

DETERMINATION OF LUTEOLIN FROM EXTRACTS OF *Helicteres hirsuta* BY HPLC

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Abstract. High-performance liquid chromatography coupled with a photodiode array detector (HPLC-DAD) has been reported to quantify isolated compounds. This work was designed, therefore, to develop an HPLC-DAD system to determine luteolin in the extract solutions from *Helicteres hirsuta*. Luteolin was analyzed on an RP-C18 column using a mobile phase including acetonitrile – 0.1% phosphoric acid (v/v) = 1:1 (v/v) with a detecting wavelength of 347 nm, a flow rate of 0.5 mL/min, and a volume of an injected sample of 10 μ L. The HPLC system was carried out at ambient temperature. The method shows linearity for luteolin in the range 0.02–1 mg/mL, and the recovery of luteolin is 94.07 ± 0.64 %. This is the first time, the contents of luteolin in methanol extracts from the plant parts of *H. hirsuta* (including branch, fruit, and aerial parts) were determined with a value of 49.06 ± 0.46 , 56.61 ± 0.62 and $91.15 \pm .42$ μ g/g, respectively.

Keywords: luteolin, *Helicteres hirsuta*, HPLC

1 Introduction

Helicteres hirsuta (An xoa) belongs to the *Helicteres* family. Sterculiaceae is widely found in Southeast Asian countries, such as Vietnam, Laos, Cambodia, Indonesia, and Thailand [1, 2]. This plant is used as a traditional medicine to treat malaria, diabetes, and cervical cancer [3]. In addition, Chin et al. reported that lignans were isolated from *H. hirsuta* with strong anti-cancer properties [2]. Studies on chemical composition and antioxidant activity of species are very limited in the literature. In Vietnam, Pham Hong Ngoc Thuy et al. reported the extraction conditions and some preliminary assessments of antioxidant activity [4, 5] and Nguyen Thanh Triet et al. reported the antioxidant activity of three compounds (3-O-acetyl betulinic, stigmasterol, and 5,8-dihydroxy-7,4'-dimethoxyflavon) [6].

Luteolin has attracted a lot of interest because of their antioxidant activity [7]. Hao Dong et al. reported enhanced antioxidant activity, antibacterial activity, and hypoglycemic effect of luteolin by complexation with manganese (II) and its inhibition kinetics on xanthine oxidase [8]. Kyoung Ah Kang et al. have reported that luteolin induces apoptotic cell death via antioxidant activity in human colon cancer cells [9]. The determination of the contents of luteolin in *H. hirsuta* is not reported. Moreover, the potential of utilization of *H. hirsuta* extracts in antioxidant activities relates to their flavonoids such as quercetin, luteolin, and rutin. The objective of this paper is to quantify luteolin in methanol extracts from the plant parts of *H. hirsuta* using the HPLC method.

2 Experimental

2.1 Materials

The plant parts of *H. hirsuta* were collected in January 2018 in Thua Thien Hue and taxonomically identified at the Department of Biology, University of Sciences, Hue University. A voucher specimen was deposited at the department.

Luteolin was purchased from Sigma – Aldrich Co. (USA) (luteolin standards were dissolved in the mobile phase yielding concentrations of 2, 4, 8, 12, and 16 $\mu\text{g/mL}$). The solutions were filtered through a 0.45 μm membrane filter. HPLC-grade solvents were purchased from Fisher Scientific (Korea).

2.2 Preparation of methanol extracts

A dried sample (10 g) was extracted with 0.1 L methanol (MeOH) three times at room temperature. The solutions were combined, filtered through Whatman No.4 paper, and evaporated under reduced pressure at 50 $^{\circ}\text{C}$, resulting in crude methanol extracts.

2.3 HPLC conditions

Preparation of sample solutions. One hundred milligrams of the given sample was accurately weighed and put into a 10 mL volumetric flask. The sample was then dissolved by adding 10 mL of methanol.

Chromatographic conditions. Chromatographic analysis (HPLC, Agilent 1260, USA) was carried

Table 1. HPLC specifications for phytochemical analysis

Chromatographic conditions	
Concentrations ($\mu\text{g/mL}$)	2 to 16
Mobile phase (v/v)	acetonitrile: 0.1% 1. Phosphoric acid = 1:1 (v/v)
Flow rate (mL/min)	0.5
Injection volume (μL)	10
Standard Rt (Min)	2.9218 ± 0.002
Detection wavelength (nm)	347

out using a C_{18} reverse-phase Inertsil ODS-3 column (150 \times 4.6 mm), packed with 5 μm diameter particles and a UV-Vis detector. The HPLC specification and chromatographic conditions are given in Table 1. All solutions and the mobile phases were filtered through a 0.45 μm membrane cellulose filter before use, and all chromatographic operations were carried out at ambient temperature.

3 Results and discussion

The HPLC profiles for luteolin indicate a single peak at a retention time of 2.9218 ± 0.002 min (Table 2 and Fig. 1). System suitability tests were carried out on a prepared luteolin standard solution ($n = 5$) with 10 μL injection volumes. All results were obtained in the acceptable range (with RSD = 0.068).

Table 2. Retention time of luteolin

Number	Retention time (min)	R_{ITB} (min)	Repeatability of retention time (RSD %)
1	2.920	2.9218 ± 0.002	0.068
2	2.920		
3	2.923		
4	2.923		

Number	Retention time (min)	R _{RTB} (min)	Repeatability of retention time (RSD %)
5	2.923		

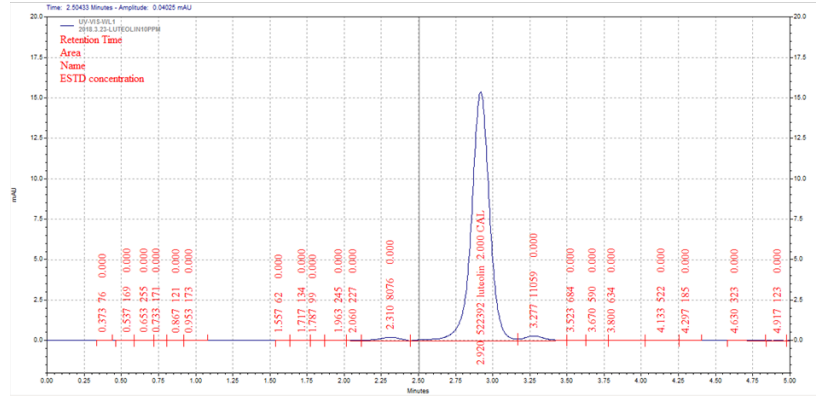


Fig. 1. HPLC chromatogram of luteolin

The linearity regression data of luteolin ($y = 347756x - 226720$) show a good linear relationship between concentrations and peak areas over a concentration range of luteolin from 2 to 16 $\mu\text{g/mL}$, and the correlation coefficient (R) is 0.9997 (Table 3) (the evaluation of each point was repeated three times).

Table 3. Regression equation, regression coefficient, LOD (limit of detection) and LOQ (limit of quantification) of luteolin

Standard solution of luteolin prepared for calculation of LOD and LOQ					
Concentration ($\mu\text{g/mL}$)	2	4	8	12	16
Peak area (mAU)	522392	1138782	2516600	3912784	5381602
Regression equation	$y = 347756x - 226720$				
Regression coefficient	$R = 0.9997$				
LOD ($\mu\text{g/mL}$)	0.448				
LOQ ($\mu\text{g/mL}$)	1.493				

The LOD (which is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified) is 0.448 $\mu\text{g/mL}$. The LOQ value (which is the lowest amount of analyte in a sample) is 1.493 $\mu\text{g/mL}$.

Accuracy was determined using a recovery test at three concentration levels (Table 4). The recovery was determined by subtracting the values obtained for the control matrix preparation from those samples that were prepared with the added standards, divided by the amount added, and then multiplied by 100%.

The distribution of luteolin in the plant parts of *H. hirsuta* is shown in Table 5 and Fig. 2. The high amount of luteolin in methanol extracts from the aerial parts of *H. hirsuta* ($91.15 \pm 0.42 \mu\text{g/g}$) is the highest in plant parts.

The luteolin contents were also compared with those of other medicinal plants. It can be seen that the luteolin content of *H. hirsuta* is higher than that of plants (the luteolin content of green pepper samples was $46.00 \pm 0.76 \text{ mg/kg}$) [10]; (without that of bird chili (*Capsicum frutescens*)) [11]; (without both that of *Raphanus sativus* Linn. $179.5 \pm 10.6 (\mu\text{g/g})$ and that of *Malus pumila* Mill. $149.5 \pm 4.5 (\mu\text{g/g})$) [12].

Table 4. Results of survey of recovery of luteolin

Amount added (µg)	Amount recoveries (µg)	Recovery (%)	$X_{TB} \pm S$
12.47	11.65	93.42	94.07 ± 1.59
15.29	14.48	94.70	
12.70	11.95	94.09	

Table 5. Luteolin contents from the plant parts of *H. hirsuta*

Plant part	Luteolin (µg/g)
Branch	49.06 ± 0.46
Fruit	56.61 ± 0.62
Aerial parts	91.15 ± 0.42

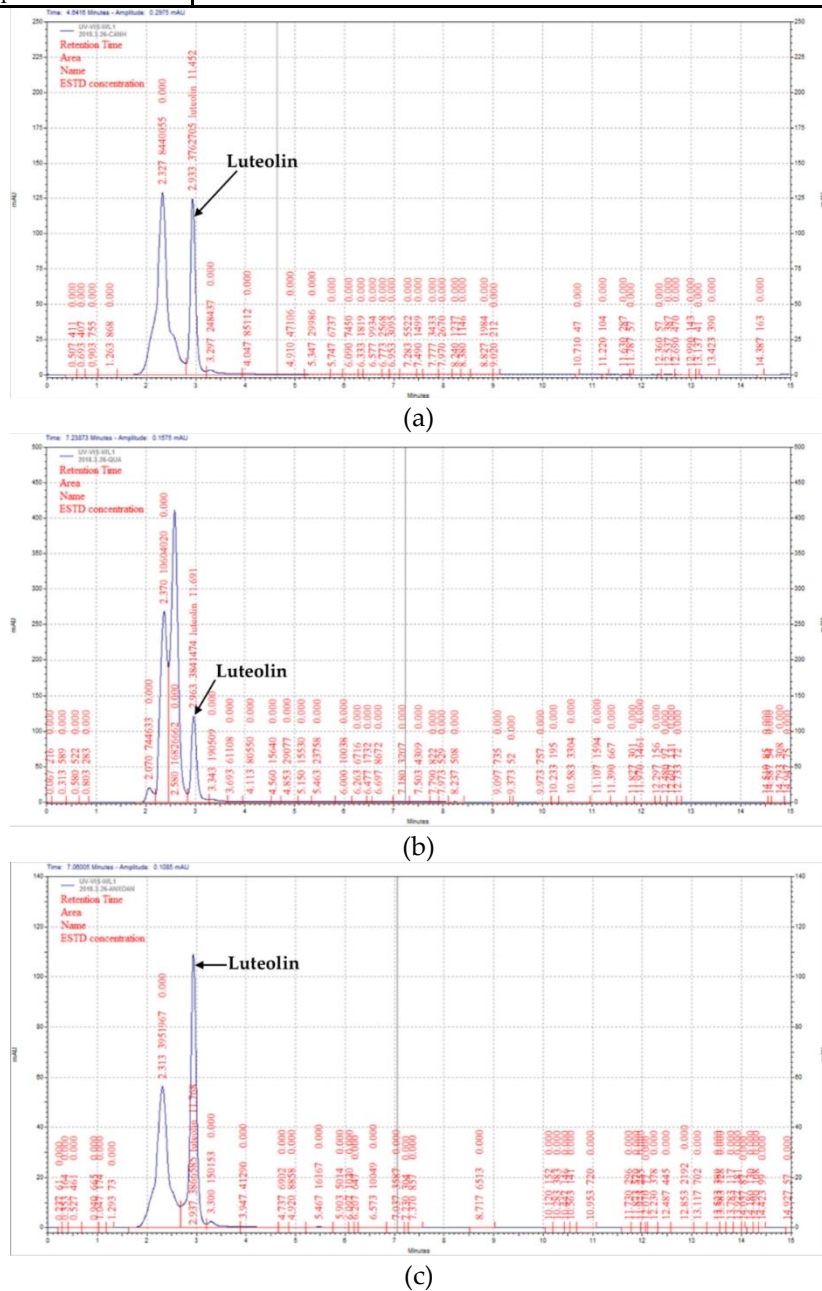


Fig. 2. HPLC chromatogram of luteolin from: (a) branch, (b) fruit and (c) the aerial parts of *H. Hirsuta*

4 Conclusions

The quantification of luteolin in the methanol extracts from the plant parts of *H. hirsuta* was determined using the HPLC method. The luteolin content from the branch, fruit, and aerial parts of *H. hirsuta* is 49.06 ± 0.46 , 56.61 ± 0.62 , and 91.15 ± 0.42 $\mu\text{g/g}$, respectively. The experimental results may provide a theoretical basis for further system research, development, and extraction of luteolin from *H. hirsuta*.

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