EFFECTS OF GLYCEMIC REGULATION AND ACETYLCHOLINESTERASE INHIBITION OF SCORPION TURMERIC (*CURCUMA RANGJUED*) IN CENTRAL HIGHLANDS, VIETNAM

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ABSTRACT

Scorpion turmeric has been traditionally grown in the Central Highlands provinces of Vietnam. Prepared medicinal products from this species (*Curcuma rangjued*) are currently used in folk medicine to treat various illnesses including bronchitis, asthma, gastritis, and typically used as an antiseptic. Despite its popular use, scientific studies into its pharmacological properties remain limited. Chemical analysis has revealed the presence of many valuable bioactive ingredients such as D-limonene, β-pinene, and caryophyllene, which exhibit high pharmaceutical potential due to antioxidant and anti-inflammatory activities. Therefore, exploiting the medicinal resource from the scorpion turmeric plant will bring various benefits to community health care.

Objectives: This study aimed to evaluate the glycemic regulation and acetylcholinesterase (AChE) inhibitory effects of the scorpion turmeric's rhizome extracts.

Methods: Blood glucose levels were measured using the method described by Sivalingam (2013), while AChE inhibitory activity was determined according to the method of Ellman et al. (1961).

Results: The results have shown that scorpion turmeric's rhizome extracts which contain D-limonene, β-pinene and caryophyllene, demonstrated significant anti-inflammatory properties. Moreover, they exhibited both *in vitro* and *in vivo* glycemic regulatory effects and inhibited AChE activity *in vitro*.

Conclusion: The findings of this study suggest that *C. rangjued* possess considerable therapeutic potential for the management of diabetes and Alzheimer's disease. Therefore, the scorpion turmeric plant should be given more attention and developed to preserve this valuable medicinal resource.

Keywords: Diabetes, Alzheimer, AchE, *Curcuma rangjued*, Scorpion turmeric.

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1. INTRODUCTION

The causes of diabetes mellitus are associated with insulin resistance, impaired insulin signaling, β-cell dysfunction, altered lipid metabolism, increased oxidative stress, and dysregulated metabolism of carbohydrate, lipid and protein, resulting from either a deficiency or diminished action of pancreatic insulin. This condition manifests as chronically elevated blood sugar levels (Testa et al., 2016), leading to typical complications, such as stroke, blindness, kidney failure, erectile dysfunction and Alzheimer's disease. Alzheimer's disease is a form of dementia, and epidemiological studies have indicated a link between type 2 diabetes and Alzheimer's disease, mediated by specific genes, autophagy and inflammatory pathways (Athanasaki et al., 2024).

Research on the phytochemical composition analysis of the rhizome of *Curcuma rangjued* (hereby used as 'Scorpion Turmeric' - locally known as '*nghệ bọ cạp*') has found D-limonene, β-pinene and caryophyllene. These compounds are known for their anti-inflammatory and antioxidant properties (Gushiken et al., 2022), implying to the ability to regulate glycemic levels and inhibit acetylcholinesterase (AChE), suggesting a therapeutic role in both type 2 diabetes mellitus and Alzheimer's disease.

2. OBJECTIVES AND METHODS

2.1. Research implementation period

The study was conducted from June 2024 to October 2024.

2.2. Research Design

Scorpion turmeric's rhizome was extracted and analyzed for the content of caryophyllene, D-limonene and β-pinene at Institute of Chemistry in Vietnam Academy of Science and Technology (VAST) and preserved at the laboratory of Institute of Health Research and Educational Development in Central Highlands. The anti-inflammatory potential, blood glucose regulation effects *in vitro* and *in vivo*, and the inhibition of acetylcholinesterase *in vitro* were evaluated at Institute of Biotechnology, VAST.

2.3. Research Subjects and Materials

Scorpion turmeric's rhizome extract, RAW264.7 cell line, alloxan-induced diabetic rats, α-glucosidase, and acetylcholinesterase (AChE) were used in the study. Male Wistar rats were used as experimental animals. Chemicals and reagents were purchased from Sigma Aldrich (USA); a mouse insulin ELISA kit was obtained from Fine Test (Wuhan, China).

2.4. Research Location and Scope

The study was carried out at Vietnam Academy of Science and Technology and Institute of Health Research and Educational Development in Central Highlands.

C. rangjued's rhizome samples were collected, extracted and analyzed for their bioactive compounds. Then, the regulatory activities of blood glucose *in vitro* and *in vivo* on diabetic rats and the AChE inhibitory activities were evaluated.

2.5. Methodologies

2.5.1. Selection of Curcuma rangjued's rhizome

C. rangjued's rhizomes were harvested and obtained from their natural habitat in Dak Ha Commune, Dak Glong District, Dak Nong Province, in November 2023.

2.5.2. Extraction and quantification of bioactive compounds from scorpion turmeric's rhizome

The rhizomes of scorpion turmeric were cleaned and the selected samples were ground into powder with a particle size of approximately 1 mm using a grinder. A 400 g sample was placed into an extraction flask that was previously added 2 liters of n-hexane, then the mixture was stirred thoroughly overnight for 16 hours. After filtration, 1.5 liters of the n-hexane extract was collected, and the remaining residue was extracted twice more. The combined extracts of all three extractions were distilled to make the n-hexane solvent completely evaporated, yielding a yellow viscous oil, which was labelled as 'Extract H'.

Gas chromatography-mass spectrometry (GC-MS) was used to determine the presence of caryophyllene, D-limonene and β-pinene in the extract.

2.5.3. Evaluation of the blood glucose regulatory effects of Extract H

- Evaluating α-Glucosidase Inhibitory Activity

A 50 µL of the diluted sample was added to each well of a 96-well plate. Subsequently, 20 µL of α-glucosidase (0.5 U/mL) and 130 µL of 100 mM phosphate buffer (pH = 6.8) were added to each well, mixed thoroughly and incubated at 37°C for 15 minutes. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate was added to each corresponding well. The mixture continued to incubate at 37°C for 60 minutes. The reaction was stopped by adding 80 µL of 0.2 M Na2CO3, and the optical density (OD) absorbance was measured at 405 nm using an ELISA Plate Reader (Biotek). The α-glucosidase inhibition of the sample was calculated using the following formula:

$$\% \text{ inhibition} = 100 - [(OD_{sample} / OD_{control}) \times 100]$$

Where:

$$OD_{control} = OD_{control'} - OD_{blank}$$

$$OD_{sample} = OD_{sample'} - OD_{blank}$$

The IC_{50} value (50% inhibitory concentration) was determined using the TableCurve2Dv4 software.

- Blood glucose regulation assay in vivo of Extract H

The hypoglycemic activity assay was conducted following the modified methods of Agila et al. (2012) and Okokon et al. (2006). Specifically, after 72 hours of the administration of Alloxan monohydrate, glycemic levels were measured using a glucose meter (T3). According to WHO (1985) guidelines, mice with blood glucose levels exceeding 8.33 mg/L were selected for the study and divided into groups (6 mice per group). The effect of the sample was evaluated by comparing decreased glucose levels in blood before and after the oral administration. The formula used to calculate the percentage change (decrease or increase) in blood glucose levels compared to pre-treatment level was as follows:

% reduction compared to the pretreatment level = 100 × (G1 – G2)/G1

% elevation compared to the pretreatment level = 100 × (G2 – G1)/G2

Where:

G1: the pre-treatment blood glucose concentration

G2: the post-treatment blood glucose concentration

2.5.4. Evaluating the AChE inhibitory activity of Extract H

The AChE inhibitory activity of the scorpion turmeric's rhizome extract was assessed using the method of Ellman et al. (1961). Specifically, the sample was dissolved in 100% DMSO, then diluted to different concentrations using ddH2O (deionized water). Each well contained a mixture of 140 µL phosphate buffer (pH 8), 20 µL of sample at different concentrations, and 20 µL of AChE enzyme (0.25 IU/mL), which was well mixed and incubated at 25°C for 15 minutes. Then, 10 µL of 2.5 mM DTNB and 10 µL of 2.5 mM ACTI were added to the wells, and the mixture was incubated for another 10 minutes at 25°C. The solution's absorbance was measured at 405 nm (the reference wavelength being 412 nm). Galantamine was used as a positive control. The blank well contains no enzyme. The negative control well contains no test sample. The percentage inhibition of AChE activity (% I) was calculated using the following formula:

%I = ((Ac-At)/(Ac))*100

Where: % I: percentage of AChE activity inhibition

Ac: absorbance of the control sample (without the 20 µL test solution), after subtracting the blank well absorbance

At: absorbance of the test sample, after subtracting the blank well absorbance.

2.5.5. Evaluation of the anti-inflammatory activity of Extract H

100 µL of cell culture medium (for sample incubation) was transferred to a new 96-well plate and 100 µL of Griess reagent was added: 50 µL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50 µL of 0.1% (w/v) N-1-naphthylethylenediamine dihydrochloride dissolved in water. The mixture was incubated at room temperature for another 10 minutes, and the nitrite content was measured using a microplate reader at 540 nm. FBS-free DMEM medium was used for the blank wells. The nitrite content of each sample was determined using a standard curve of $NaNO_2$ and the percentage of inhibition was compared to the negative control (LPS). The percentage inhibition of NO production was calculated using the following formula:

% inhibition = 100% - [(content of NO_{sample} / content of NO_{LPS}) * 100]

The experiment was repeated three times to ensure reproducibility. The IC_{50} value (concentration required for 50% inhibition of NO production) was determined using TableCurve 2Dv4 software.

2.6. Data analysis

Data were analyzed using Excel and presented as Mean ± SE. Student's t-test, F-test and one-way ANOVA were used to assess significant differences compared to the pathological control group, with $p < 0.05$ considered statistically significant. Parameters were calculated and processed using PK Solver software (Zhang Y., et al., 2010).

3. RESULTS

3.1. Extraction and quantification of active ingredients in the *C. rangjued's* rhizome

Turmeric rhizomes were extracted with n-hexane. The combined extracts of all three extractions were distilled to completely remove the solvent, yielding a 1.05% extract (w/w) compared to the fresh rhizome of scorpion turmeric. GC-MS analysis of the extract showed that the contents of β-pinene, D-limonene and caryophyllene accounted for 14.5%, 10.5% and 8.8%, respectively.

3.2. Evaluation of the glycemic regulatory effects of Extract H

3.2.1. The α-glucosidase inhibition by Extract H

Table 1. Inhibitory effects of Extract H to α-glucosidase

Concentration (µg/mL)	Extract H		Acarbose	
	% inhibition	SD	% inhibition	SD
500	100.53	2.13	73.70	0.78
100	73.91	1.42	51.67	0.91
20	5.29	0.37	17.26	1.24
4	1.86	0.11	8.36	0.21
IC_{50}	52.78 ± 2.32		125.95 ± 5.04	

Table 1 presents the results of the α-glucosidase inhibitory activity assay. The Extract H exhibited significant inhibitory activities with an IC_{50} value of 52.78 µg/mL, which was more potent compared to the positive control, acarbose.

*3.2.2. In vivo **glycemic regulation by Extract H***

The results of the glycemic level of different experimental lots are presented in Table 2.

Table 2. Blood glucose concentration in mice before and after the oral administration of Extract H

Experimental Lot	Blood glucose level (mmol/L)			
	Before oral administration (T3)	After 10 days (T10)	% change compared to day 0 (baseline)	% reduction vs. the control
Lot 1: Physiological Control	4.52 ± 0.38	4.69 ± 0.42		
Lot 2: Pathological Control	15.67 ± 2.77	14.64 ± 2.56	↓ 6.57	-
Lot 3: Reference Control	15.70 ± 2.92	7.42 ± 0.98	↓52.74*	↓49.32*
Lot 4: Extract H at the dosage of 200 mg/kg	15.72 ± 2.85	7.08 ± 0.72	↓54.96*	↓51.84*

*Note: * p<0.05; **p<0.01 compared to pathological control*

As shown in Table 2, the oral administration of the Extract H at a dose of 200 mg/kg/day after 7 days reduced blood glucose levels from 15.72 mmol/L to 7.42 mmol/L (p<0.05) compared to the diabetic control group. Metformin, a positive control, also significantly reduced blood glucose levels from 15.72 mmol/L to 7.08 mmol/L after 7 days of treatment. This reduction compared with the pathological control group was at a statistically significant level (p<0.05).

3.3. Evaluation of the AChE inhibitory effect of Extract H

Table 3. Acetylcholinesterase enzyme inhibitory effect of Extract H

Concentration (µg/mL)	Extract H		Galantamine	
	% inhibition	SD	% inhibition	SD
100	55.72	2.87	96.10	0.07
20	28.42	1.38	51.15	0.89
4	26.71	1.86	25.07	0.60
0.8	18.44	0.60	4.35	0.60
IC_{50}	78.88 ± 1.32		1.49 ± 0.02	

The Extract H demonstrated acetylcholinesterase (AChE) inhibitory activity with an IC_{50} value of 78.88 µg/mL. The positive control, galantamine, showed consistent activity in the assay.

3.4. Evaluation of the anti-inflammatory mechanism of Extract H

Table 4. Inhibitory activity of Extract H on nitric oxide (NO) production

Concentration (µg/mL)	Extract H				Dexamethasone			
	% NO inhibition		% live cell		% NO inhibition		% live cell	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
100	138.86	2.49	3.31	0.43	88.02	2.34	93.53	2.23
20	101.14	2.15	90.68	1.21	53.19	1.02	100.31	1.51
4	29.31	1.17			42.21	0.91		
0.8	18.86	0.85			32.66	0.82		
IC_{50}	11.38 ± 0.39		-		13.85 ± 1.39		-	

Note: For IC_{50} calculation, Extract H was tested at the concentration range of 80-20-4-0.8 µM meanwhile Dexamethasone was tested at the concentration range of 100-20-4-0.8 µM;

Table 4 indicates that the Extract H inhibited nitric oxide (NO) production with an IC_{50} value of 11.38 ± 0.39 µg/mL, which was not significantly different from the positive control, dexamethasone, at concentrations ranging from 80 to 0.8 µM ($p>0.05$).

4. DISCUSSION

The chemical composition of turmeric (*Curcuma longa*) is primarily comprised of curcuminoids, including curcumin, demethoxycurcumin, and bisdemethoxy curcumin, with curcumin being the most abundant. Ultrasonic-assisted extraction of curcumin from turmeric using 96% ethanol followed by silica gel column chromatography and reverse-phase HPLC yielded highly purified curcumin (>99%) with a yield of approximately 2.2% (Hà & Hiền, 2011). Unlike turmeric, *Curcuma rangjued* (or 'Scorpion turmeric') contains D-limonene as its major bioactive compound, along with β-pinene, caryophyllene, and other substances contributing to its unique pharmacological properties. Analysis of the Extract H revealed D-limonene as the primary constituent. A previous study demonstrated that D-limonene at a dose of 100 mg/kg body weight effectively countered diabetes in an alloxan-induced diabetic rat model, comparable to the positive control glibenclamide (Murali et al., 2012). Consequently, the extract from scorpion turmeric rhizome exhibited potent α-glucosidase inhibitory activity with an IC_{50} value of 52.78 µg/mL, higher compared to the positive control, acarbose (IC_{50} = 125.95 µg/mL). Furthermore, oral administration of the Extract H at a dose of 200 mg/kg/day after seven days significantly lowered glycemic levels in diabetic rats from 15.72 mmol/L to 7.42 mmol/L. The reduction has statistical significance ($p<0.05$) when compared to the pathological control and is comparable to the positive control, metformin. Additionally, the extract demonstrated acetylcholinesterase (AChE) inhibitory activity with an IC_{50} value of 78.88 µg/mL, which is likely attributable to the ingredient content of D-limonene. Eddin et al. (2021) reported that limonene inhibits AChE activity and protects neurons from damage by preventing the reduction of mitochondrial dehydrogenase activity, ROS production, and KV3 channels.

The Extract H also showed potent anti-inflammatory activity, with an IC_{50} value of 11.38 ± 0.39 µg/mL compared to dexamethasone (13.85 ± 1.39 µg/mL), likely due to the presence of β-pinene and caryophyllene. Santos et al. (2022) demonstrated that β-pinene possessed anti-inflammatory properties and lowered blood glucose levels in an alloxan-induced diabetic rat model, while Gushiken et al. (2022) reported caryophyllene held the potent anti-inflammatory and antioxidant properties. These findings suggest that the potent glycemic regulation and AChE inhibitory activities of Extract H from the scorpion turmeric rhizome might be thanks to three important active ingredients, D-limonene, β-pinene and caryophyllene, with their combined anti-inflammatory properties.

A recent study by Rolandsson et al., (2024) at Umeå University, Sweden, showed that there was a strong correlation between type 2 diabetes and Alzheimer's disease, suggesting that individuals with diabetes may face more difficulty to eliminate a protein associated with Alzheimer's disease. Therefore, in this study, the ability of Extract H to simultaneously treat both conditions highlights its significant potential of this medicinal plant as a therapeutic agent.

5. CONCLUSION

The *C. rangjued* rhizome is a potential medicinal resource possessing strong efficacy in supporting diabetes treatment. *In vitro* experiments demonstrated that Extract H exhibited significantly higher activity compared to the positive control, while *in vivo* experiments showed comparable efficacy to the positive control, with statistically significant differences when compared to the negative control ($p<0.05$). Additionally , Extract H inhibited AChE with an IC_{50} of 78.88 µg/mL, indicating its potential for Alzheimer's disease treatment. The observed efficacy is likely due to the strong anti-inflammatory properties of Extract H (IC_{50} = 11.38±0.39 µg/mL), which can be ascribed to its key active compounds, such as D-limonene, β-pinene, and caryophyllene. Therefore, it is essential to develop appropriate cultivation regions to facilitate the future production of health-promoting products from this plant.

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