

Cyperus rotundus cyperaceae: a study of phytochemistry, total polyphenol content, flavonoid content, and antioxidant activity

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Abstract. *Cyperus rotundus* is a folk medicine with many benefits such as anti-inflammatory, anti-cancer, antioxidant, etc. It is widely used in many parts of the world. This study provided data on the phytochemical composition of *Cyperus rotundus* rhizome, including the active groups of alkaloids, flavonoids, phenols, tannins, and triterpenoids. The polyphenols content in the ethanolic and aqueous extracts of *Cyperus rotundus* were 26.89 ± 4.33 (mgGAE/g) and 51.84 ± 6.46 (mgGAE/g), respectively. While, the flavonoids content in the ethanolic and aqueous extracts of *Cyperus rotundus* were 78.03 ± 3.11 (mgQE/g) and 20.28 ± 2.25 (mgQE/g), respectively. According to the ABTS assay, the IC₅₀ values of the aqueous and ethanolic extracts were 117.8 ± 19.85 (µg/ml) and 162.02 ± 11.43 (µg/ml), respectively. According to the DPPH assay, the IC₅₀ values of *Cyperus rotundus* extracted by water and alcohol were 337.42 ± 22.84 (µg/ml) and 447.53 ± 33.8 (µg/ml), respectively. With the rich presence of secondary compounds, antioxidant activity confirmed in many studies, *Cyperus rotundus* is evaluated as having potential applications in many different fields such as pharmaceuticals, cosmetics, and functional foods.

1 Introduction

Nowadays, herbs and natural active ingredients are more and more popular due to their availability and minimal side effects. With characteristics of easy cultivation, high reproductive rate, good adaptation to many adverse climatic conditions, *Cyperus rotundus* is widely distributed and cultivated in many parts of the world, even, in someplace, it is regarded as weeds [1, 2]. Moreover, *Cyperus rotundus* is a medicinal herb used in folk medicine in many countries such as India, Iran, China, Japan, Vietnam, etc. [3, 4]. The results of phytochemical analysis showed that the leaves and rhizomes of *Cyperus rotundus* contain a variety of compounds with high biological activity such as glycosides, tannins, reducing sugars, alkaloids, flavonoids, polyphenols, terpenoids, saponins, and phlorotannin. [5]. In 2014, Jeyasheela and colleagues analyzed the phytochemical components of *Cyperus*

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rotundus in different fractions of methanol extract, ethyl acetate extract, and evaluated the activities of those bioactive compounds. Similar to other studies, many groups of compounds present in this plant have good biological activity, such as polyphenols, flavonoids, and tannins [6].

The isolated compounds from methanol extract of *Cyperus rotundus* showed inhibitory activity against α -glucosidase and α -amylase, which were considered as a potential medicine diabetes treatment[4]. According to the review article of Kamala in 2018, many studies had demonstrated the analgesic, anti-allergic, anti-inflammatory, antioxidant effects of *Cyperus rotundus* and its chemical components. However, the exact mechanism of action was still unclear and needed further evaluation [7]. In 2019, the study of F. Wang and colleagues demonstrated the role of *Cyperus rotundus* alcohol extract in the treatment of breast cancer [8]. Especially, the ethanolic extract from *Cyperus rotundus* induced apoptosis by promoting the expression of BAX, inhibiting the expression of BCL-2. These results demonstrated that it inhibited the proliferation and induction apoptosis in human Triple-negative breast cancer. In Vietnam, the study by Dr. Tran Thi Hong Hanh showed that *Cyperus rotundus* had the ability to treat hyperglycemia with α -glucosidase and α -amylase models. [4].

2 Material and methodology

2.1 Material

In May 2020, *Cyperus rotundus* rhizome was collected in Buon Ma Thuot city, Dak Lak province. After its damaged parts were removed and cleaned, the rhizomes were cut into thin slices with a thickness of 0.5 to 0.7 mm and dried at $45^{\circ}\text{C} \pm 5^{\circ}\text{C}$. When the moisture content was below 15%, the herbs were ground until the powder diameter was in the range of 0.25 - 0.5 mm. Next, the medicinal powder was stored in a vacuum bag at -18°C .

2.2 Solvent extraction

Cyperus rotundus raw powder was extracted with two different solvents (96% alcohol and water), the obtained products were ethanolic extract and aqueous extract. About 20g of dry powder was weighed into a conical flask and added 600ml of solvent. Extraction was carried out with solvent recovery at 60°C for 60 min with a stirring rate of 300 rounds per minute. Next, the solvent was removed to obtain a dried extract with a moisture content of less than 15%. Before use, the extract was refrigerated at -18°C , protected from light[9].

2.3 Methodology

2.3.1 Phytochemistry screening

Following the methods of Aug *et al* [10] and Nguyen *et al* [11], the chemical composition of *Cyperus rotundus* was determined by observing specific reactions performed in vitro. The main groups of active substances identified include alkaloids, anthraquinones, coumarin, flavonoids, phenols, reducing compounds, saponins, tannins, and triterpenoids (Table 1). If either alcohol or water extract shows a positive reaction when testing the active substance, the raw material *Cyperus rotundus* is considered to have that group of active ingredients.

Table 1. The groups of chemical active ingredients investigated in this study.

Compounds	Positive reactions
Alkaloids (General reagents)	Dragendorff's (orange or orange-red precipitate), Mayer's (yellow precipitate), Bouchardat (Reddish-brown precipitate).
Antraquinons (Borntrager's test)	Pink, red or violet colour.
Coumarins (UV Test)	An intense fluorescence under UV light (λ 365 nm).
Flavonoids (Shinoda's test)	Orange to magenta.
Phenols (Ferric Chloride test)	Blue, green, red or purple color.
Reducing compounds (Fehling's test)	Red precipitate.
Saponin (Frothing test)	Foam.
Tannins (Ferric Chloride test)	Greenish-brown or black precipitate.
Triterpenoids (Liebermann Burchard test)	Reddish violet colour.

2.3.2 Total polyphenol content

The total polyphenol content was determined following the methods in research of Nhut and colleagues [12]. All processes were performed protected from light. The extract was dissolved with the extraction solvent itself to obtain the experimental solution. The mixture of 0.5ml of the test solution and 2.5ml of 10% Folin-Ciocalteu solution was mixed by a vortex machine. After 5 min of rest, the mixture was added to 2.0 ml of 7.5% Na_2CO_3 and incubated for another 60 min. Finally, the mixture was photometrically measured at 765 nm and evaluated against the standard gallic acid [12].

2.3.3 Total flavonoid content

Similar to the method for the determination of polyphenols, the procedure was also protected from light and the extract was mixed with its extraction solvent to obtain the test extract. A mixture of 0.1 mL 10% AlCl_3 and 0.1 mL 1M CH_3COOK was added to 0.5 mL of the test extract, stirred, and allowed to stand for 5 min. Next, enough amount of distilled water was added to obtain a mixture with a volume of 5 mL. The mixture after reaching an incubation time of 30 minutes will be photometrically measured at 415 nm with quercetin as standard [13].

2.3.4 The antioxidant activity

The antioxidant activity of *Cyperus rotundus* rhizome was determined by two methods: DPPH and ABTS scavenging activities.[14]. The working solution of each DPPH and ABTS assay was adjusted so that the absorbance was 1.1 ± 0.02 at 517 nm and 734 nm, respectively. Within 30 minutes after the working solution was prepared, the survey was immediately conducted. Specifically, 0.5ml sample extract and 1.5ml working solution were mixed well. After incubation for 30 min, the mixture was photometrically measured at 517 nm (DPPH) or 734 nm (ABTS) and evaluated based on the concentration of sample required to scavenge 50% of the free radicals (IC 50% value).[15].

3 Results and discuss

3.1 Phytochemical analysis

Analysis of secondary metabolites showed that *Cyperus rotundus* contains many groups of active ingredients (Table 2), such as alkaloids, flavonoids, phenols, tannins, and triterpenoids. This result was similar to the study by Abo *et al.*, using GC-MS methods, which analyzed the presence of 10 alkaloids and 25 phenolic compounds.[16].

Table 2. Phytochemical analysis of *Cyperus rotundus* rhizome's extracts.

Compounds	<i>Cyperus rotundus</i> Cyperaceae		
	Aqueous extract	Ethanollic extract	Conclusion
Alkaloids	+	+	+
Anthraquinones	—	—	—
Coumarins	—	—	—
Reducing compounds	—	—	—
Flavonoids	+	+	+
Phenols	+	+	+
Saponin	—	—	—
Tannins	+	+	+
Triterpenoids	+	+	+

3.2 Total polyphenols and flavonoids content

The polyphenols content in the ethanolic and aqueous extracts of *Cyperus rotundus* were 26.89 ± 4.33 mgGAE/g and 51.84 ± 6.46 mgGAE/g, respectively (Figure 1). Although, the flavonoids content in the ethanolic and aqueous extracts of *Cyperus rotundus* were 78.03 ± 3.11 mgQE/g and 20.28 ± 2.25 mgQE/g, respectively. This result was similar to the study of Kamala *et al* [3]. In 2018, the authors tested the optimal conditions for extracting from *Cyperus rotundus* rhizome. The results for polyphenols of 70% acetone extract ranged from 0.036 ± 0.002 to 118.924 ± 5.946 μ g/mg extract. The flavonoids content value were $7,196 \pm 0.359$ to 200.654 ± 10.032 μ g/mg extract [3].

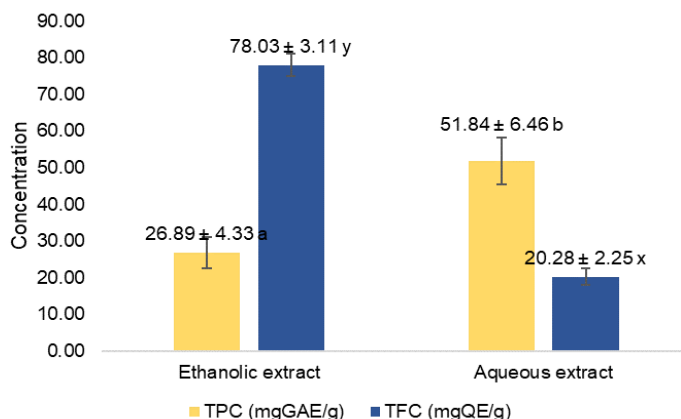


Fig. 1. The TPC and TFC of ethanolic and aqueous extracts of *Cyperus rotundus*

3.3 The antioxidant activity

The higher the antioxidant capacity, the lower the IC₅₀ value of that substance. Specifically in our study, the aqueous extract exhibited stronger antioxidant activity than the ethanolic extract. According to the ABTS method, the IC₅₀ values of the aqueous and ethanolic extracts were 117.8 ± 19.85 (µg/ml) and 162.02 ± 11.43 (µg/ml), respectively. According to the DPPH assay, the IC₅₀ values of *Cyperus rotundus* extracted by water and alcohol were 337.42 ± 22.84 (µg/ml) and 447.53 ± 33.8 (µg/ml), respectively (Figure 2).

Based on the high antioxidant capacity, the methanol extract of *Cyperus rotundus* rhizome was conducted to investigate the ability of anticancer activity in the different cancer cell lines. The results were very promising when the antioxidant activity was directly proportional to the anticancer activity with ranged from 4.52 ± 0.57 to 9.85 ± 0.68 µg ml. According to a study by Kadum in 2019 in Iraq, *Cyperus rotundus* extract after fermentation was demonstrated stronger antioxidant activity for DPPH (49.08 µg/mL) and FRAP (46.95 mmol Fe(II)/g) [17].

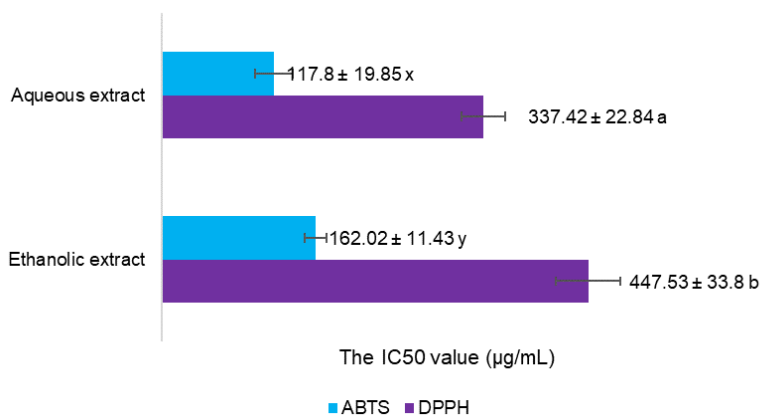


Fig. 2. The IC₅₀ values of the aqueous and ethanolic extract from *Cyperus rotundus* rhizome

4 Conclusions

The results of our study had provided preliminary data on the chemical composition of *Cyperus rotundus*, such as alkaloids, flavonoids, phenols, tannins, and triterpenoids. According to folk documents, this medicinal herb is often used due to its ability to resist oxidation and fight cancer cells. The study evaluated the polyphenol and flavonoid content of both ethanolic and aqueous extracts. The results showed that *Cyperus rotundus*, when extracted with alcohol, obtained higher polyphenol content, while the aqueous extract resulted in higher flavonoid content. After evaluating the antioxidant activity by two different assays, the results showed that *Cyperus rotundus* had good antioxidant activity, similar to the studies in the world. With the rich presence of secondary compounds, antioxidant activity confirmed in many studies, *Cyperus rotundus* is evaluated as having potential applications in many different fields such as pharmaceuticals, cosmetics, and functional foods.

This research is funded by Nguyen Tat Thanh University, Ho Chi Minh city, Vietnam.

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