

IN VITRO ANTIOXIDANT ACTIVITY AND CONTENT OF COMPOUNDS FROM *CURCULIGO ORCHIOIDES* RHIZOMES

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Abstract. *Curculigo orchioides* Gaertn. is used in traditional medicine in Vietnam. Its antioxidant potential was evaluated through DPPH radical scavenging and the total antioxidant capacity method. The data resulted from DPPH radical scavenging activity indicate that *Curculigo orchioides* display high activity with a low IC₅₀ value (22.78 μg/mL), approximately 1.5 times less than that of curcumin (34.34 μg/mL). The total antioxidant capacity of the extract is equivalent to 132.48 ± 1.48 mg GA/g or 264.45 ± 2.34 μmol AS/g. The composition of *Curculigo orchioides*, including the total phenolic, total flavonoid, polysaccharides, and triterpenoid saponins, was examined by using the colorimetric method with reagents, and their quantity is equivalent to 196.24 ± 1.45 mg GAE/g, 78.49 ± 1.78 mg QE/g, 4.34 ± 0.08 %, 47.60 ± 0.24 mg Rb1/g (Rb1: Gypenoside III), respectively. Specifically, the total triterpenoid saponins content of *Curculigo orchioides* has been reported for the first time.

Keywords: *curculigo orchioides*, antioxidant activity, polysaccharide, triterpenoid saponins, total phenolic content, total flavonoid content

1 Introduction

One of the most important ways to detect bioactive compounds is from indigenous knowledge. The research is normally 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 time, it reduces time, effort, and money, compared with screening in the laboratory [1].

Curculigo orchioides Gaertn. (Sam cau) belongs to the family Hypoxidaceae and is a

precious medicinal plant commonly used in traditional medicine in India, Pakistan, China, and Vietnam [2, 3]. This medicinal plant is used in treating jaundice, asthma, making tonics, preventing osteoporosis [4], treating diabetes [5], anti-cancer [6], and antioxidant [7]. Chemical investigations of *Curculigo orchioides* led to the isolation of numerous phenols and phenolic glycosides, lignans, lignan glycosides, and polysaccharides [2, 4, 6].

From the literature review, it is proven that *Curculigo orchioides* has potent antioxidant activity. However, there is little research on *in vitro* antioxidant activity and chemical constituents of *Curculigo orchioides* rhizome in Vietnam.

This study aims to evaluate the antioxidant potential of *Curculigo orchioides* by using the total antioxidant capacity and DPPH radical scavenging methods. The content of compounds from *Curculigo orchioides*, including the total phenolics, total flavonoids, polysaccharides, and triterpenoid saponins was examined by using the colorimetric method.

2 Experimental

2.1 Plant material, chemicals, and equipment

Materials

Curculigo orchioides rhizome was collected in Son Tay district, Quang Ngai province, Vietnam.

Chemicals and equipment

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

2.2 Preparation of ethanol extract

The powder sample (100 g) was dispersed in 500 mL ethanol three times at 78 °C for 90 min. The solutions were combined, filtered, and evaporated under reduced pressure at 50 °C, yielding a crude

ethanol extract (approximately 3.18% w/w). The resulting crude extract was then stored at –20 °C until further analysis (without polysaccharide) [8].

2.3 Determination of total antioxidant activity with the phosphor-molybdenum method

The total antioxidant activity of the sample was determined according to Nair et al. [9] with certain modifications. In brief, a 0.3 mL of aliquot of the sample was mixed with 3 mL of a reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and then the mixture was incubated at 95 °C for 90 min. The mixture was then cooled to 25 °C, and the absorbance was measured at a wavelength of 695 nm against a blank containing 3 mL of the reagent solution. The antioxidant activity was calculated from the optical density of the sample. The high optical density value indicates that the sample possesses high antioxidant activity [9]. The antioxidant capacity is expressed as the number of equivalents of gallic acid [10] or ascorbic acid [11] (the standard curve equation of gallic acid: $Abs = 0.7820 \times C_{GA} + 0.1648$, $R = 0.9966$; and the standard curve equation of ascorbic acid: $Abs = 4.5974 \times C_{As} - 0.3231$, $R = 0.9952$).

2.4 Determination of DPPH radical scavenging activity

The DPPH radical scavenging activity of the sample was determined according to Wong et al. with minor modifications [12]. In brief, 1.5 mL of each extract of various concentrations (10, 20, 30, 40, and 50 µg/mL) was dissolved in 1.5 mL of 100 µM DPPH in ethanol. The reaction mixture was shaken for one minute and incubated at room temperature for 30 minutes to determine the optical density (OD). The optical density was then measured at a wavelength of 517 nm. Ethanol was used as a blank sample. Ascorbic acid was used as a positive control (reference standard). Inhibition

of DPPH radical in percentage was calculated according to the following formula

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

Radical scavenging activity expressed as the IC_{50} value, which represents the concentration of the extraction that causes 50 % of deactivation of the DPPH radicals, was calculated from the graph plotting the percentage of inhibition against the concentration of the sample.

2.5 Total phenolic content

Total phenolic content was determined by using the Folin-Ciocalteu method. Typically, 0.5 mL of the ethanol extract solution was mixed with 2.5 mL of Folin-Ciocalteu (1:10) and 2 mL of the saturated Na_2CO_3 solution. The tubes were incubated for 2 hours at room temperature for color development. The optical density was then measured at 760 nm wavelength. The results were expressed as the amount (mg) of gallic acid equivalents (GAE) per one gram of the sample [13].

2.6 Total flavonoid content

The total flavonoid content was determined according to Neto et al. with minor modifications [13]. Briefly, 1 mL of the ethanol extract solution was diluted with a mixture of 4 mL of deionized water and 0.3 mL of 5% NaNO_2 . After 5 minutes, 0.3 mL of 10% AlCl_3 solution was added into the above solution. Then, 2 mL of 1 M NaOH solution was also added before filling to 10 mL with deionized water. Optical density was then measured at 510 nm wavelength. The results were expressed as quercetin equivalents (QE) on a dry weight (DW) basis [13].

2.7 Qualitative and quantitative analysis of water-soluble polysaccharides

The polysaccharides were extracted as follows: the powder samples (3 g) were dispersed in 150 mL of distilled water at 100 °C for 3 h, and 3 replications were carried out. The solutions were combined and filtered. Ethanol 96% was added to the concentrated extract solution to precipitate polysaccharides completely (the ratio of ethanol 96% to the extract volume is 4:1) [14].

Qualitative and quantitative analysis of polysaccharides

Polysaccharides were examined by using the phenol-sulfuric acid colorimetric method with D-glucose as a standard at a wavelength of 490 nm [15]. The standard curve equation of D-glucose is $Y = 0.0082 \times X - 0.0004$, $R = 0.9993$. The content of pure polysaccharides was calculated as follows:

$$\text{Content of pure PS (\%)} = \frac{\text{OD} + 0.0004}{0.0082} \times V \times \frac{100}{m \times (1 - W)} \times \frac{162}{180}$$

where OD is the optical density of the sample; V is the volume of the sample; m is the mass of the sample; W is the moisture content of the sample [16].

2.8 Qualitative and quantitative analysis of triterpenoid saponins

The triterpenoid saponins content was determined via the coloring reaction of saponin-triterpenoids with vanillin/ HClO_4 reagent [17]. A 1.0 mL aliquot of the sample in a cuvette was evaporated to remove the solvent then added with 0.3 mL of a 5% vanillin solution in CH_3COOH and 1 mL of HClO_4 . The mixture was incubated at 60 °C for 15 min. The mixture was then cooled to 25 °C and added with 3.7 mL CH_3COOH . The optical density was measured at a wavelength of 540 nm against a blank that contains the reagent solution. The saponin-triterpenoids content is expressed as the

number of equivalents of oleanolic acid or Rb1 (Gypenoside III). The total content of triterpenoid saponins compounds was determined from two calibration curves: the standard curve equation of Rb1: $Abs = 0.0036 \times C_{Rb1} + 0.0014$, $R = 0.9980$ and the standard curve equation of oleanolic acid: $Abs = 0.0212 \times C_{AS} + 0.0451$, $R = 0.9962$.

3 Results and discussion

3.1 *In vitro* evaluation of antioxidant potential of ethanol extract

The DPPH radical scavenging activity

The appearance of yellow spots bleaching the purple color of the DPPH confirms the antioxidant activity of the extract. The antioxidant activity of the ethanol extracts was compared with that of ascorbic acid and curcumin (Table 1).

As seen from Table 1, the higher the concentrations of the extract of *Curculigo orchioides* are, the better the DPPH inhibition becomes. At the concentration of 50 $\mu\text{g/mL}$, the DPPH radical scavenging activity of the ethanol extract from *Curculigo orchioides* (88.36%) is much higher than that of curcumin (69.38%). The extract of *Curculigo orchioides* ($IC_{50} = 22.78 \mu\text{g/mL}$) has 1.5 times higher activity than curcumin ($IC_{50} = 34.34 \mu\text{g/mL}$) but lower activity than ascorbic acid. The DPPH radical scavenging activity of the ethanol extract of *Curculigo orchioides* is higher than that of ethyl acetate fraction of *Curculigo orchioides* ($IC_{50} = 52.93 \mu\text{g/mL}$) [18]. The *Curculigo orchioides* in this study has 4.5 times higher activity ($IC_{50} = 22.78 \mu\text{g/mL}$) than *Curculigo orchioides* from India ($IC_{50} = 105.94 \mu\text{g/mL}$) [19]. Thus, the ethanol extract of *Curculigo orchioides* from Vietnam is a potent antioxidant.

Table 1. DPPH radical scavenging activity rates of ethanol extract of *Curculigo orchioides*

	Ascorbic acid				
Concentrations ($\mu\text{g/mL}$)	2	4	6	8	10
Inhibited DPPH (%)	25.82	42.32	68.35	85.25	93.45
IC_{50} ($\mu\text{g/mL}$)	4.54				
	Curcumin				
Concentrations ($\mu\text{g/mL}$)	10	20	30	40	50
Inhibited DPPH (%)	18.25	30.64	45.25	58.26	69.38
IC_{50} ($\mu\text{g/mL}$)	34.34				
	Ethanol extract of <i>Curculigo orchioides</i>				
Concentrations ($\mu\text{g/mL}$)	10	20	30	40	50
Inhibited DPPH (%)	22.37	46.24	66.12	82.25	88.36
IC_{50} ($\mu\text{g/mL}$)	22.78				

Total antioxidant capacity

The total antioxidant capacity was determined by assessing the electron-donating capacity of the sample with the phospho-molybdenum method. As shown in Figure 1, the ethanol extract of *Curculigo orchioides* exhibits a high total antioxidant activity in the electron transfer mechanism. At low concentration (0.1–0.2 mg/mL), the ethanol extracts of *Curculigo orchioides* show higher antioxidant activity than curcumin. However, at high concentrations (0.3–0.5 mg/mL), their antioxidant activities are lower than those of curcumin and ascorbic acid.

The antioxidant capacity is expressed as the number equivalents of gallic acid or ascorbic acid. The study reveals that the antioxidant capacity of the extracts increases with their concentration, and the highest capacity is observed at the concentration of 0.5 mg/mL [11]. Here, the total antioxidant capacity of the extract of *Curculigo orchioides* is equivalent to 132.48 ± 1.48 mg GA/g or 264.45 ± 2.34 μ mol AS/g, which is significantly higher than that of a sample grape seeds (from 233.2 to 337.1 μ mol AS/g) [20] and tea (115 mg GA/g)

[11]. This result suggests that the ethanol extract of *Curculigo orchioides* is a potent antioxidant.

3.2 Content of compounds from *Curculigo orchioides*

In previous studies, the antioxidant potential of medicinal plants was attributed to phenolics and flavonoids [21, 22]. According to Wu et al., phenolic compounds are major contributors to the antioxidant activity of *Curculigo orchioides* [23]. In this study, the total phenolic content determined by using the Folin–Ciocalteu’s reagent is expressed as the gallic acid equivalent, and the content of flavonoids in the ethanol extract was determined by using the spectrophotometric method with aluminum chloride. The content of phenolic and flavonoid compounds found in *Curculigo orchioides* is equivalent to 196.24 ± 1.45 mg GAE/g and 78.49 ± 1.78 mg QE/g, respectively. In the study, the total phenolics from *Curculigo orchioides* is higher than that from both the ethyl acetate fraction of *Curculigo orchioides* (176.58 mg GAE/g) [18] and the sample of *Curculigo orchioides* in India (192.56 mg GAE/g) [7]. It should be noted that *Curculigo orchioides* from Vietnam is rich in phenolics.

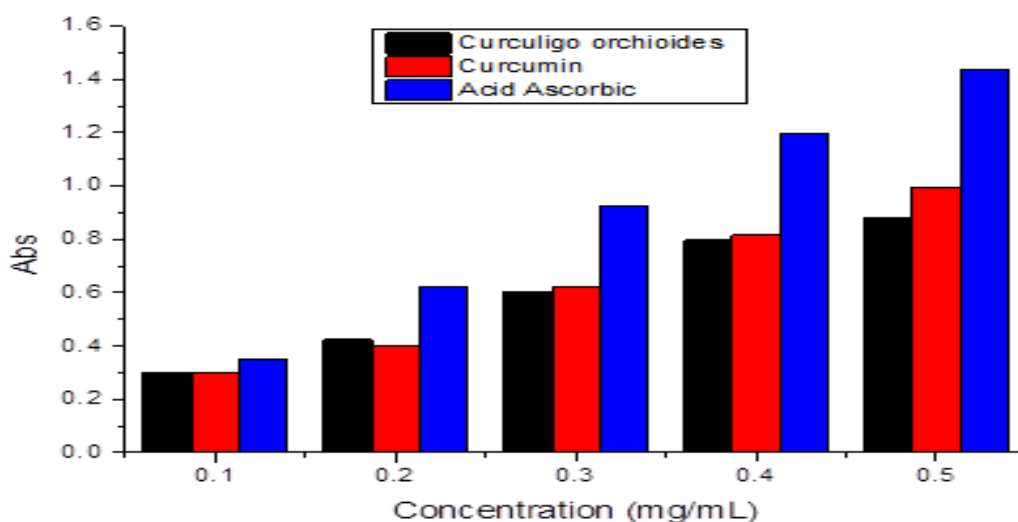


Fig. 1. Antioxidant activity of extracts of *Curculigo orchioides* in total antioxidant capacity model

According to Nie Yan et al. [2], Wang Xueqian et al. [4], and Xia Ling-fang et al. [6], the components that make up the valuable biologically active substances in the *Curculigo orchioides* are saponin triterpenoid compounds and polysaccharides. In fact, the polysaccharides obtained from *Curculigo orchioides* have anti-osteoporosis activities [4]. The anticancer effect of polysaccharides from the rhizome of *Curculigo orchioides* on HeLa (human cervical cancer) cells, such as caspase-3, caspase-9, and P53 cells, has been reported [6]. Four cycloartane-type triterpene glycosides named curculigo saponins G, H, I, and J were isolated from rhizomes of *Curculigo orchioides* and showed excellent immunological activity [24]. The content of polysaccharides and triterpenoid saponins in *Curculigo orchioides* is presented in Table 2. The polysaccharide content is lower than that of *Curculigo orchioides* in Shanghai, China (5.89%) [6]. Specifically, the total triterpenoid saponins content of *Curculigo orchioides* has been reported for the first time in this study.

4 Conclusions

In this study, the antioxidant properties of the ethanol extract of *Curculigo orchioides* have been investigated. This extract displays good activities with low IC₅₀ values, approximately 1.5 times less than that of curcumin. The total antioxidant capacity of the extract is equivalent to 132.48 ± 1.48 mg GA/g or 264.45 ± 2.34 μ mol AS/g, and the content of polysaccharides is 4.34 ± 0.08 %. The content of phenolic and flavonoid compounds found in *Curculigo orchioides* is equivalent to 196.24 ± 1.45 mg GAE/g and 78.49 ± 1.78 mg QE/g, respectively, indicating that *Curculigo orchioides* is rich in phenolics. Specifically, this study has reported the total triterpenoid saponins content of *Curculigo orchioides* for the first time. *Curculigo orchioides* is a promising resource of natural antioxidants.

Table 2. Content of compounds from *Curculigo orchioides* ($n = 3$)

Sample	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Polysaccharides (%)	Total triterpenoid saponins	
				(mg Rb1/g)	(mg oleanolic acid/g)
Curculigo orchioides	196.24 ± 0.45	78.49 ± 1.23	4.34 ± 0.08	47.60 ± 0.24	78.48 ± 1.89

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