Scientific paper

# Phytochemical Composition, Antioxidant and Enzyme Inhibitory Effects of Stahlianthus thorelii Gagnep. Rhizomes

Pham M. Tuan, Danh C. Vu, Sy Vo Van, Ngo Thi Thuy, Vo Mong Tham and Nguyen Thi Ngan 1,\* ©

<sup>1</sup> Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam

<sup>2</sup> McCampbell Analytical Inc., Pittsburg, California, the United States

<sup>3</sup> Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Hai Chau District, Da Nang, Vietnam

<sup>4</sup> Hanoi Medical University Hospital, Dong Da District, Hanoi, Vietnam

<sup>5</sup> Faculty of Pharmacy, Hong Bang International University, Binh Thanh District, Ho Chi Minh City, Vietnam

\* Corresponding author: E-mail: nguyenthingan\_vsh@iuh.edu.vn Tel: +84 2838940390

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#### **Abstract**

Stahlianthus thorelii Gagnep., traditionally used for treating various ailments, is a promising source of bioactive compounds. This study investigated the ethyl acetate extract of *S. thorelii* rhizomes for its phytochemicals, antioxidant properties, and inhibitory effects on key enzymes related to diabetes and Alzheimer's disease. The extract contained significant levels of phenolic compounds such as ferulic acid, catechin, and quercetin, and its major volatile components included  $\beta$ -patchoulene, (E)-nerolidyl isobutyrate, and aristolene. The extract exhibited strong antioxidant activity (DPPH IC<sub>50</sub> = 86.9 µg/mL) and enzyme inhibition, including acetylcholinesterase (IC<sub>50</sub> = 246.4 µg/mL) and  $\alpha$ -amylase (IC<sub>50</sub> = 789.8 µg/mL). These findings highlight the potential of *S. thorelii* as a natural source for developing therapeutics targeting neuro-degenerative and metabolic disorders, warranting further investigation into its pharmacological applications.

Keywords: Stahlianthus thorelii; HPLC; GC-MS; phenolics; bioactivities

#### 1. Introduction

Stahlianthus thorelii (English name: Ginger Panax pseudoginseng) is a plant belonging to the family Zingiberaceae and has a wide distribution in Asian countries, including China, India, Laos, Myanmar, Sikkim, Thailand, Vietnam, and Cambodia.<sup>1</sup> In Vietnam, S. thorelii was also known by the vernacular name "Tam thất gừng" and has been widely used as folk medicine to treat a wide range of diseases. According to traditional Vietnamese medicine, S. thorelii rhizomes possess a pungent characteristic with a slightly bitter and warm nature and has traditionally been used to address hemorrhage, rheumatism, irregular menstruation, digestive issues, and joint pain.<sup>2</sup>

Although it has been used by humans for a long time, indepth research on the composition and activity of *S. thorelii* has been very limited. Recently, a study has reported the volatile chemical composition of essential oils from the leaves and rhizomes of *S. thorelii*, revealing that the monoterpene group (accounting for 9.4–14.2%) and sesquiterpenes (accounting for 60.0–63.9%) are the two main classes of volatile compounds, in which two compounds, α-copaene and allo-aromadendrene, are the major constituents.<sup>3</sup> The rhizome essential oil showed antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *and Aspergillus niger*, with MIC ranges between 150 and 200 μg/mL. while the leaf essential oil was effective only against *B. subtilis* at MIC 200 μg/mL.<sup>3</sup> In addition, isolated compounds from the rhizomes of *S. thorelii*, including thorechalcone A and

flavonoid derivatives, also showed antiproliferative activity, with IC $_{50}$  values < 40  $\mu$ M of inhibitory activities against colon, lung, and liver cancer cell lines.<sup>4</sup>

To provide more information on the chemical compositions and bioactivities of this plant, in this study, we first analyzed the chemical constituents of the ethyl acetate extract of *S. thorelii* rhizomes using gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography system equipped with a diode-array detector (HPLC-DAD). Second, we assayed its antioxidant and enzyme inhibition activities to evaluate its potential health benefits. These findings have the potential to broaden the medicinal applications of *S. thorelii*.

## 2. Experimental

#### 2. 1. Sample Collection

Fresh rhizomes of *S. thorelii* were collected from Kon Ka Kinh National Park, Kbang District, Gia Lai Province, Vietnam in June 2020. The identification of this plant was conducted by Assoc. Prof. Dr. Nguyen Hoang Tuan (Faculty of Pharmacognosy and Traditional Medicine, Hanoi University of Pharmacy, Vietnam). A voucher specimen (No. SVV-031) was deposited in the Department of Pharmacy, Da Nang University of Medical Technology and Pharmacy, Danang, Vietnam.

#### 2. 2. Chemicals

HPLC-grade extraction solvents were obtained from Fisher Scientific (Pennsylvania, USA). Ferulic acid, kojic acid, and galantamine were purchased from Sigma-Aldrich (Missouri, USA). Catechins and quercetin were obtained from Chengdu Biopurify Phytochemicals (Sichuan, China), while kaempferol (purity > 99%) and acarbose were procured from the National Institute of Drug and Quality Control (Hanoi, Vietnam).

#### 2. 3. Preparation of Extract

Rhizomes of *S. thorelii* were harvested and subsequently dried in a desiccator (CYF EL040 model, Chin Ying Fa Mechanical Ind. Co., Ltd, Taiwan) at a low temperature (42–45 °C) to protect bioactive constituents in plant materials from degradation. The dried materials were then ground into powder form using a grinder (DF-20 model, VINASTAR Company, Hanoi, Vietnam). The sample was mixed with methanol at a ratio of 1:10 (g/mL). The extraction was performed at room temperature using ultrasound-assisted method (Model STE-17CSA, Taiwan). After the mixture was filtered using filter paper and the solvent was removed under reduced pressure, a crude methanol extract (165.8 g) was obtained. The extract was resuspended in water and successively partitioned with n-hexane (5 times) and ethyl acetate (5 times).<sup>5</sup> After-

wards, the ethyl acetate fraction was concentrated under reduced pressure, resulting in 62.1 g of an extract (EtOAc). The extract was kept in an amber vial at 4 °C until analysis.

# 2. 4. High-performance Liquid Chromatography Analysis

Phenolics present in diluted solutions of the EtOAc extract were examined using a Shimadzu LC-2030C high-performance liquid chromatography system equipped with a diode array detector (HPLC-DAD). Separation of the analytes was carried out on an Agilent Zorbax Eclipse XDB-C18 column (4.6  $\times$  150 mm, 5  $\mu$ m), employing a mobile phase consisting of solvent A (0.1% formic acid) and solvent B (100% acetonitrile). The mobile phase composition underwent the following changes: 0 – 0.1 min, 6% B; 0.1 – 5 min, 6 – 17% B; 5 – 10 min, 17 – 19% B; 10 – 20 min, 19 – 40% B; 20 – 25 min, 40 – 60% B; 25 – 30 min, 60 – 80% B; 30 – 33 min, 80 – 6%; 33 – 41 min, 6% B. Detection of phenolics was carried out at three wavelengths (295, 340, and 360 nm), and quantification relied on external standards, as previously reported.

## 2. 5. Gas chromatography-Mass Spectrometry (GC-MS) Analysis

The volatile constituents of the EtOAc extract of S. thorelii rhizomes were determined by GC-MS system. The GC-MS analysis was performed on Agilent 7890B GC System equipped with Agilent 5977B MSD model, using HP-5MS Ultra Inert column (30 m length  $\times$  0.25 mm i.d.  $\times$ 0.25 µm film thickness, Agilent Technologies, USA). The carrier gas was helium and flow rate of 1.2 mL/min. A volume of 1.0 μL solution (1000 ppm, dissolved in EtOAc, Merck) was injected with a split ratio of 25:1. The temperature of the inlet-F was set at 300 °C, of MS Quad was set at 150 °C, while the MS source was heated at 230 °C. The oven temperature started from 80 °C for 1 min, ramped at a rate of 20 °C/min to 300 °C, then kept at this point for 15 min. The EI mass spectra (70 eV) were acquired over the range 50-550 amu (2 scans/s). Retention indices (RI) were experimentally determined as previously described.<sup>7, 8</sup>

The volatile constituents were identified via comparison of their RI and their mass spectra data base with those in the literature (NIST 17 search program and Adams data). Relative percentages (%) of the components were computed from the peak area per total area in the GC chromatogram.

#### 2. 6. Antioxidant Activity

The antioxidant activity of the EtOAc extract of *S. thorelli* rhizomes was determined through ABTS and DPPH radical scavenging assays. <sup>10</sup> For DPPH, three volumes of a 3 mM DPPH solution were finely mixed with two volumes of diluted extracts. The mixtures were then

incubated at 37 °C in the dark before measuring absorbance at 517 nm. As for the ABTS assay, ABTS solution (7 mM) and potassium persulfate (2.45 mM) were mixed at a ratio of 1:1 (v/v), followed by a 12-hour incubation in the dark to prepare the ABTS working solution. Before testing, the absorbance of the solution at 420 nm was adjusted to  $1.70 \pm 0.02$  using phosphate-buffered saline (pH 7.4). The working solution (3 mL) was then mixed with diluted samples (100  $\mu$ L) and incubated for 15 min before measuring the absorbance at 734 nm using a spectrophotometer. In both assays, ascorbic acid was used as the positive control. IC<sub>50</sub> values-the concentrations ( $\mu$ g/mL) at which the extract could scavenge 50% of the free tested radicals-were calculated to determine the extract's antioxidant activity.

## 2. 7. Anti-acetyl Cholinesterase (AChE) Activity

This assay followed the a previously described method, which relies on AChE acting as a catalyst for the hydrolysis of acetylthiocholine iodide (ACTI), producing thiocholine.<sup>11</sup> Thiocholine then reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid) to form a yellow-colored compound, 5-thio-2-nitrobenzoic acid. The generation of this colored compound directly correlates with AchE activity. In detail, the samples were initially dissolved in 100% DMSO and subsequently diluted to various concentrations using ion-deionized water. Experimental wells were prepared with a mixture consisting of 140 µL of phosphate buffer (pH: 8), 20 μL of test samples at different concentrations, and 20 μL of 0.25 IU/mL AChE. This mixture was thoroughly mixed and incubated at 25 °C for 15 minutes. Subsequently, 10 μL of 2.5 mM DTNB and 10 µL of 2.5 mM ACTI were added to the wells and further incubated at 25 °C for 10 minutes. Following this, the absorbance of the solution was measured at 405 nm (with a reference wavelength of 412 nm). Galantamine was used as the positive control. The blank well did not contain the enzyme, whereas the negative control well did not include the test sample. The percentage inhibition of AChE activity was calculated using the formula:

Percentage of inhibition (%) =  $[1 - At/Ac] \times 100$  (%)

where: Ac denotes the absorbance of the control sample (without 20  $\mu$ L of the test solution) minus the absorbance of the blank well; At represents the absorbance of the test sample minus the absorbance of the blank well.

#### 2. 8. Anti-α-amylase Activity

The experiment followed a previously described method with minor modifications.  $^{12}$  A diluted EtOAc extract was mixed with 10  $\mu L$  of  $\alpha$ -amylase solution (0.14 U/mL) in phosphate buffer (pH 6.9) and incubated for 15 min at 37 °C. The reaction was initiated by adding starch solution (0.25%), followed by another 15-min incubation at 37 °C. A blank sample

underwent the same steps without the inclusion of  $\alpha$ -amylase. To stop the reaction, 50  $\mu L$  of 1 M HCl was added, followed by 100  $\mu L$  of KI $_3$  solution. Absorbance was measured at 595 nm using a spectrophotometer. The percentage of enzymatic activity inhibition was calculated using the formula:

Percentage of inhibition (%) =  $[1 - As/Ab] \times 100$  (%)

where, As and Ab represent the absorbance of the sample and blank, respectively. Acarbose was employed as the reference standard.  $IC_{50}$  values (µg/mL) were used to evaluate the EtOAc extract's activity. Concentrations of the EtOAc extract tested ranged from 0 to 500 µg/mL.

#### 2. 9. Anti-α-glucosidase Activity

Firstly, 50  $\mu$ L volume of EtOAc extract, diluted in 5% DMSO, was mixed with 40  $\mu$ L of  $\alpha$ -glucosidase (0.05 U) in phosphate buffer (pH 6.8), and then incubated at 37°C for 20 minutes. Subsequently, 40  $\mu$ L of 5 mM 4-nitrophenyl- $\beta$ -D-glucopyranoside (p-NPG) was added to the mixture, which underwent another 20-minute incubation at 37 °C. To stop the reaction, 130  $\mu$ L of 0.2 M sodium carbonate solution was used, and the changes in absorbance were measured at 405 nm. Acarbose was used as the reference standard in this assay, while the concentrations of the EtOAc extract employed ranged from 0 to 2000  $\mu$ g/mL. <sup>13</sup>

#### 2. 10. Antityrosinase Activity

The tyrosinase inhibitory potential of the EtOAc extract was evaluated using the previously described method. Different concentrations of diluted EtOAc extract (125, 250, and 500  $\mu g/mL$ ) were combined with tyrosinase (80 U/mL) and 2 mM L-DOPA at a 5:2:2 (v/v/v) ratio. This mixture was then incubated at 37 °C for 20 minutes, and the absorbance was recorded at 490 nm. Kojic acid was used as the reference standard in this experiment.

#### 2. 11. Statistical Analysis

All experiments were carried out in triplicate. The data are shown as mean  $\pm$  standard deviation (n = 3). Microsoft Excel was used to calculate IC<sub>50</sub> values. Analysis of variance (ANOVA) was used to statistically process the data and determine the significance level (p < 0.05). Minitab 19 (Pennsylvania, USA) was employed for data analysis.

#### 3. Results and Discussion

# 3. 1. Phenolics and Volatile Organic Compounds of the EtOAc Extract

In the present study, a total of six compounds (No. 1–6) were found to be present in the EtOAc extract of *S*.

thorelli rhizomes (Figure 1, Table 1) by using an HPLC-DAD analysis. Ferulic acid was found at an average concentration of 14.7 µg/g, and it was the only phenolic acid detected. Three catechins, namely catechin, epicatechin, and epigallocatechin gallate (EGCG), were found in the S. thorelli extract, with average levels ranging from 169.3 to 2449.6 μg/g. Other flavonoids detected in the S. thorelli extract are quercetin and kaempferol, with much lower concentrations than catechins. No study has reported the presence of these flavonoids in *Stahlianthus* species. Previously, one study reported this compound was also identified in Stahlianthus involucratus through use of high resolution mass spectrometry.<sup>14</sup> However, they were found in rhizome extracts of species in the Zingiberaceae family, such as Zingiber roseum, Z. officinale, Z. Zerumbet and Curcuma longa. 15-18 Interestingly, catechins were also identified as major constituents in these Zingiber plants. Phenolics are known as contributors to a variety of potential health benefits of plants, such as antioxidant, anti-inflammatory, antidiabetic, and anticancer properties. 19-21 Investigating the phenolic content in S. thorelli as part of this study will give an initial insight into the potential applications of this plant for preventing and treating diseases.

Based on GC-MS analysis (Figure 2), a total of 16 volatile compounds (No. 1–16) (representing 68.3% of its composition) in the EtOAc extract of *Stahlianthus thorelii* rhizomes were identified and are presented in Table 2. Of these volatile components,  $\beta$ -patchoulene (23.1%), (E)-nerolidyl isobutyrate (11.9%) and aristolene (10.8%) were present in significant amounts. Among the volatile organic compounds detected,  $\beta$ -patchoulene (9) has been widely used in traditional Chinese medicine for treatment of inflammatory diseases. Meanwhile, the essential oil from *Croton heliotropiifolius* leaves, with its main component being aristolene at 22.43%, demonstrates significant cyto-

toxic activity against all tested cancer cell lines, including HL-60 (leukemia), HCT-116 (colon), MDA-MB435 (melanoma), and SF295 (glioblastoma).<sup>23</sup>

 Table 1. Phenolic contents in the EtOAc extract of Stahlianthus

 thorelii rhizomes

No.	Retention time	Phenolics	Concentrations, μg/g
1	9.59	Catechin	467.0 ± 12.1
2	11.52	Epicatechin	$2450 \pm 40$
3	11.98	EGCG	$169.3 \pm 12.2$
4	17.07	Ferulic acid	$14.7 \pm 0.6$
5	22.65	Quercetin	$14.7 \pm 0.6$
6	25.09	Kaempferol	$75.1 \pm 1.0$

EGCG: epigallocatechin gallate

#### 3. 2. Antioxidant Activity of the Extract

Natural antioxidant compounds are important as they can stabilize free radicals, thus protecting cells and maintaining physiological functions of the body. <sup>24</sup> In this study, we first determined the antioxidant activities of *S. thorelii* by DPPH and ABTS free radical scavenging assays. The results are presented in Table 3. The extract demonstrated scavenging activity against both DPPH and ABTS free radicals; however, its effectiveness varied significantly between the two assays. In the ABTS assay, the extract exhibited only weak activity, with no measurable inhibition observed at lower concentrations (15.6–62.5  $\mu$ g/mL). Activity increased steadily at higher concentrations, resulting in an IC<sub>50</sub> of approximately 743.6  $\mu$ g/mL. The extract showed moderate antioxidant activity in the DPPH assay, with an IC<sub>50</sub> of approximately 86.9  $\mu$ g/mL. Despite its ac-

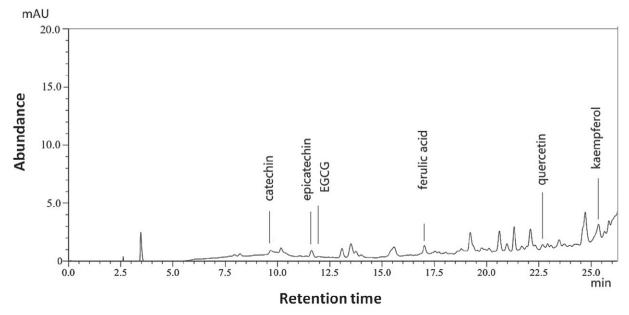


Figure 1. HPLC-DAD chromatogram of the phenolics detected in the extract

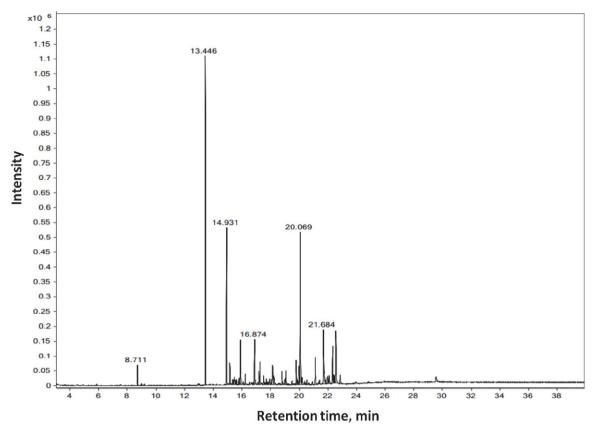


Figure 2. Total ion chromatogram of the EtOAc extract of Stahlianthus thorelii rhizomes

Table 2. Identification of the volatile constituents the EtOAc extract of Stahlianthus thorelii rhizomes using GC-MS analysis

No.	RT	Compounds	RIa	$RI^b$	Formula	Percentage (%)
1	8.711	3-Bornanone	1154	1149	C <sub>10</sub> H <sub>16</sub> O	1.9
2	8.985	Isoborneol	1165	1160	$C_{10}H_{18}O$	0.2
3	13.446	$\beta$ -Patchoulene	1384	1381	$C_{15}H_{24}$	23.1
4	14.931	Aristolene	1453	1453	$C_{15}H_{24}$	10.8
5	15.157	Alloaromadendrene	1463	1461	$C_{15}H_{24}$	1.7
6	15.786	Germacrene D	1487	1481	$C_{15}H_{24}$	0.6
7	15.896	$\beta$ -Guaiene	1493	1490	$C_{15}H_{24}$	3.5
8	16.874	Viridiflorol	1592	1591	$C_{15}H_{26}O$	3.9
9	17.182	Zingiberenol	1617	1616	$C_{15}H_{26}O$	1.1
10	17.264	Eudesm-4(14)-en-6 $\alpha$ -ol	1623	1617	$C_{15}H_{26}O$	1.8
11	17.935	10-Hydroxycalamene, cis-	1670	1666	$C_{15}H_{22}O$	0.9
12	18.071	10-Hydroxycalamenene, trans-	1679	1676	$C_{15}H_{22}O$	0.4
13	18.147	Cedr-8-en-15-ol	1685	1682	$C_{15}H_{24}O$	2.7
14	18.797	Oplopanone	1732	1730	$C_{15}H_{26}O_2$	1.0
15	20.069	(E)-Nerolidyl isobutyrate	1827	1825	$C_{19}H_{32}O_2$	11.9
16	21.109	Myrrhanolide C	1908	1904	$C_{15}H_{18}O_4$	2.8
		Total identified				68.3

Abbreviations: RT: Retention time (min); RIa: Retention indices on HP-5MS Ultra Inert column; RIb: Retention indices in literature.

tivity in both assays, the extract's scavenging power was consistently lower than that of ascorbic acid, highlighting its relatively moderate antioxidant potential. The difference in antioxidant activity measured by two different methods may be attributed to its different solubility in the reaction medium, leading to an alteration in their capability to donate electrons. However, taken together, these findings confirmed the antioxidant capacity of the studied extract. There has been limited research on the antioxidant activity of *Stahlianthus* species. Recently, the aqueous and methanol extracts of *S. involucratus* were reported to possess free radical scavenging activities. <sup>14</sup> The antioxidant

activity depended on the solvent used for extraction; the methanolic extract resulted in higher antioxidant activity than the aqueous extract. The DPPH and ABTS for the methanolic extract of *S. involucratus* were 21.09 and 89.62 mg Trolox equivalent/g dried extract, higher than those of the aqueous extract, with activities of 6.86 and 67.58 mg Trolox equivalent/g, respectively. It is widely known that antioxidant activity is mostly associated with the content of phenolic groups. In this study, we determined the major proportion of flavonoids in the extract (Table 2). These compounds could partly contribute to the antioxidant activity of the extract.

## 3. 3. Enzyme Inhibitory Activities of The Extract

The EtOAc extract of *S. thorelli* rhizomes exhibits moderate inhibition against AChE and  $\alpha$ -amylase with IC<sub>50</sub> values of 246.4  $\pm$  11.4  $\mu$ g/mL and 789.8  $\pm$  8.3  $\mu$ g/mL, respectively, as shown in Table 4. It can be observed that the AChE inhibitory activity is the strongest and approximately three times stronger than the  $\alpha$ -amylase inhibitory activity for this extract. Additionally, tests on  $\alpha$ -glucosidase and tyrosinase enzymes for the EtOAc extract of *S. thorelli* were also conducted but showed no activity on these tested enzymes.

**Table 3.** Antioxidant activity of the EtOAc extract of *Stahlianthus thorelii* rhizomes and ascorbic acid

Concentra	a-	DPPH		BTS	
tions,	Extract	Ascorbic acid		Ascorbic acid	
μg/mL Percentage of inhibition, %					
15.63	$16.3 \pm 0.6$	n.a.	n.i.	n.a.	
31.25	$21.6\pm1.6$	n.a.	n.i.	n.a.	
62.5	$40.5\pm1.4$	n.a.	n.i.	n.a.	
125	$64.7 \pm 0.9$	n.a.	$6.7 \pm 1.1$	n.a.	
250	$94.1 \pm 0.3$	n.a.	$17.8\pm1.3$	n.a.	
500	$95.5 \pm 0.6$	n.a.	$37.6 \pm 1.1$	n.a.	
1000	$96.0 \pm 0.6$	n.a.	$60.1 \pm 0.5$	n.a.	
$IC_{50}$ (µg/mL)86.9 ± 2.9 a10.1 ± 0.4 b743.6 ± 56.5 a 55.3 ± 2.4 b					

Values are shown as mean of triplicate experiments ± standard deviation. Different letters (a, b) indicate statistically significant differences in antioxidant activity between the extract and reference standard. n.a.: not available n.i.: no inhibition

In chemical analysis, major phenolic compounds were detected using HPLC-DAD analysis, specifically ferulic acid, catechin, epicatechin, EGCG, quercetin, and kaempferol, which may contribute significantly to the observed AChE and α-amylase inhibitory activities. Among the detected compounds, quercetin and catechin, known flavonoids found in Eugenia dysenterica, exhibit effective AChE inhibitory activity with IC<sub>50</sub> values of 46.6 μg/mL and 42.4 µg/mL, respectively, as reported previously by Gasca and colleagues.<sup>25</sup> Another flavonoid, EGCG, found in tea, demonstrates outstanding AChE inhibitory activity with an IC<sub>50</sub> value of 4.4 μg/mL.<sup>26</sup> Regarding α-amylase, kaempferol isolated from the leaves of Cassia bakeriana shows potent  $\alpha$ -amylase inhibitory activity with an IC<sub>50</sub> value of  $1.5 \pm 0.1 \,\mu\text{g/mL}$ . Additionally, ferulic acid has been reported to significantly inhibit  $\alpha$ -amylase with an  $IC_{50}$  value of 622 µg/mL. To our knowledge, there have been no reports on the anti-AChE and anti-α-amylase activities of Stahlianthus species extracts and chemical constituents. However, within the Zingiberaceae family, there are several reports demonstrating inhibitory activities. For example, extracts from two Aframomum species, A. melegueta and A. danielli show significant AChE activity with IC<sub>50</sub> values of 373.3 μg/mL and 417.1 μg/mL, respectively.<sup>28</sup> The dichloromethane extract of Curcuma aromatica exhibits notable  $\alpha$ -amylase inhibitory activity (IC<sub>50</sub> = 9.0 ± 0.3 μg/mL).<sup>29</sup> In summary, the EtOAc extract obtained from S. thorelli demonstrates significant AChE and α-amvlase inhibitory activities, and the main phenolics may play a crucial role in these enzyme inhibitory activities.

#### 4. Conclusion

The study e described the characteristics of the EtOAc extract of *Stahlianthus thorelii* concerning its chemical composition and biological activity. The non-volatile fraction of this extract was found to contain phenolic compounds such as ferulic acid, catechin, epicatechin, epigallocatechin gallate, quercetin, and kaempferol. The volatile fraction, on the other hand, was dominated by  $\beta$ -patchoulene, (E)-nerolidyl isobutyrate, and aristolene as major components. Additionally, the extract demonstrated

Table 4. Enzyme inhibition of the EtOAc extract of Stahlianthus thorelii rhizomes

Samples	IC <sub>50</sub> (μg/mL)			
	Anti-AChE activity	Anti-α-amylase activity	Anti-α-glucosidase activity	Anti-tyrosinase activity
EtOAc extract	246.4 ± 11.4 a	789.8 ± 8.3 a	n.a.	n.a.
Galantamine	$1.7 \pm 0.1 \text{ b}$	n.t.	n.t.	n.t.
Acarbose	n.t.	$88.8 \pm 1.8 \text{ b}$	$72.0 \pm 8.4$	n.t.
Kojic acid	n.t.	n.t.	n.t.	$62.0 \pm 1.3$

Values are shown as mean of triplicate experiments ± standard deviation. Different letters (a, b) indicate statistically significant differences in antioxidant activity between the extract and reference standards. n.a.: not available n.t.: not tested.

moderate antioxidant activity, as measured by DPPH and ABTS assays, and inhibitory effects on two enzymes, acetylcholinesterase (AChE) and  $\alpha$ -amylase. These findings suggest that *S. thorelii* could serve as a valuable source of bioactive phytochemicals and holds potential for further exploration as a therapeutic candidate for managing diabetes and Alzheimer's-related conditions in humans.

#### Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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### **Povzetek**

Stahlianthus thorelii Gagnep., ki se tradicionalno uporablja za zdravljenje različnih bolezni, je obetaven vir bioaktivnih spojin. V tej študiji smo proučevali etil acetatni izvleček koreninic *S. thorelii* z vidika fitokemikalij, antioksidativnih lastnosti in zaviralnih učinkov na ključne encime, povezane z diabetesom in Alzheimerjevo boleznijo. Izvleček je vseboval znatne količine fenolnih spojin, kot so ferulna kislina, katehin in kvercetin, njegove glavne hlapne sestavine pa so bile  $\beta$ -pačulen, (E)-nerolidil isobutirat in aristolen. Izvleček je imel močno antioksidativno aktivnost (DPPH IC50 = 86,9  $\mu$ g/ml) in je inhibiral encime, vključno z acetilholinesterazo (IC50 = 246,4  $\mu$ g/ml) in  $\alpha$ -amilazo (IC50 = 789,8  $\mu$ g/ml). Te ugotovitve poudarjajo potencial *S. thorelii* kot naravnega vira za razvoj terapevtikov, usmerjenih v nevrodegenerativne in presnovne motnje, ter upravičujejo nadaljnje raziskave njegove farmakološke uporabe.



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