

Determination of methyl gallate and rutin from *Helicteres hirsuta* by HPLC and using methyl gallate content as a marker for the evaluation of antioxidant capacity

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Abstract

The aim of study was to investigate a relationship between the antioxidant activity and the total phenolic and some phenolic content in methanol extract from *Helicteres hirsuta*. Methyl gallate and rutin are extensively interested because of their antioxidant activity and they were quantified by HPLC. Antioxidant potential of *Helicteres hirsuta* through total phenolic content using Folin - Ciocalteu's method has been studied and their antioxidant activity was evaluated using DPPH radical scavenging and total antioxidant activity. The data resulted from DPPH radical scavenging activities indicated that *Helicteres hirsuta* displayed the good activities with low IC₅₀ values (17.07 µg/mL), approximately 2 times less than that of curcumin (38.50 µg/mL). Contents of methyl gallate and rutin in methanol extract from *H. hirsuta* were determined using HPLC with value of 8.569±0.462 and 6.687±0.534 mg/g, respectively. Moreover, the amount of methyl gallate from *H. hirsuta* could be used as a marker for the evaluation of the total antioxidant capacity or total phenolic content.

Keywords. *Helicteres hirsuta*, antioxidant activity, methyl gallate, rutin, HPLC.

1. INTRODUCTION

One of the most important properties of disease prevention and cure for food or pharmaceutical purposes is their antioxidant activity. The antioxidative compounds are usually able to scavenge the free radicals, slowing down the ageing process in the body, protecting liver function and preventing some health complications: Alzheimer, Parkinson.^[1-3] One of the most important ways to detect bioactive compounds is from indigenous knowledge. The research will be based on the experience of using medicinal plants through biological screening, long-term accumulation and the impartation from one generation to another in the ethnic community. As thousands of *in vivo* tests on the human body over a very long period of time, it reduces time, effort and money compared to screening in the laboratory.

In recent years, people have spoken about the treatment of diseases associated with antioxidant effects, liver cancer and anti-inflammatory effect of *Helicias hirsuta* Lour., which has created a fever in internet community about using of this plant.

Helicteres hirsuta (An xoa) belongs to the

Helicides family. Sterculiaceae is wildly found in Southeast Asian countries such as Vietnam, Laos, Cambodia, Indonesia and Thailand.^[4-5] This plant was used as a traditional medicine to treat malaria, diabetes and cervical cancer.^[6] In addition, Chin et al. reported that lignans have been isolated from *H. hirsuta* with strong anti-cancer properties.^[5] Through the literature review, the number of studies on chemical composition and antioxidant activity of species is very limited. In our country, Pham Hong Ngoc Thuy et al. reported on the extraction conditions and some preliminary assessments of antioxidant activity^[7-8] and Nguyen Thanh Triet et al. have reported the antioxidant activity of four (3-O-acetyl betulinic, stigmaterol, 5,8-dihydroxy-7,4'-dimethoxyflavon and 5,8-dihydroxy-7,4'-dimethoxyflavone).^[9-10]

The aim of study was to evaluate antioxidant potential of *H. hirsuta* by total antioxidant capacity and DPPH radical scavenging methods, the total phenolic content, to determine amounts of methyl gallate and rutin and to calculate the correlation coefficients between the components having antioxidant capacity.

2. EXPERIMENTAL

2.1. Plant materials

The aerial parts of *H. hirsuta* were collected in January 2018 in Thua Thien Hue province of Vietnam and were taxonomically identified by Nguyen Viet Thang (Department of Biology, College of Sciences; Hue University). A voucher specimen was deposited at the department of Biology, College of Sciences; Hue University.

2.2. Preparation of methanol extracts

A dried sample (100 g) was extracted with 0.5 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 °C, resulting of the crude methanol extract.

2.3. Evaluation of the total antioxidant activity using the phospho-molybdenum method

The total antioxidant activity of studied samples was determined according to the method described by Gopi et al.^[11] The total antioxidant activity was expressed as number of equivalents of gallic acid (GA)^[12] and ascorbic acid (AS)^[13] (with concentrations of between 0.1÷0.5 mg/mL) and as the absorbance of the sample. The higher absorbance value indicates the higher antioxidant activity.

2.4. Evaluation of DPPH radical scavenging activity

The DPPH free radical scavenging activity of each sample was determined using the Jasco V-630 Spectrophotometer according to the method described by Gopi and coworkers^[11] and Wong et

al.^[14] Radical scavenging activity was evaluated using the IC₅₀ value.^[15-16]

2.5. Total phenolic content

Total phenolic content was determined by the Folin - Ciocalteu method. Gallic acid was used to calculate the standard curve (with concentrations of between 0.05÷3 mg/mL) and the results were expressed as mg of gallic acid equivalents (GAE) per g of sample.^[14,17]

2.6. Total flavonoid content

The total flavonoid content was determined using the method of Meda et al. (2005). The total flavonoid content was determined using a standard curve of quercetin at 0-50 mg/mL. The results were expressed as quercetin equivalents (QE) on a dry weight (DW) basis.^[18]

2.7. HPLC conditions

Preparation of standard solutions: Methyl gallate standard solutions were prepared in 10 mL methanol at 5 levels varied from 5 to 50 mg, rutin from 0.5 to 20 mg.

Preparation of sample solutions: One hundred milligrams of given sample were accurately weighed and put into 10 mL volumetric flask. The sample was then dissolved by adding 10 mL of methanol to obtain 10 mg/mL sample solution.

Chromatographic conditions: Chromatographic analysis was carried out by C₁₈ reversed phase Inertsil ODS-3 column (150x4.6 mm) packed by 5 µm diameter particles, detector UV-Vis. The HPLC specification and chromatographic conditions are given in table 7. All of the solutions and the mobile phases were filtered through a 0.45 µm membrane cellulose filter before used and all chromatographic operations were carried out at ambient temperature.

Table 1: HPLC specifications for phytochemical analysis

Compounds	Mobile phase (v/v)	Flow rate (mL/min)	Injection volume (µL)	Standard Rt (Min)	Detection wavelength (nm)
methyl gallate	0.5 % orthophosphoric acid (A): Methanol (B) (0 ~ 10 min, 10 → 30 % A; 10 ~ 20 min, 30 → 50 % A)	1.0	20	11.23±0.07	370
rutin				14.55±0.04	

All of the solutions and the mobile phases were filtered through a 0.45 µm membrane cellulose filter

before used and all chromatographic operations were carried out at ambient temperature.

3. RESULTS AND DISCUSSION

3.1. Total antioxidant capacity

The total antioxidant capacity was determined by assessing the electron-donating capacity of the sample using the phospho-molybdenum method. In principle, this method based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex at a low pH with a maximal absorbance at 695 nm. A high absorbance value indicates that the sample possesses high antioxidant activity.^[11]

As shown in figure 1, methanolic extract of *H. hirsuta* exhibited having antioxidant activity in the electron transfer model. However, their antioxidant activities were lower than that of curcumin and ascorbic acid.

The antioxidant capacity was expressed as the number of equivalents of gallic acid or ascorbic acid (the standard curve equation of gallic acid : $Abs = 0.7820 C_{GA} + 0.1648$, $R = 0.9966$; and the standard curve equation of ascorbic acid : $Abs = 4.5974 C_{AS} -$

0.3231 , $R = 0.9952$). Total antioxidant capacity of *H. hirsuta* plants showed contained 132.89 ± 0.16 mg GA/g or 251.69 ± 1.27 μ mol AS/g, which was higher than sample of grape seeds (from 233.2 to 337.1 μ mol AS/g),^[13] and that of *Piper betle* and tea.^[19]

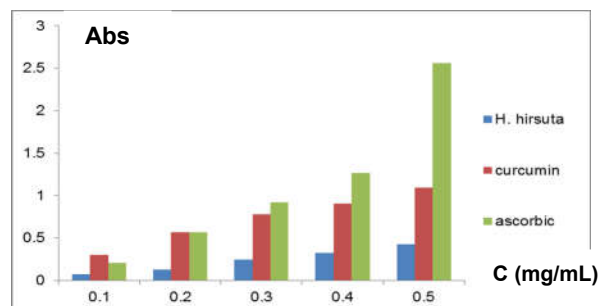


Fig. 1: Antioxidant activity of methanolic extract of *H. hirsuta*

3.2. DPPH radical scavenging activity

The values of DPPH radical scavenging activity is presented in table 2.

Table 2: The DPPH radical scavenging activity rates of *H. hirsuta*

Concentration	<i>H. hirsuta</i>	Curcumin	Ascorbic acid
100.0	82.78	81.26	96.65
20.0	68.27	40.64	93.80
4.0	12.74	29.07	88.81
0.8	1.32	20.19	37.08
IC ₅₀ (μ g/mL)	14.69	38.50	1.60

It can be seen that the DPPH radical scavenging activities of methanol extract of *H. hirsuta* enhanced along with the increasing of concentration (in table 2). The DPPH radical scavenging activity at the concentration of 100 μ g/mL of *H. hirsuta* (82.78 %) was higher than that of curcumin (81.26 %). Importantly, IC₅₀ of the methanol extracts of *H. hirsuta* showed the highest activities which is 2.5 times than that of curcumin. However, methanol extracts of *H. hirsuta* had the lower activities which is 9 times than those of ascorbic acid.

From two models used to evaluate the antioxidant potential of *H. hirsuta*, they exhibited a good antioxidant activity complying the hydrogen donor mechanism in which the DPPH free radical scavenging are significantly higher than that of curcumin.

3.3. Total phenolic and flavonoid contents

The antioxidant activity of medicinal plants were

attributed by phenolic compounds.^[20] The total phenolic content using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $A (Abs) = 7.026 C_{GA} - 0.019$, $R = 0.999$). The total flavonoid content was expressed in terms of quercetin equivalent (the standard curve equation: $A (Abs) = 17.231 C_{QU} - 0.059$, $R = 0.9985$). The content of phenolic and flavonoid compounds found in *H. hirsuta* were 26.718 ± 0.862 mg GAE/g, 9.090 ± 0.242 mg QUE/g, respectively. The total phenolic content was also compared with the same species in the literature.^[21] The total phenolic content of *H. hirsuta* was 2.9 times higher than that the same species in Khanh Hoa (8.99 mg GAE/g). The differences between phenolic contents could be originated from the characteristics of the samples. Geographical location and weather conditions in Thua Thien Hue maybe favorable than that of Khanh Hoa.

3.4. Quantification of the methyl gallate and rutin from methanol extraction of *H. hirsuta* by HPLC

HPLC profiles for both methyl gallate and rutin indicated a single peak at retention time of 11.23 ± 0.07 min and 14.55 ± 0.04 min, respectively. System suitability tests were carried out on prepared methyl gallate and rutin standard solutions ($n = 5$) with 20 μ L injection volumes. Linearity regression data (methyl gallate ($y = 59648698.76x + 20722.99$), rutin ($y = 27371495.33x + 15425.25$)) showed a good linear relationship between concentrations and peak areas over a concentration range of methyl gallate from 5 to 50 mg, rutin from 0.5 to 20 mg. The correlation coefficients (R) were found to be 0.999 and recovery of samples were from 96 % to 98 %. Total content of compounds owning the antioxidant activity in *H. hirsuta* is 15.283 mg/g while the amount of methyl gallate and rutin is 8.596 ± 0.018 mg/g and 6.687 ± 0.044 mg/g, respectively.

3.5. Correlation between methyl gallate or rutin and antioxidant capacity of *H. hirsuta*

Several studies have reported on the relationships between phenolic content and antioxidant capacity, or bioactive compound content and antioxidant capacity. Some authors found a correlation while others found no such relationship. In the publication,^[22] we had published the phenolic content and antioxidant capacity of some medicinal herbs such as *H. parasitica*, *A. clypearia*, *M. caseariafolia*, *S. oleracea*, *P. venusta*. Combined with obtained results of methyl gallate content, rutin content and antioxidant capacity of *H. hirsuta*. Thus, the amount of methyl gallate was strongly correlated with total phenolic content and total antioxidant capacity with high coefficients from 0.8909 to 0.9341. Therefore, the amount of methyl gallate could be used as a marker for the evaluation of the total antioxidant capacity and total phenolic content.

Table 3: Methyl gallate, rutin, total antioxidant capacity and phenolic contents from some medicinal plants

Sample	methyl gallate (mg/g)	rutin (μ g/g)	TAC	TPC	References
<i>A. clypearia</i>	14.469 ± 0.133	86.895 ± 0.104	280.27 ± 1.32	74.49 ± 1.08	[22]
<i>M. caseariafolia</i>	0.229 ± 0.002	6.964 ± 0.008	146.78 ± 1.15	21.13 ± 0.40	
<i>P. venusta</i>	0.157 ± 0.001	3.248 ± 0.004	139.63 ± 1.11	21.35 ± 0.43	
<i>S. oleracea</i>	0.574 ± 0.005	10.897 ± 0.013	143.72 ± 1.52	18.17 ± 0.79	
<i>H. parasitica</i>	18.335 ± 0.001	41.876 ± 0.049	301.47 ± 1.68	93.22 ± 0.34	
<i>H. hirsuta</i>	8.596 ± 0.018	6687.45 ± 44.124	132.89 ± 0.16	26.718 ± 0.862	

Table 4: Correlation between methyl gallate or rutin and antioxidant capacity of *H. hirsuta*

Statistical Correlations	Regression equation	Pearson correlation coefficient R
Methyl gallate and TPC	$y = 3.8484x + 15.343$	0.9341
Methyl gallate and TAC	$y = 8.6701x + 129.58$	0.8909
Rutin and TPC	$y = 0.0027x + 45.635$	0.2278
Rutin and TAC	$y = -0.0102x + 202.39$	0.3547

4. CONCLUSION

The experimental results showed that *H. hirsuta* in Thua Thien Hue province of Vietnam were strongly contained the phenolic content, which is greater than those of other in Khanh Hoa reported before. *H. hirsuta* showed the high activities which is strongly roughly 2.5 times than that of curcumin. The amount of methyl gallate, which was strongly correlated with total phenolic content or total antioxidant capacity, could be used as a marker of the evaluation of antioxidant activity in some medicinal plants. The first time, antioxidant potential, total phenolic content, methyl gallate content and rutin content had been reported and the experimental results showed

that *H. hirsuta* seem to be promising making a new resource of natural antioxidant.

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