

In vitro antioxidant activity and content of bioactive compounds from *Homalomena occulta*

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Abstract. *Homalomena occulta*, a plant widely used in traditional Vietnamese medicine, was assessed for its antioxidant potential through DPPH radical scavenging and total antioxidant capacity methods. The results showed that *Homalomena occulta* exhibited significant antioxidant activities with a low IC₅₀ value of 40.27 µg/mL, which is comparable to that of curcumin (38.50 µg/mL). Furthermore, the total antioxidant capacity of the *Homalomena occulta* extract was determined to be 77.48 ± 2.34 mg GA/g. To investigate the composition of the plant, various compounds including total phenolic, total flavonoid, polysaccharides, and triterpenoids were quantified using colorimetric methods with specific reagents. The content of phenolic compounds, flavonoid compounds, polysaccharides, and triterpenoids in *Homalomena occulta* was found to be 36.87 ± 0.42 GAE/g, 26.83 ± 0.29 mg QUE/g, 5.06 ± 0.06%, and 52.09 ± 1.77 mg Olenanoic/g, respectively. Notably, this study provides the first-ever report on the polysaccharide and total triterpenoid content of *Homalomena occulta*.

Keywords: *Homalomena occulta*, antioxidant activity, polysaccharide, triterpenoids, total phenolic content, total flavonoid content

1 Introduction

Indigenous knowledge plays a crucial role in identifying bioactive compounds, especially in the context of medicinal plants. Such research is grounded in the experiences and wisdom gained from the use of these plants in traditional medicine, which has been passed down through generations within ethnic communities. This long-term accumulation of knowledge serves as a valuable resource for the identification of bioactive compounds. Harnessing indigenous knowledge enables researchers to leverage the extensive in vivo testing conducted on humans over an extended period. This approach offers

several advantages, including significant time, effort, and cost savings compared to laboratory-based screening methods. By tapping into the rich traditional knowledge of medicinal plants, researchers can streamline the process of identifying and studying bioactive compounds, leading to more targeted and efficient research endeavors [1].

Homalomena, a large genus belonging to the Araceae family, is primarily distributed in Vietnam, China, Japan, various Asian countries, and South America [2]. Among its species, *Homalomena occulta* holds significant importance in Traditional Medicine and Ethnic Medicine in

Vietnam, China, Japan, and other Asian regions [3, 4]. This plant has been traditionally used to address various health conditions, including wound healing, diarrhea, coughs, abdominal pain, stomach disorders, rheumatoid arthritis, inflammatory conditions, and central nervous system disorders [5-7]. Modern pharmacological investigations have revealed the diverse chemical composition of *Homalomena occulta*, comprising essential vitamins, minerals, essential oils, and triterpenoids, phenolic compounds, among others [8-10]. These compounds have demonstrated various biological activities, such as antibacterial, antioxidant, anticancer, and anti-inflammatory properties [3, 11, 12]. The therapeutic potential of *H. occulta* makes it a subject of considerable interest in the realm of natural medicine and pharmacology.

From the literature review, it is proven that *H. occulta* has been potent antioxidant activity [13-15]. However, there is little research on *in vitro* antioxidant activity and chemical constituents of *H. occulta* in Vietnam.

The primary objective of this study was to assess the antioxidant potential of *H. occulta* using two methods: the total antioxidant capacity and DPPH radical scavenging assays. Additionally, we analyzed the content of various compounds present in *H. occulta*, including total phenolic, total flavonoid, polysaccharides, and triterpenoids. These analyses were carried out using colorimetric methods with specific reagents to provide comprehensive insights into the antioxidant properties and chemical composition of *Homalomena occulta*.

2 Experimental section

2.1 Plant material, chemicals and equipments

Materials: The aerial parts of *Homalomena occulta* were collected in Thua Thien Hue province,

Vietnam. They were identified and deposited by the University of Sciences, Hue University.

Chemicals and equipment: Curcumin, ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma - Aldrich Co. (USA). Gallic acid, quercetin, sulfuric acid, ammonium molybdate, and sodium phosphate were purchased from Shandong Chemical Co. (China). The ethanol used in all experiments was food grade and was purchased from local suppliers. Other reagents and solvents were analytical grade. The major equipment used was Jasco V-630 Spectrophotometer (Japan Spectroscopic Company, Japan).

2.2 Preparation of ethanol extract of *Homalomena occulta*

The powder samples (20 g) were subjected to extraction using 500 mL of ethanol 96% (v/v), and the process was repeated three times at a temperature of 78 °C for 3 hours each time. Subsequently, the extracted solutions were combined, filtered, and further concentrated under reduced pressure at 50 °C, resulting in a crude ethanol extract with an approximate yield of $6.88 \pm 0.14\%$ w/w (n=3). The resulting crude extract was then stored at -20 °C, until further analysis (without polysaccharide).

2.3 Evaluation of the total antioxidant activity using the phosphor-molybdenum method

The total antioxidant activity of the samples was assessed following the method described by Nair et al. [16] with slight modifications. In summary, a 0.3 mL aliquot of the sample was combined with 3 mL of a reagent solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was then incubated at 95 °C for 90 minutes. After the incubation period, the mixture was cooled to 25 °C, and its absorbance was measured at a

wavelength of 695 nm against a blank containing 3 mL of the reagent solution without the sample. The antioxidant activity was evaluated based on the optical density of the sample. The antioxidant capacity is expressed as the number of equivalents of gallic acid. (*the standard curve equation of gallic acid: $Abs = 1.951 \times C_{GA} + 0.2373$, $R = 0.9994$*).

2.4 Evaluation of DPPH radical scavenging activity

The DPPH radical scavenging activity of the samples was determined following a method from the literature with slight modifications [17, 18]. Briefly, 1.5 mL of each extraction at various concentrations (0.8, 4, 20, and 100 $\mu\text{g/mL}$) was mixed with 1.5 mL of 100 μM DPPH solution in ethanol. The reaction mixture was shaken for 1 minute and allowed to incubate at room temperature for 30 minutes. After the incubation period, the optical density (OD) changes were measured at a wavelength of 517 nm. Ethanol was used as the blank sample, and ascorbic acid, gallic acid, and curcumin served as the positive control (reference standard). *Inhibition* of DPPH radical in percent (%) was calculated by the formula:

$$\text{Inhibition of DPPH (\%)} = \frac{[(OD_{DPPH} - OD_{\text{sample} + DPPH}) / OD_{DPPH}] \times 100}{}$$

Radical scavenging activity was evaluated using the IC_{50} value which represented the concentration of the extract that caused 50 % neutralization of DPPH radicals, was calculated from the graph plotting between percentage of inhibition and concentration of the sample.

2.5 Total phenolic content

The total phenolic content (TPC) of the ethanolic extract was quantified using the Folin-Ciocalteu method. In brief, 0.5 mL of the ethanolic extract solution was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted at a 1:10 ratio) and 2 mL of saturated Na_2CO_3 solution. The tubes were

then incubated for 2 hours at room temperature to facilitate color development. Subsequently, the optical density was measured at a wavelength of 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of the sample [19].

2.6 Total flavonoid content

The total flavonoid content (TFC) was determined using a modified method from Ribarova et al [20]. In summary, 1 mL of the ethanolic extract solution was diluted with a mixture of 4 mL of deionized water and 0.3 mL of 5% NaNO_2 . After a 5-minute incubation, 0.3 mL of 10% AlCl_3 solution was added to the above solution, followed by the addition of 2 mL of 1M NaOH solution. The volume was then adjusted to 10 mL with deionized water. Subsequently, the optical density was measured at a wavelength of 510 nm. The results were expressed as quercetin equivalents (QE) on a dry weight (DW) basis [19].

2.7 Qualitative and quantitative analysis of water-soluble polysaccharides

The extraction of polysaccharides involved dispersing 3 grams of the powder samples in 150 mL of distilled water. The extraction process was conducted at a temperature of 100 $^\circ\text{C}$ for a duration of 3 hours, with a total of 3 replications. After extraction, the solutions were combined and filtered. To precipitate the polysaccharides completely, ethanol 96% was added to the concentrated extract solution at a ratio of 4:1 (ethanol 96% to extract volume) [21].

Qualitative and quantitative analysis of polysaccharides: polysaccharides (PS) were examined by using the phenol-sulfuric acid colorimetric method with D-glucose as a standard at a wavelength of 490 nm [22]. The standard curve equation of D-glucose is $Y = 0.0082X -$

0.0082 với $R = 0,9999$. The content of pure polysaccharides was calculated as follows:

$$\text{Content of pure PS (\%)} = \frac{OD+0.0082}{0.0082} \times V \times \frac{100}{m \times (1-W)} \times \frac{162}{180}$$

where OD is the optical density of the sample; V is the volume of sample; m is the mass of the sample; W is the moisture content of the sample.

2.8 Qualitative and quantitative analysis of total triterpenoids

The content of triterpenoids (TT) was determined through a coloring reaction with vanillin/ HClO_4 reagent [23, 24]. Initially, a 1.0 mL aliquot of the sample in a cuvette was subjected to solvent evaporation. Subsequently, 0.3 mL of 5% vanillin solution in 99% CH_3COOH and 1 mL of HClO_4 were added to the cuvette. The mixture was then incubated at 60 °C for 15 minutes. After incubation, 3.7 mL of CH_3COOH was added to the mixture, and it was allowed to cool to 25 °C. The optical density was measured at a wavelength of 540 nm against a blank containing the reagent solution without the sample. The triterpenoid content was quantified in terms of equivalents of oleanolic acid. The total content of triterpenoid compounds was determined based on calibration curves: $\text{Abs} = 0.0212 \times \text{COA} + 0.0451$, $R = 0.9962$.

3 Results and discussion

3.1 *In vitro* evaluation of the antioxidant potential of ethanol extractions

Free radical scavenging is a crucial mechanism for inhibiting lipid oxidation and is commonly employed to assess the antioxidant activity of the studied sample. The DPPH free radical scavenging capacity serves as an excellent method to determine the antioxidant activity of substances in the sample based on their ability to donate

hydrogen atoms or electrons [25]. The appearance of yellow spots, which bleach the purple color of the DPPH, confirms the antioxidant activity of the extractions. To evaluate the antioxidant activity of the ethanol extract, they were compared with gallic acid, ascorbic acid, and curcumin, well-known antioxidant references. The results of this comparison are presented in Figure 1.

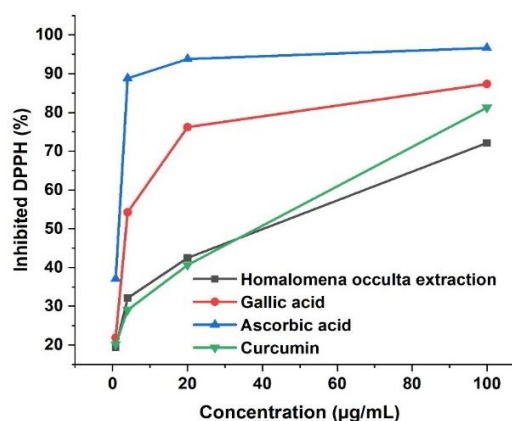


Fig. 1. The DPPH antioxidant activities of the sample and standards

The Figure 1 indicated that the higher the concentrations of the extracts of *H. occulta* extraction were, the better the DPPH inhibitions were. At the concentration of 20 µg/mL, the DPPH radical scavenging activity of ethanol extract from *H. occulta* extraction (42.48%) was higher than that of curcumin (40.64%). IC_{50} of the extract of *H. occulta* ($\text{IC}_{50} = 40.27$ µg/mL) was comparable to that of curcumin (38.50 µg/mL) but lower activity than that of ascorbic acid and gallic acid ($\text{IC}_{50} = 1.60$ µg/mL and $\text{IC}_{50} = 3.86$ µg/mL, respectively). It can be seen that the DPPH radical scavenging activity of the extract of *H. occulta* is higher than that of *H. occulta* Rhizoma from Chinese ($\text{IC}_{50} = 110$ µg/mL). Furthermore, the achieved IC_{50} value 40.27 µg/mL, considerably outperforms those of variously reported plants, as shown in Table 1. Thus, the ethanol extract of *H. occulta* had potential antioxidant properties.

Table 1. IC₅₀ values obtained from DPPH free radical scavenging activity of some medicinal plants

No.	Plants	IC ₅₀ DPPH (µg/mL)	Ref
1	<i>H. occulta</i>	40.27	This study
2	<i>H. occulta</i> Rhizoma	110	[13]
3	<i>H. aromatica</i> leaves	199.51	[26]
4	<i>H. aromatica</i> tuber	61.65±1.12	[27]
5	<i>Dureup</i>	119.4	[28]
6	<i>Physalis minima</i> Linn	280.23 ± 5.75	[29]
7	<i>Galanthus transcaucasicus</i> Fomin	From 125.07 to 171.07	[30]
8	<i>Persicaria odorata</i> Polygonaceae	311.26 ± 3.06	[31]
9	<i>Conyza blinii</i> H.Lév	(0.11 ± 0.01) × 10 ³	[32]

Moreover, the total antioxidant capacity was determined by evaluating the electron-donating capacity of the sample using the phospho-molybdenum method, a widely accepted approach for assessing antioxidant activity in chemical and biological samples. The total antioxidant capacity was expressed as milligrams of gallic acid equivalents per gram of dried sample. Notably, the total antioxidant capacity of the extract from *H. occulta* was found to be 77.48 ± 2.34 mg GAE/g, which significantly surpassed that of other both *O. sobolifera* [33] and *Calocybe indica* [34]. This result highlights the remarkable antioxidant potential of *H. occulta*.

3.2 Content of compounds from *Homalomena occulta*

Previous studies have indicated that the antioxidant potential of medicinal plants can be attributed to their phenolic and flavonoid content

[35, 36]. According to Tian et al. [8], phenolic compounds play a significant role in the antioxidant activity of *H. occulta*. In this study, the total phenolic content using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalent and the content of flavonoids in the plant extracts was determined using the spectrophotometric method with aluminum chloride. The content of phenolic and flavonoid compounds found in *H. occulta* were 36.87 ± 0.42 mg GAE/g, 26.83 ± 0.29 mg QUE/g, respectively (in Table 2). Remarkably, the total phenolic from *H. occulta* is higher than that of both sample *H. occulta* in China (1.14 ± 0.03 mg GAE/g) [15] and some medical plants in Table 3 (without *Curculigo orchoides*, *Bidens pilosa* flowers and *H. aromatica* leaves). It should be noted that *H. occulta* is the phenolic rich sources, which may contribute significantly to its potent antioxidant properties.

Table 2. Content of compounds from *H. occulta*

STT	Total phenolic (mg GAE/g)	Total flavonoid (mg QUE/g)	PS (%)	Total triterpenoids (mg oleanolic acid/g)
1	37.31	27.14	5.00	53.82
2	36.48	26.75	5.08	52.17
3	36.83	26.59	5.11	50,28
X _{TB} ± S	36.87 ± 0.42	26.83 ± 0.29	5.06 ± 0.06	52.09 ± 1.77

Previous research has demonstrated that triterpenoids and polysaccharides are constituents with remarkable biological activity in medicinal plants [37-39]. In our study, we evaluated the content of polysaccharides and triterpenoids in the extract obtained from *H. occulta*, and the results are presented in Table 2. The contents of polysaccharide and total triterpenoid found in *H. occulta* were 5.06 ± 0.06 %, and 52.09 ± 1.77 mg oleanolic acid/g, respectively. The content of

polysaccharides and total triterpenoids found in *H. occulta* are remarkably higher than those reported in the literature, as shown in Table 3. This observation suggests the potential utilization of *H. occulta* for various biomedical applications. Notably, this study is the first to report the total triterpenoid content and polysaccharide content of *H. occulta*, which further adds to the significance of our findings in the field of medicinal plant research.

Table 3. The content of TAC, TPC, TFC, PS and TT in the plants.

No.	Plant	TAC (mg GA/g)	TPC (mg GA/g)	TFC (mg QE/g)	PS (%)	TT (mg oleanolic acid/ g)	Ref
1	<i>H. occulta</i>	77.48 ± 2.34	36.87 ± 0.42	26.83 ± 0.29	5.06 ± 0.06	52.09 ± 1.77	This study
2	<i>Curculigo orchioides</i>	132.48 ± 1.48	196.24 ± 1.45	78.49 ± 1.78	4.34 ± 0.08	47.60 ± 0.24	[23]
3	<i>Ophiocordyceps sobolifera</i>	18.25 ± 0.15	18.08 ± 0.52	10.48 ± 0.02			[33]
4	<i>Calocybe indica</i>	64.94 ± 1.03	29.33 ± 0.16	17.84 ± 0.11	5.04 ± 0.04	4.96 ± 0.04	[40]
5	<i>Bidens pilosa</i> flowers	85.05 ± 0.28	59.35 ± 0.83	42.35 ± 1.50	4.44 ± 0.02	32.88 ± 0.66	[34]
6	<i>H. occulta</i>	-	1.14 ± 0.03	-	-	-	[15]
7	<i>Curcuma aromatica</i>	-	0.61 ± 0.02	-	-	-	
8	<i>Curculigo orchioides Gaerte</i>	-	11.98 ± 0.09	-	-	-	
9	<i>Mentha haplocalyx Briq</i>	-	20.92 ± 0.20	-	-	-	
10	<i>Paeonia lactiflora</i> Pall. (red)	-	31.48 ± 1.52	-	-	-	
11	<i>H. aromatica</i> leaves	-	From 91.21 ± 0.19 to 247.98 ± 4.75	From 17.79 ± 1.60 to 43.64 ± 1.28	-	-	[41]

4 Conclusions

The ethanol extract of *Homalomena occulta* was evaluated for its antioxidant properties, and the results demonstrated its remarkable activity with low IC₅₀ values, comparable to that of curcumin. The total antioxidant capacity of the extract was found to be 77.48 ± 2.34 mg GA/g. Additionally, the content of polysaccharides and triterpenoids in the extract was 5.06 ± 0.06 % and 52.09 ± 1.77 ,

respectively. Furthermore, *H. occulta* exhibited abundant phenolic and flavonoid compounds with contents of 36.87 ± 0.42 mg GAE/g and 26.83 ± 0.29 mg QUE/g, respectively, making it a valuable source of phenolics. Notably, this study also reports, for the first time, the total triterpenoid content and polysaccharide content in *H. occulta*, further enhancing the significance of our findings. These results collectively establish *H. occulta* as a promising natural resource for potent antioxidants, contributing to its potential

applications in various fields of biomedical research.

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