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CHEMICAL COMPOSITION AND HERBICIDAL ACTIVITY OF FLOWER EXTRACT FROM SPIKED SPIRALFLAG GINGER (*Costus spicatus*)

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Abstract

This study aimed to detemine the chemical composition and evaluate the herbicidal potential of flower extracts from Costus spicatus. The ethanol extract exhibited markedly higher total phenolic and flavonoid contents (0.754 mg GAE/g FW and 0.470 mg QE/g FW) than the aqueous extract (0.182 mg GAE/g FW and 0.038 mg QE/g FW). Notably, the phenolic and flavonoid contents of the ethanol extract (0.754 mg GAE/g FW and 0.470 mg QE/g FW) was four and twelve times greater than that of the aqueous extract (0.182 mg GAE/g FW and 0.038 mg QE/g FW). Bioassays showed that the ethanol extract strongly inhibited seed germination, root elongation, and shoot growth of mustard greens, with the greatest effect on shoot length (IC_{50}=1.431 mg/mL). The herbicidal property increased proportionally to its concentrations. Besides, the ethanol extract, at a concentration of 1.5 mg/mL, markedly lowered chlorophyll a, total chlorophyll, and carotenoid levels in mustard seedlings relative to the control. Regarding pigment biosynthesis, treatment with ethanol extract at 1.5 mg/mL significantly reduced chlorophyll a, total chlorophyll, and carotenoid contents in mustard green seedlings compared to the control. These results indicate that Costus spicatus *flowers possess potent allelopathic properties, reducing germination, growth, and photosynthetic pigment levels. These findings indicate that Costus spicatus flowers possess strong inhibitory potency on seed germination, growth, and reduce the photosynthetic capacity of plants. Further studies are needed to isolate and identify active compounds for sustainable weed management.*

Keywords: *Costus spicatus, flavonoids, flower extract, herbicidal activity, phenolic, pigment biosynthesis.*

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THÀNH PHẦN HÓA HỌC VÀ KHẢ NĂNG ỨC CHẾ THỰC VẬT CỦA CAO CHIẾT HOA GỪNG XOẮN ỐC GAI (*Costus spicatus*)

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Tóm tắt

Nghiên cứu được thực hiện nhằm xác định thành phần hóa học và đánh giá khả năng ức chế thực vật của cao chiết hoa gừng xoắn ốc gai (Costus spicatus). Kết quả nghiên cứu cho thấy hàm lượng phenolic và flavonoid tổng có trong cao chiết ethanol (0,754 mg GAE/g FW và 0.470 mg QE/g FW) cao hơn gấp 4 và 12 lần so với cao chiết nước (0,182 mg GAE/g FW và 0.038 mg QE/g FW). Ngoài ra, cao chiết ethanol có khả ức chế mạnh lên sự nảy mầm, chiều dài rễ và thân của hạt cải bẹ xanh, và khả năng ức chế tăng dần theo nồng độ khảo sát. Tác động mạnh nhất của cao chiết thể hiện lên sự phát triển của thân cải bẹ xanh với giá trị IC_{50} là 1,431 mg/mL. Về tổng hợp sắc tố, hàm lượng chlorophyll a, a và carotenoid của cải bẹ xanh bị giảm mạnh so với đối chứng khi được xử lý với cao chiết ethanol ở nồng độ 1,5 mg/mL. Kết quả nghiên cứu cho thấy hoa gừng xoắn ốc gai có tiềm năng ức chế sự nảy mầm, quá trình sinh trưởng phát triển và đặc biệt làm giảm khả năng quang hợp của thực vật. Nghiên cứu tiếp theo cần phân lập tìm ra những chất hay hợp chất tự nhiên có khả năng kiểm soát cỏ dại bền vững trong tương lai.

Từ khóa: *Cao chiết, Costus spicatus, flavonoid, phenolic, sắc tố quang hợp, ức chế thực vật.*

1. Introduction

Weeds are considered among the four most dangerous factors in paddy fields, along with pests, diseases and rats. They affect the growth of rice by competiting for nutrients, water and light and being potential reservoir for pathogens and pest (Antranlina et al., 2015; Sharshar et al., 2022). In addition, statistic analysis presented that weeds cause much higher losses in rice yield than insects and pests (Khang et al., 2016).

In developed countries, herbicides are one of the most commonly used methods to kill weeds for half a century (Davis and Frisvold, 2017; Dennis et al*.,* 2018). The application of herbicides has contributed significantly to the development of crop productivity. However, the continuous use of them pollutes the environment, affects human health, ecosystems and negatively impacts on natural enemies (Gonzalo et al*.,* 2011; Hagner et al*.,* 2019; Hussain et al., 2021). Because of herbicides risks in agricultural production, scientists and researchers have recently concentrated on applying of biological controls by using an allelopathy approach (Quy et al., 2019). Allelophathy is a biological process in which a plant can produce substances (allelochemicals) that are inhibitory to surrounding plants. (Xuan et al*.,* 2005). In recent years, some allelochemicals are emerging as an alternative to the use of pesticides for weed control (De Albuquerque et al*.,* 2011). Therefore, utilization of allelochemicals from many organisms for weed control in agricultural production has received increasing attention (Khanh et al*.,* 2008).

Costus spicatus (Zingiberaceae) is one of medicinal plants that widely distributed in coastal moist forests and tropical rainforests (Quintans Júnior et al., 2010). They are grown as ornamental plants in gardens, roadsides and parks (Gonçalves et al., 2005). This species is commonly used as a nutritional food and medicine in many countries. The phytochemical studies have shown the presence of polyphenols, flavonoids, alkaloids, saponins, terpenoids and tannins in the extract of *Costus spicatus* (Devendran and Sivamani, 2015). *Costus spicatus* exhibits a wide spectrum of pharmacological properties, including chronic headaches, gastroenteritis, diarrhea, hyperlipidemia, diabetes, cancer, ascariasis and dermatitis (Keller et al., 2009; Shediwah et al., 2019; Ali et al., 2022). Thi et al. (2025) reported that Zingiberaceae family (*Lanxangia tsao-ko*, *Meistera vespertilio*, *Wurfbainia schmidtii*) significantly inhibited the growth of *Brassica juncea* and *Echinochloa crus-galli* at various concentrations. Besides, The leaf extract of *Costus speciosus* reduced the seed germination and seedling growth of wheat (Mandal et al., 2015). However, the herbicidal activity of *Costus spicatus* has not comprehensively examined. Based on previous reports of high phenolic and flavonoid contents in *C. spicatus* and their known allelopathic effects, we hypothesize that the flower extract of *C. spicatus* exhibits significant inhibitory effects on seed germination, seedling growth, and pigment biosynthesis in mustard greens.

2. Materials and research methods

2.1. Materials and standard chemicals

The flowers of *Costus spicatus* were collected from Phuoc Thoi ward, O Mon district, Can Tho city. They were dried by freeze-drying machine at 40^{o}C. The dried samples were

packaged in a sealed container and deposited at 4°C for further analysis. Mustard greens seeds were purchased at Xuan Nong company, Can Tho city. Standard chemicals for the analysis of individual acetone, ethanol, methanol, Folin-Ciocalteu, gallic acid, quercetin, Na_2CO_3 $AlCl_3$, and dimethyl sulfoxide (DMSO).

2.2. Research methods

2.2.1. Preparation of water and ethanol extracts

Water extract: The fresh flowers (180 g) were cleaned, finely chopped, and subjected to extraction with distilled water at a ratio of 1:1 (w:v) using a laboratory blender. The ground liquid is filtered through cloth and filter paper. The water extract is put into a glass bottle and stored in a refrigerator at 4°C.

Ethanol extract: The powder (flower, 100g) was extracted with 1000 mL of ethanol. The mixture was filtered through filter paper to remove particulate matter. The extraction solution was concentrated by rotary evaporator to obtain the ethanolic extract. This extract was also stored in a refrigerator at 4°C for further analysis.

2.2.2. Qualitative determination of chemical phytochemical constituents

The qualitative phytochemical components was tested using colormetric methods (Harborne, 1973). The determination was investigated by both ethanol extract and water extract to make sure which compounds dissolved in water or in ethanol would be detected clearly. There were nine compounds would be identified including polyphenol, coumarin, tannin, flavonoid, carotenoid, saponin, alkaloid, quinone and terpenoid in spiked spiralflag flower extract of *Costus spicatus*.

2.2.3. Quantitative analysis of total phenolic and flavonoid contents

a. Determination of total phenolic content

The total phenolic content of *Costus spicatus* flower extract was measured by the Folin–Ciocalteu method described previously (Yadav & Agarwala, 2011). A volume of 100 µL sample was added into the wells then 250 µL Folin-Ciocalteu reagent was added and mixed. It was put at room temperature for 30 minutes. The absorbance of phenolic at 765 nm was recorded by spectrophotometer. A gallic acid calibration curve was established to calculate the total phenolic content of the samples. The total phenolic content of *Costus spicatus* flower extract was displayed by mg of gallic acid equivalent per gram of fresh weight (mg GAE/g FW) or per gram of extract (mg GAE/g extract).

b. Determination of total flavonoid content

The content of flavonoid was measured by the description of Pękal and Pyrzynska (2014). A volume of 100 µL of the extract was mixed well with 100 µL of the 2% aluminum chloride solution. After 15 minutes at ambient temperature, the absorption was read at 430 nm by spectrophotometer. The content of flavonoid in leaf extract was measured based on the quercetin standard curve equation (20 - 100 µg/mL). The total flavonoid content of

Costus spicatus flower extract was represented in milligrams of quercetin equivalent per gram of fresh weight (mg QE/g FW) or per gram of extract (mg QE/g extract).

2.2.4. Determination of herbicidal activity

The herbicidal property were carried out by using the protocol of Andriana et al. (2018). The extract solutions (10 mL) were put in a petri dish. Ten seeds of mustard greens (*Brassica juncea)* were also put in petri dish. The petri dish was put in ambient temperature. The germination, root and shoot length were recorded. The ratio of treatments over the control were shown as the inhibition percentage (%). Germination and growth inhibition of mustard greens were also evaluated by the IC_{50} value, which was milligram to inhibit 50% of germination and growth of mustard greens.

2.2.5. Determination of chlorophyll and carotenoid content

The contents of chlorophyll (a, b, and total chlorophylls) and carotenoid of mustard greens seedlings were determined by the protocol of Ladhari et al. (2014). Amount of 0.5 g of leaves was placed in a tube, ground, and was put 1.5 mL of acetone (80%). It was incubated at 4ºC for 30 minutes and then centrifuged at 15,000 rpm. The supernatant was read at 440, 645 and 663 nm by a microplate reader. The contents of photosynthetic pigments were displayed as µg/g fresh weight of leaf:

Chlorophyll a (µg/g FW) = $12.7 \times A_{663} - 2.69 \times A_{645}$

Chlorophyll b (µg/g FW) = $22.9 \times A_{645} - 4.68 \times A_{663}$

Total chlorophylls (µg/g FW) = $20.2 \times A_{645} + 8.02 \times A_{663}$

Carotenoid (µg/g FW) = $(4.7 \times A_{440} - (1.38 \times A_{663} + 5.48 \times A_{645})$

2.3. Statistical Analysis

Data was expressed as the average ± standard deviation (SD) values. The statistical analysis was run by one-way ANOVA using Minitab 16.0. The significant difference between means were tested by using Turkey's test at $p < 0.05$.

4. Results and discussion

4.1. The chemically qualitative analysis of *Costus spicatus* flower extracts

The results of phytochemical screening from *Costus spicatus* flower extract were displayed in (Table 1). The result revealed that some constituents present in the extracts, while some are not. The phytochemical investigation in water and ethanol extracts showed the presence of coumarin, flavonoid, polyphenol, quinone, tannin and terpenoid. Alkaloid, carotenoid and saponin were not present in ethanol extract. However, there are higher flavonoid and polyphenol contents in the ethanol than water extract. Flavonoid and polyphenol are structural components of cell walls and can exert inhibitory effects on plant growth (Thepphakhun et al., 2020; Mushtaq & Fauconnier, 2024). Therefore, both constituents was used for further quantification by using spectrophotometric method.

Table 1. Qualitative phytochemical analysis of flower extracts from *Costus spicatus*

No.	Compounds	Ethanol extract	Water extract
1	Polyphenol	++	+
2	Terpenoid	+	+
3	Coumarin	+	+
4	Quinone	+	+
5	Tanin	+	++
6	Flavonoid	+++	+
7	Carotenoid	-	+
8	Saponin	-	+
9	Alkaloid	-	+

Note: (+++) = present in high amounts; (++) = moderately present; (+) = present; - = not present. Modified from Harborne (1973).

4.2. Quantitative results of chemical components contained in *Costus spicatus* flower extracts

The contents of phenolic and flavonoid of both extracts are presented in Table 2. The phenolic content was measured on standard curve of gallic acid and the flavonoid content was quantified based on standard equation curve of quercetin.

Table 2. Quantitative analysis of total phenolic and flavonoid content of *Costus spicatus* flower extracts

Treatments	Phytochemical constituents			
	Phenolic		Flavonoid	
	(mg GAE/g extract)	(mg GAE/g fresh weigh)	(mg QE/g extract)	(mg QE/g fresh weigh)
Ethanol extract	86.56 ± 2.627	0.754 ± 0.023[a]	53.97 ± 2.122	0.470 ± 0.019[a]
Water extract	-	0.182 ± 0.003[b]	-	0.038 ± 0.001[b]

Data are presented as means ± standard deviations (SD) value. Values assigned by the same alphabets ([a,b]) in a column were not significantly different at $p < 0.05$ (Turkey's test). (-): measurement was not conducted. Phenolic content determined according to Pekal & Pyrzynska (2014).

In general, both phenolic and flavonoid contents of ethanol extract were higher than those of water extract. Specifically, phenolic content in ethanol extract (0.754 mg GAE/g fresh weigh) was 5 times higher than that of water extract (0.182 mg GAE/g fresh weigh). Similarly,

flavonoid content in ethanol extract (0.470 mg QE/g fresh weigh) was 12 times higher than that of water extract (0.038 mg QE/g fresh weigh). Besides, phenolic and flavonoid contents of *Costus spicatus* ethanol extract (86.56 mg GAE/g extract & 53.97 mg QE/g extract) were higher than those of *Gynandropsis gynandra'*leaf (16.821 mg GAE/g extract & 21.956 mg QE/g extract) (Widodo et al., 2022), *Mitragyna speciosa*'s stem (23.59 mg GAE/g extract & 10.65 mg QE/g extract), and *Melastoma malabathrium*'s flower (43.97mg GAE/g extract & 11.26 mg QE/g extract.) (Hanifah et al., 2022).

The contents of phenolic and flavonoid were higher than those of water extract, indicating that these contents in *Costus spicatus* flowers depend on the type of extraction solvents. These results coincides with the study of Sun et al. (2015) and Maulana et al. (2019), total phenolic and flavonoid content of ethanol extract had a higher amount than the water extract. Many of the researchers reported that phenolics and flavonoids can inhibit the germination and growth of other plant species (Alford et al., (2009); Ladhari et al., (2020); Anh et al., (2021)).

4.3. Inhibitory effects of *Costus spicatus* flower extract

The inhibition of *Costus spicatus* flower extract on *Brassica juncea*'s seed germination was recorded after 7 days within in-vitro conditions. The inhibitory activity impacts on germination and plant growth increased proportionally to its concentrations, which was significantly different compared to the control (Table 3). Specifically, flower's extract gave stronger suppression of shoot elongation than germination and shoot growth of green mustard (*Brassica juncea)*. This experiment gave better inhibitory activity than the study of Nam et al. (2021), *Cosmos bipinnatus* extract inhibited 23.01% of shoot length and 56.45% of root length at 0.03 g/ml respectively for mustard green.

Table 3. Effect of water extracts on germination rate, root and shoot length of *Brassica juncea*.

Treatments	Concentration (mg/mL)			IC_{50} value (mg/mL)
	1	2	3	
Root length (%)	12.058 ± 1.759^{c}	37.090 ± 3.162^{b}	70.380 ± 4.211^{a}	2.651 ± 0.137^{b}
Shoot length (%)	24.190 ± 2.760^{b}	82.670 ± 5.080^{a}	99.120 ± 7.540^{a}	1.431 ± 0.184^{c}
Germination rate (%)	3.330 ± 0.270^{c}	16.670 ± 2.280^{b}	46.670 ± 3.820^{a}	3.412 ± 0.271^{a}

Data are presented as means ± standard deviations (SD) value. Values assigned by the same alphabets (^{a,b,c}) in a row (column with IC_{50} value) were not significantly different at $p < 0.05$ (Turkey's test). Bioassay method adapted from Andriana et al. (2018).

There is a correlation between the IC_{50} value and the phytochemical inhibitory potential of the extract. It means that the plant inhibitory ability of the extract is high, the IC_{50} value is

small. The flower extract inhibited 50% of shoot length at the concentration of 1.431 mg/mL while the root and germination were 2.651 mg/mL and 3.412 mg/mL. It could be elucidated that the ethanol extract was more effective in shoot length of green mustard. Inhibition of germination rate, stem and root length are expressions reflecting inhibition of plant growth and development (Zhang et al., 2015). The results also delineated that the *Costus spicatus* flower extract influenced growth of *Brassica juncea* in-vitro conditions.

4.4. Chlorophyll and carotenoid contents

The ethanol extract reduces the chlorophyll a, b, and carotenoid accumulations of green mustard at 1,5 mg/mL, which was significantly different in statistics as compared with the control (Figure 1).

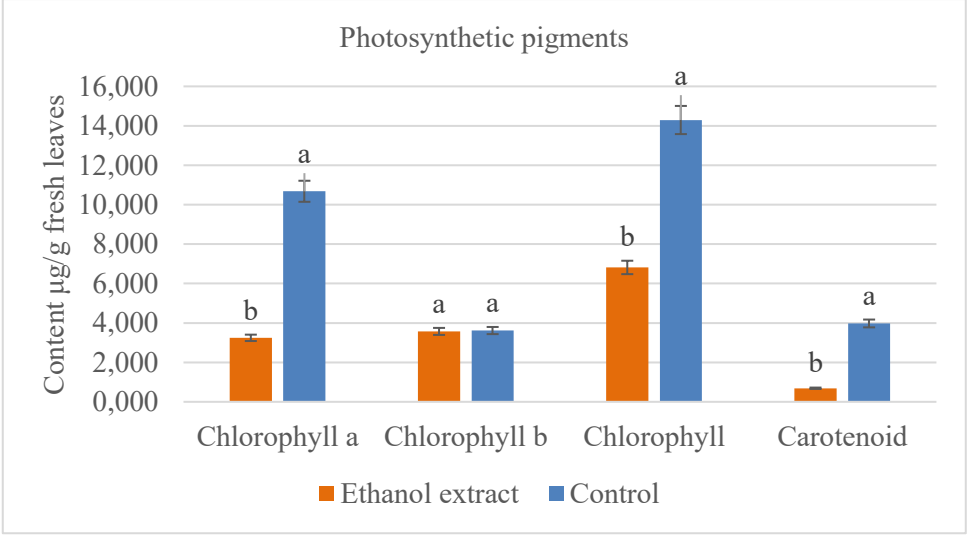

Figure 1. Pigment contents of mustard greens treated with ethanol extract at 1,5 mg/mL. Columns with different superscript letters in bars showed significant differences at $p < 0.05$ by Tukey's test

The extract presented the strong inhibition on content of chlorophyll a and total chlorophyll (3.245 µg/g fresh leaves and 6.816 µg/g fresh leaves). Besides, carotenoid was also inhibited dramatically by the ethanol flower's extract, which was 0.688 µg/g fresh leaves. The flower extract significantly reduced the chlorophyll a about 3.29 times as compared to the control. Chlorophyll a is the main pigment in photosynthesis, the main electron donor in the electron transport chain (Martin, 2019). In the study of Men et al. (2022), the content of chlorophyll a in radish's leaves when treated with the extract of *Lantana camara* decreased about 1.2 times as compared to the control. *Costus spicatus* flower extract inhibits the photosynthesis to provide essential organic substances for cell metabolism. These changes directly affect the growth and development of plants.

Many previous studies presented that a lot of natural compounds that possessed germination and plant growth inhibition belonged to flavonoids and phenolic (John & Sarada, 2012; Cheng & Cheng, 2015). They affect plants directly by changing their morphology and

biochemistry. The decline of photosynthetic pigments under allelochemicals affect may be caused by impaired chlorophyll biosynthesis and pigment degradation and (Batish et al., 2006). A number of plants can produce thousands of chemicals for self defence against pests, insects and neighbouring plants. The ethanol extract contained high levels of flavonoids and phenolics, which may be allelochemicals responsible for the herbicidal activity of *Costus spicatus* flower extract. In summary, the findings of this study highlight the strong allelopathic potential of Costus spicatus flowers, with ethanol extracts showing particularly high phenolic and flavonoid contents that correlate with significant inhibition of seed germination, seedling growth, and pigment biosynthesis in mustard greens. These results not only contribute new phytochemical and bioactivity data for C. spicatus but also suggest its potential as a natural source of bioherbicides for sustainable weed management. Further field trials and compound isolation studies are recommended to validate efficacy and identify the specific active constituents. This process may help to develop plant-based herbicides for sustainable agricultural production in the future.

5. Conclusion

The extract of *Costus spicatus* flowers was evaluated for its chemical constituents, quantification of phenolics and flavonoids, germination and growth bioassays. The ethanol extract contains high content of phenolics and flavonoids which is correlated with allelopathic property. Thus, *Costus spicatus* flowers extract can inhibits the germination of seeds and the growth of shoot and root of green mustard as well as reduced the photosynthetic pigments of indicator plant.

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