

Analysis of a Major Phenolic Glucoside and Biochemistry Compounds from *Curculigo Orchioides* Gaertn

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Abstract

Background: Orcinol- β -D-glucoside, which is also known as orcinol glucoside, is a major phenolic glucoside compound in the rhizome of the *Curculigo orchioides* Gaertn. This compound has many medicinal properties such as being antioxidant, immunomodulatory, antiosteoporosis, stress relief, antidepressant, etc.

Methods: Determination of reducing sugar content by Bertrand's method, determination of lipid content by Soxhlet method, determination of vitamin C content by iodine titration, determination of enzyme activity catalase by titration with KMnO₄. Quantification of Orcinol- β -D-glucoside was conducted by HPLC analysis.

Results: The Orcinol- β -D-glucoside of *C. orchioides* in Thuy Bang mountain was highest. Besides, the content of reducing sugars, vitamin C, enzyme catalase, and lipids of *C. orchioides* differed significantly among sites. In which, the reducing sugar and vitamin C of *C. orchioides* in Ngu Binh mountain was highest. Whereas, enzyme catalase was also highest in Thuy Bang mountain. However, the lipid content of *C. orchioides* was also highest in Huong Tho mountain.

Conclusions: The result will contribute to providing the scientific basis for the selection of breeding, planting, development of *C. orchioides* in Thua Thien Hue province, as well as other provinces in Vietnam and opening new research directions for applications in the future.

Keywords: Antioxidants, Hypoxidaceae, Medicinal Plants, Vietnam.

Introduction

Curculigo orchioides Gaertn (*C. orchioides*) belongs to the family Hypoxidaceae, which is one of the important medicinal plants in Vietnam and is popularly recognized by various names such as Sam cau, Ngai cau, Co noc lan (1-3). It is often used for traditional medicine in China, India and Southeast Asia, including Vietnam. The rhizomes of this plant were used for treatments of impotence, enuresis, cold sperm, back and knee pains, limb numbness, decline in physical strength, diarrhea. They were also used as a potent immunomodulatory

and aphrodisiac, etc (2,4). Besides, *C. orchioides* was also used for treatments of leukorrhea, anemia, neurasthenia, diuretics, rheumatism, and movement difficulty in Vietnam (3,5).

There was some previous research on the pharmacological effects of this compound have shown that Orcinol- β -D-glucoside have antioxidant effects and might be useful to treat skin aging immunomodulatory, antiosteoporosis, anxiolytic effect, antidepressant and it is potential cytotoxic agent

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against gastric, colon and hepatoma carcinoma cell lines (6-14). In Vietnam, the study of Nguyen and Nguyen also showed that the antioxidant ability of pharmaceutical materials was quite high with 66.25% (1). Additionally, there were also several quantitative studies of phenolic glucoside compounds including Orcinol- β -D-glucoside but their quantitative methods are different (15,16). In Vietnam, this was a research project to build a qualitative and quantitative method of Orcinol- β -D-glucoside in rhizomes of *C. orchioides* but its work only evaluated on some samples collected in a limited number of site (17). While the studies on genetic diversity of *C. orchioides* populations from Vietnam in 2018 shows that there were 7 provinces (including Lai Chau, Quang Binh, Quang Tri, Thua Thien Hue, Quang Nam, Kon Tum and Lam Dong) with high different genetic diversity, therein, the

populations originate from Thua Thien Hue had the highest genetic identity (0.9407) (18).

Further, some studies on *C. orchioides* focus on breeding by rhizome stem cutting and *in vitro* in Vietnam (19,20). Thus, researching on Orcinol- β -D-glucoside and biochemistry compound in the rhizomes of *C. orchioides* in sites of Vietnam is needed.

Materials and Methods

Sample Collection

C. orchioides was collected in 4 sites in Thua Thien Hue province, Vietnam including Phu Bai mountain of Phu Bai town, Huong Tho Mountain of Huong Tra district, Ngu Binh mountain of Hue city, and Thuy Bang Mountain of Huong Thuy district (Fig. 1). In each study locality, we randomly collected 30 trees at a distribution.

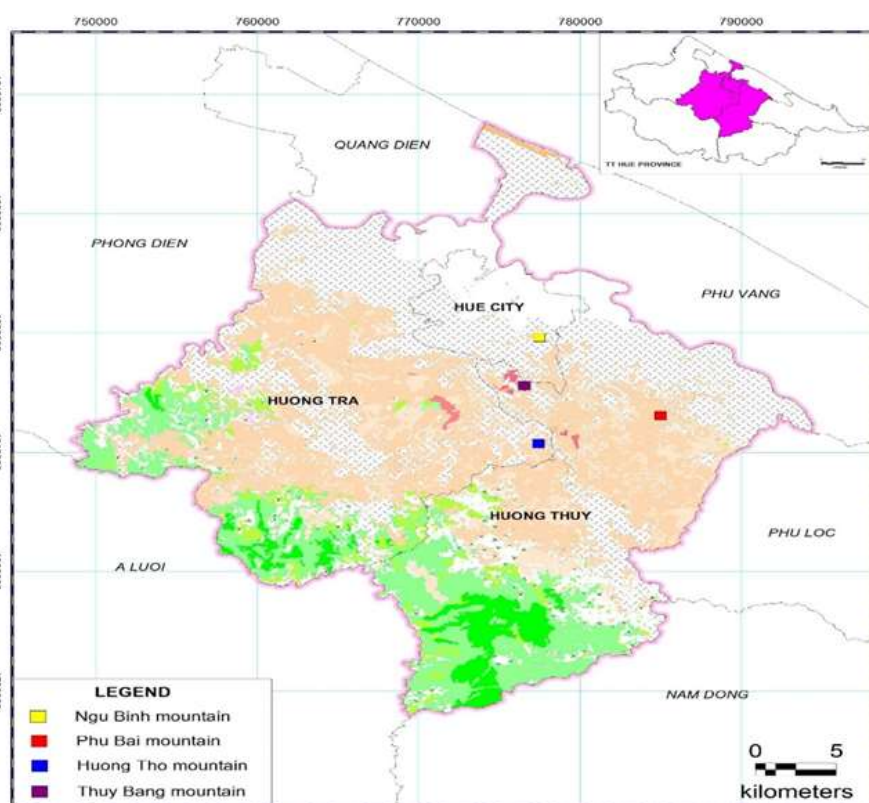


Fig. 1. The sample of *Curculigo orchioides* in different site of Thua Thien Hue province, Vietnam.

Sample Treatment

Samples collected from the wild are washed. The secondary roots were removed, using only rhizomes for analysis. Next, the samples are

crushed in porcelain mortar with liquid nitrogen and dried at 60 °C for 48 hours. Finally, the samples were crushed into fine

powder and put in glass jars and stores in the refrigerator compartment.

Analysis of biochemistry compounds (reducing sugar, lipid, vitamin C, iodine titration and enzyme activity catalase) of Curculigo orchioides

Determination of reducing sugar content by Bertrand's method (21), determination of lipid content by Soxhlet method, determination of vitamin C content by iodine titration, determination of enzyme activity catalase by titration with KMnO₄.

Analysis of Orcinol-β-D-glucoside of Curculigo orchioides

One gram of sample was weighed accurately and with 100mL solvent into a 300mL round-bottomed flask. To investigate the effect of

solvents and extraction methods on the results of Orcinol-β-D-glucoside quantification, we use the formulas in Table 1. Then, the extract was transferred to centrifugal tube and centrifuged (6000 rpm) for 20 min. The solution was filtrated through a 0.45 μm cellulose acetate membrane filter before HPLC analysis HPLC Fingerprinting. Using HPLC - LC 20AD (Shimadzu) high performance liquid chromatography system (HPLC InertSustain C18 GL Sciences column). Conditions for running HPLC according to Tu (16). Extracts (10μ l) were analyzed using a phase device: ACN: H₂O (10:90, v/v) with HPLC column (250 x 4.6mm; 5 μm), helium carrier gas flow at 0.5 ml-1. UV-VIS detector was detected wavelength of 220 nm. Using standard substances with Orcinol-β-D-glucoside of Solarbio (China) has purity of over 98%.

Table 1. Formulas used to investigate the effect of solvents and extraction methods on the results of Orcinol-β-D-glucoside of quantification.

STT	Treatment	Solvent	Extraction method
1	CT-1	Methanol 100%	Soxhlet in 2 hours
2	CT-2	Methanol 70%	Soxhlet in 2 hours
3	CT-3	Methanol 16%	Soxhlet in 2 hours
4	CT-4(Control)	Dissolve 1mg of Orcinol-β-D-glucoside standard into 1ml methanol 100%	

Data processing

The collected data is processed on a computer using the SPSS 20 software.

Results

Effect of solvent and extraction method on quantitative results of Orcinol-β-D-glucoside in HPLC fingerprinting

The results showed that the retention time of Orcinol-β-D-glucoside in all treatments around the 20th minute, indicating that this compound is present in 4 samples (Table 2, Fig. 3). The results of quantitative analysis of Orcinol-β-D-glucoside in the formulas showed that this substance content was different when using different extraction and solvent methods with statistical significance ($p < 0.0001$, $F = 3.415E+11$, $df = 4$). The Tukey's test indicated

that the CT 1 was significantly highest compared with the other treatment (CT-2, CT-3), except CT4 (control). Therefore, we use 100% methanol solvent to extract Orcinol-β-D-glucoside from the sample base (Table 2).

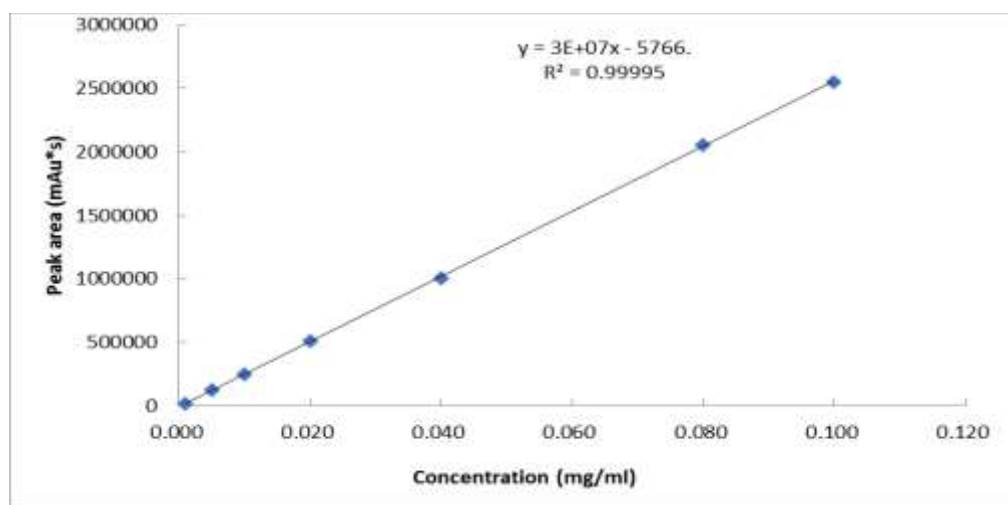
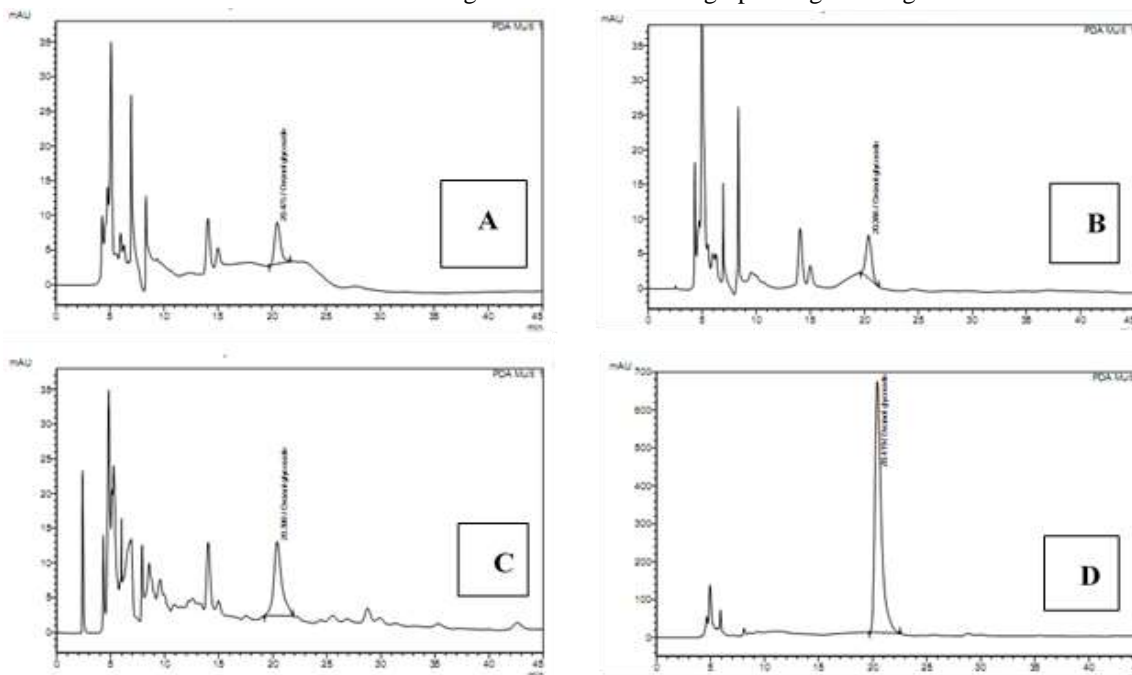
Constructing a calibration curve and linear regression equation

The linear dependence of the chromatographic peak area on the concentration of Orcinol-β-D-glucoside are shown at Fig. 3. There is a close correlation between peak area and concentration of Orcinol-β-D-glucoside. The coefficient of determination $R^2 = 0.99995$ is content with condition $0.99 \leq R^2 \leq 1$ and the bias Δ does not exceed $\pm 15\%$ for all concentrations being used to build the standard line. Therefore, the standard curve that was built is acceptable.

Table 2. Effect of solvent and extraction methods on quantitative results of Orcinol- β -D-glucoside in HPLC Fingerprinting.

Order	Treatment	Peak area (mAU*s)	Retention time (r.t) (min)	Concentration (mg/ml)
1	CT-1	544635\pm34.21c	20.390 \pm 0.0115	0.0200\pm0.00c
3	CT-2	225021 \pm 10.15a	20.386 \pm 0.0003	0.0083 \pm 0.00a
4	CT-3	229150 \pm 19.50b	20.475 \pm 0.00145	0.0084 \pm 0.00b
5	CT-4	27228355 \pm 19.16d	20.419 \pm 0.00176	1 \pm 0.00e
F			3.415E+11	
P			<0.0001	

Lower-case letters in a cell show the results of significant differences between treatments using Tukey's multiple range test ($p < 0.05$). The values represent the mean (\pm SE) of three independent experiments. The highest statistically significant mean value was bold. The signal E+11 means 10^{11} .

**Fig. 2.** The calibration curve of Orcinol - β - D - glucoside in HPLC fingerprinting: The signal E+7 means 10^7 .**Fig. 3.** HPLC chromatogram of the dry rhizome of *Curculigo orchioides* Gaertn. extract visualized by A) CT-3, B) CT-2, C) CT-1, and D) CT-4. The vertical axis is intensity (mAU), the horizontal axis is retention time (min). The retention time of Orcinol- β -D-glucoside compound in 4 formulas are all around the 20th minute.

Effect different site on Orcinol- β -D-glucoside content of *C. orchioides*

The results of quantitative analysis of Orcinol- β -D-glucoside in the rhizome samples of *C. orchioides* showed that the content of this substance has a difference between the distribution site with statistical significance ($p < 0.0001$, $F = 8529.239$, $df = 10$). Using

Tukey's test shown that the content of Orcinol- β -D-glucoside of *C. orchioides* distributed in Thuy Bang – Thua Thien Hue was highest (Table 3, Fig. 4). The results of quantitative analysis by HPLC in the rhizome samples of *C. orchioides* in sites showed that the Orcinol- β -D-glucoside content ranges from 1.27 ± 0.0020 to 9.16 ± 0.0189 mg/g.

Table 3. Content of Orcinol- β -D-glucoside in *C. orchioides* Gaertn in different sites Thua Thien Hue province, Vietnam.

Locality	Content (mg/g dried)
Ngu Binh mountain	$3.66 \pm 0.0038c$
Phu Bai mountain	$1.35 \pm 0.0056a$
Huong Tho mountain	$6.41 \pm 0.0422d$
Thuy Bang mountain	$9.16 \pm 0.0189e$
F	8529.239
P	< 0.0001

Lower-case letters in a cell show the results of significant differences between treatments using Tukey's multiple range test ($p < 0.05$). The same letters within each column indicates no statistically significant differences. The values represent the mean (\pm SE) of three independent experiments. The highest statistically significant mean value was bold.

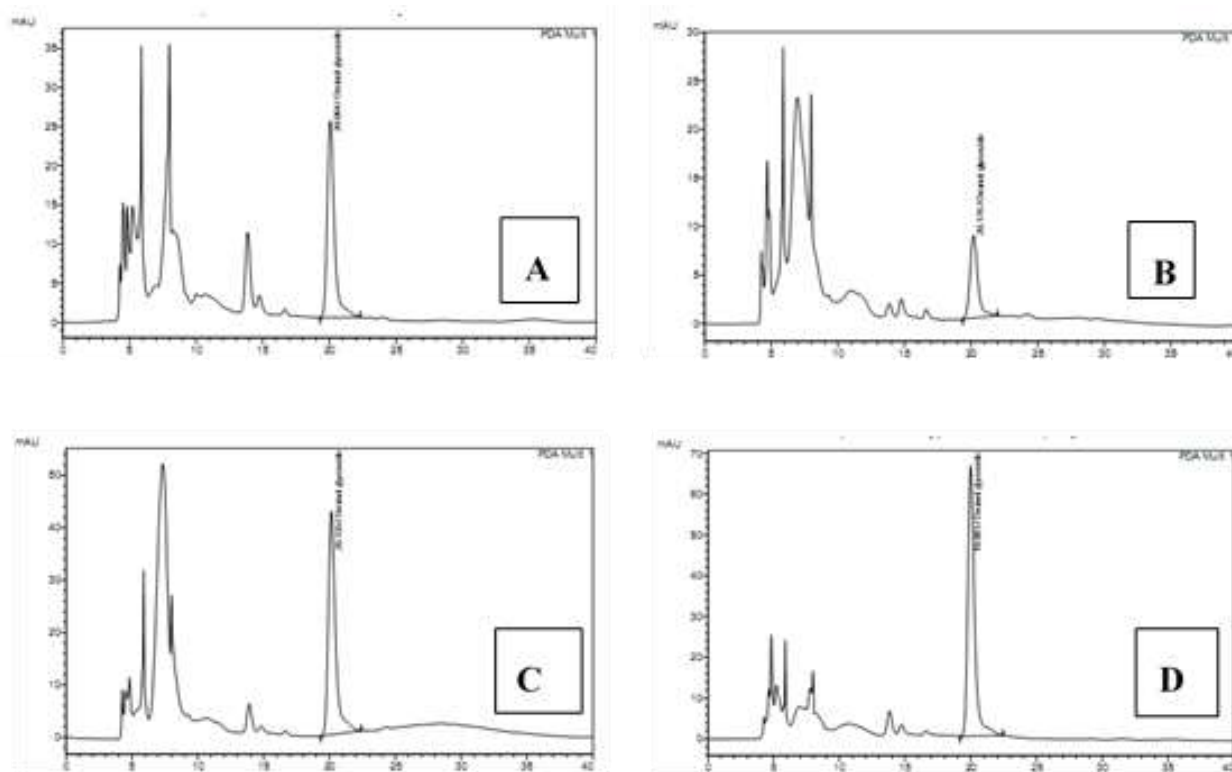


Fig. 4. HPLC Fingerprint of 04 samples by A) Ngu Binh mountain (Hue city), B) Phu Bai mountain (Phu Bai town), C) Huong Tho mountain (Huong Tra district), D) Thuy Bang mountain (Huong Thuy district). The vertical axis is intensity (mAU), the horizontal axis is retention time (min). The retention time of Orcinol- β -D-glucoside compound in 4 samples are all around the 20th minute.

Analysis of biochemistry compounds of *Curculigo orchioides*

The results of quantitative determination of reducing sugars, lipids, vitamin C, and catalase activity in rhizomes of *C. orchioides* in the sites were statistically significantly different (Table 2). Using Tukey test shows that the reducing sugar and vitamin C of *C. orchioides*

distributed in Ngu Binh mountain was highest, while the highest lipid of *C. orchioides* was Huong Tho mountain. The activity of the catalase enzyme of *C. orchioides* distributed in Thuy Bang mountain was highest (Table 4).

Quantitative results of some biochemical parameters in rhizomes of *C. orchioides* showed a difference between sites.

Table 4. Biochemistry compounds of *C. orchioides* Gaertn in different sites of Thua Thien Hue province, Vietnam.

Sites	Average content			
	Reducing sugar (%)	Lipid (%)	Catalase (U/g)	Vitamin C (%)
Ngu Binh mountain	4.65±0.03d	2.05±0.04a	168.06±1.39c	3.74±0.023c
Thuy Bang mountain	4.35±0.03c	2.97±0.033c	172.55±2.00c	2.42±0.13a
Huong Tho mountain	3.88±0.04b	3.05±0.02d	152.22±1.70b	3.30±0.13ab
Phu Bai mountain	2.85±0.03a	2.53±0.02b	112.47±1.46a	2.35±0.07a
F	278.70	419.10	272.01	213.68
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Discussion

In our study, the results of quantitative analysis by HPLC in the rhizome samples of *C. orchioides* in four sites showed that the Orcinol-β-D-glucoside content ranges from 1.27 to 9.16 mg/g. This result was higher than that of He *et al* (16), in which Orcinol-β-D-glucoside content only ranges from 0.333 to 2.854 mg/g.

When using 100% methanol solvent and Soxhlet extraction method, Orcinol-β-D-glucoside quantitative result was higher than when using 70% ethanol solvent and extraction method under reduced pressure at 60 °C.

This result was similarly to the study of Bian *et al.* in which the Orcinol-β-D-glucoside content ranges from 1.43 to 11.58 mg/g (15). The samples collected at Thuy Bang mountain (9.16±0.0189 mg/g) were only lower than Yulin - Guangxi (11.58 mg/g) but higher than the 9 remaining sites in China. In comparing this result with the research results in Vietnam, Orcinol-β-D-glucoside content in *C. orchioides* sample distributed at Thuy Bang mountain was highest (16). This proves that, when using 100% methanol as a solvent, it will yield higher quantitative results than 60% ethanol. From this, we have found that among the sites in Vietnam, *C. orchioides* distribution in Thuy

Bang mountain should be selected for planting and developing and for constructing a product branding later.

Around the world, rhizomes of *C. orchioides* distribution site was used in making supplements in various types such as tea, powder, extract, and tablet tonic. Besides, the rhizomes extract of *C. orchioides* distribution was also being used as the main ingredient in the production of cosmetics for women thanks to its anti-inflammatory properties, wound healing, stimulating cell regeneration.

In particular, the reducing sugar content (glucose) in the experimental samples is higher than the results in Joy's study (22). Lipid content in the experimental samples is similar to the results of Joy and Arinathan *et al.* In particular, the lipid content in the sample of Cucumber originating in Ngu Binh is higher than that of two sites in India (21). The content of vitamin C is much different among sites and lower than the research results of Arinathan *et al.* (23). The enzyme catalase activity in *C. orchioides* samples from different origins is very high, proving that the antioxidant capacity of medicinal herbs is very good.

According to Pham *et al.* when studying the

distribution of *C. orchioides* in Thua Thien Hue, the condition where the distribution of the species is under pine forest with canopy coverage of 0 - 50%, distributed in height is from 30 – 130 m above water level, the slope is up to 30 °C, but it is concentrated in the height of 30 – 60 m and canopy cover is from 0-5%, temperature is 24 - 25.2 °C, average rainfall year 2,600 - 2,800 mm and humidity 83 - 84%. In addition, the soil in the natural distribution of *C. orchioides* is usually brown to reddish brown or pale yellow Leptosols, or Acrisols are gray to grayish yellow, soil mixed with gravel and

surface soil often eroded, gravel from 20 - 70%; rotten floor mats are very thin and usually only about 0.1 - 1.5 cm. In particular, soil moisture in the surface layer is from 0-30 cm quite high, ranging from 30-40% (5).

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