0.9995$ (standard sample) and $R^2 = 0.9994$ (sample) was linear. The linear range of the standard gallic acid solution is 0.25 - 5 μg / ml. The linear range of the extract solution is 0.2 - 1.5 mg / ml.

Precision

Table 2. Result of precision

	Intra-day precision	**Inter-day precision**
Mean (mg GAE/ 100g)	460.41	463.41
SD	12.15	10.63
RSD	2.64	2.29

The repeatability and the intermediate precision were of no significant difference between them. (RSD $< 7.3\%$)

Accuracy (Recovery)

Accuracy was calculated as the percentage recovery of a known amount of standard added to the sample. Standard gallic acid solution was added to sample solution and analyzed by the proposed method.

Table 3. Result of accuracy

Levels	**Recovery %**	**RSD %**
80%	90.26	2.90
100%	92.33	0.78
120%	94.01	1.45

These percentages were within the range of 80 - 115% established in published reports.

3.4. Method application

Method was applied to determination of total polyphenol content in the bulbs of C. latifolium (L.). Standard solution was used at the concentration of 75 μg/mL, total polyphenol content of ethanolic extracts was 475.58 mg GAE/100 g dry weight.

4. Conclusions

The phytochemical analysis revealed that the bulb extract of *C. latifolium (L.)* contains triterpenoids, alkaloids, polyphenols, saponins, and polyuronids.

From that result, analytical method was developed to determine the content of total polyphenols (TP) in an extract isolated from *C. latifolium (L.)* bulbs using the Folin-Ciocalteu method. Optimal conditions such as analysis time, wavelength and ratio of reagents were identified. The process meets

the verification requirements, and the collected values are within the allowable limits.

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