Natural Product Sciences
30(4) : 300-303 (2024)
https://doi.org/10.20307/nps.2024.30.4.300

Sesquiterpenes from *Curcuma zedoaria* (Christm.) Rosc. Rhizomes and Their Alpha-Glucosidase Inhibitory Effects

Ngan Thi Tran[1,2], Phuong Nguyen Thi Thu[1,2], Mai-Ngan Thi Le[1], and Quynh-Mai Thi Ngo[1,2,*]

[1]*Faculty of Pharmacy, Haiphong University of Medicine and Pharmacy, Haiphong 180000, Vietnam*
[2]*Biomedical – Pharmaceutical Sciences Research Group, Hai Phong University of Medicine and Pharmacy, Haiphong 180000, Vietnam*

Abstract – *Curcuma zedoaria* (Christm.) Rosc. is a popular traditional herb to treat digestive disorders in Asian tropical countries. Previous studies indicated the presence of sesquiterpenoids, diterpenoids, and curcuminoids with various bioactivities. To enrich the phytocomposition data of this plant, this investigation was conducted. The dried rhizomes of *C. zedoaria* were collected in Hai Phong City (Vietnam), extracted with methanol and fractionated with *n*-hexane, CH_2Cl_2, and EtOAc. Compounds were isolated from *n*-hexane soluble fraction by open column chromatography combined with thin layer chromatography from fraction *n*-hexane. Their chemical structures were elucidated by 1D, and 2D NMR spectra and comparison with reported data. As a result, a phytochemical investigation was conducted to isolate six sesquiterpenes from *C. zedoaria*. Their chemical structures were elucidated to be curcumenol (**1**), procurcumenol (**2**), neoprocurcumenol (**3**), 13-hydroxygermacrone (**4**), zederone (**5**), and curcumalactone (**6**). Among isolated compounds, compounds **1**, **2**, **4**, and **5** were reported from *C. zedoaria*. Meanwhile, neoprocurcumenol (**3**) and curcumalactone (**6**) are isolated from this species for the first time. Compound **5** exhibited a mild inhibitory effect on α-glucosidase with an IC_{50} of 99.45 ± 0.50 μg/mL.
Keywords – *Curcuma zedoaria*, Sesquiterpene, Antidiabetic, Glucosidase

Introduction

Curcuma zedoaria (Christm.) Rosc. is a popular traditional herb in India, China and Vietnam. It is also widely distributed in other tropical countries, including Malaysia, Thailand, and India. In Vietnam, it is locally known as "Nga truật", an herb for treating digestive disorders, menstrual disorders, and cough, as well as a tonic.[1] In Indian Ayurveda and Chinese medicine, *C. zedoaria* has been used as an herbal remedy for a long time.[2] Previous studies indicated the presence of sesquiterpenoids, diterpenoids, and curcuminoids in *Curcuma* species. In *C. zedoaria*, the main component is sesquiterpenes with various bioactivities such as neuroprotective,[2] hepatoprotective,[3] antioxidant, and cytotoxic activities.[3–5]

Reducing carbohydrate absorption through the inhibition of α-glucosidase represents a pivotal mechanism employed by pharmaceutical agents targeting type 2 diabetes.[6] Various *Curcuma* species have been identified as possessing inhibitory activity against α-glucosidase. The rhizome extract derived from *Curcuma longa* demonstrated noteworthy α-glucosidase inhibitory activity with an IC_{50} of 17.1 μg/mL.[7] Additionally, compounds isolated from this species, as well as its volatile components, displayed potent inhibitory effects.[8–9] Another *Curcuma* species, *C. manga*, exhibited substantial α-glucosidase inhibitory effect through its fractions, with IC_{50} values ranging from 1.55 to 22.61 μg/mL.[10] In contrast, *C. zedoaria* has not been sufficiently studied for its α-glucosidase inhibitory potential. To identify potential candidates for the development of antidiabetic drugs from medicinal plants, the present study was conducted to isolate and identify organic compounds from *C. zedoaria* and evaluate their α-glucosidase inhibitory effects.

Experimental

General experiment procedures – The 1D and 2D NMR spectra were recorded using a Varian Unity Inova 400 MHz spectrometer with tetramethylsilane as an internal standard; the chemical shifts were recorded in δ values (ppm). Open column chromatography method was conducted by

*Author for correspondence
Quynh-Mai Thi Ngo, Ph. D., Faculty of Pharmacy, Haiphong University of Medicine and Pharmacy, Haiphong 180000, Vietnam
Tel: +84-369581891; E-mail: ntqmai@hpmu.edu.vn

silica gel (Merck, 63-200 μm) and RP-18 (Merck, 75 μm). Merck silica gel 60 F254 and RP-18 F254 plates were employed for TLC. All the chemicals and solvents utilized were of analytical grade quality.

Plant materials – The rhizomes of *C. zedoaria* (Christm.) Rosc. (Zingiberaceae) used in this study were collected in Haiphong City, Vietnam in December 2021. Botanists authenticated samples and a voucher specimen was deposited at the Herbarium of Pharmacognosy Department, College of Pharmacy, Haiphong University of Medicine and Pharmacy.

Extraction and isolation – The dried rhizomes of *C. zedoaria* (2.0 kg) were extracted three times with 5 L methanol in 3 h under reflux conditions. The solvent in the combined extract was removed by rotary evaporation *in vacuo*. The resulting extract (350 g) was suspended in a minimum amount of hot distilled water and then partitioned with *n*-hexane, CH_2Cl_2, and EtOAc. Open column chromatography (OCC) of dried *n*-hexane-soluble fraction (59.7 g) over silica gel eluted with a mobile phase of *n*-hexane:EtOAc (20:1 → 0:1) yielded 26 fractions (CZ1–CZ26). Subfraction CZ8 was subjected to silica gel OCC with a mixture of CH_2Cl_2:MeOH (20:1) to obtain **6** (3.3 mg). Fraction CZ16 was subjected to RP-18 silica gel OCC with a mobile solvent mixture of MeOH:H_2O (2:1) to obtain **5** (5.4 mg) and **1** (3.3 mg). Fraction CZ16 was subjected to RP-18 silica gel column, eluted with MeOH:H_2O (2:1) to obtain compounds **4** (3.2 mg) and **3** (11.9 mg) and 12 subfractions. Subsequently, CZ26.7 was further purified by silica gel OCC with a mixture of *n*-hexane:EtOAc (10:1) to yield compound **2** (5.0 mg).

Curcumenol (1) – Colourless gum; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 6.96 (1H, s, H-9), 3.87 (1H, d, J = 15.0 Hz, H-6a), 3.33 (1H, d, J = 15.0 Hz, H-6b), 3.03 (3H, s, CH_3-13), 2.87 (3H, s, CH_3-15), 2.82 s (3H, s, CH_3-12), 2.24 (3H, s, CH_3-14); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 51.2 (C-1), 27.5 (C-2), 31.2 (C-3), 40.4 (C-4), 85.8 (C-5), 37.2 (C-6), 138.6 (C-7), 101.5 (C-8), 126.2 (C-9), 137.0 (C-10), 122.0 (C-11), 22.3 (C-12), 18.9 (C-13), 11.9 (C-14), 21.0 (C-15).

Procurcumenol (2) – Colourless gum; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.88 (1H, dd, J = 2.7, 1.3 Hz, H-9), 2.61 (1H, d, J = 15.9, H-6a), 2.38 (1H, d, J = 10.6 Hz, H-1), 1.88 (3H, s, CH_3-15), 1.78 (3H, s, CH_3-13), 1.76 (3H, s, CH_3-12), 1.25 (3H, s, CH_3-14); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 50.7 (C-1), 27.1 (C-2), 40.1 (C-3), 80.5 (C-4), 54.8 (C-5), 28.8 (C-6), 136.9 (C-7), 199.3 (C-8), 129.4 (C-9), 155.1 (C-10), 136.5 (C-11), 21.5 (C-12), 22.6 (C-13), 24.5 (C-14), 23.6 (C-15).

Neoprocurcumenol (3) – Colourless gum; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.42 (1H, d, J = 15.4, H-9a), 2.89 (1H, d, J = 15.4, H-9b), 2.72 (1H, d, J = 13.2 Hz, H-3a), 2.20 (1H, m, H-3b), 1.87 (3H, s, CH_3-13), 1.77 (3H, s, CH_3-12), 1.63 (3H, s, H-15), 1.10 (3H, s, H-14); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 122.5 (C-1), 27.9 (C-2), 28.2 (C-3), 80.2 (C-4), 54.1 (C-5), 39.1 (C-6), 135.3 (C-7), 204.2 (C-8), 51.2 (C-9), 137.2 (C-10), 135.3 (C-11), 22.8 (C-12), 22.0 (C-13), 21.9 (C-14), 21.5 (C-15).

3-Hydroxygermacrone (4) – Colourless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.96 (1H, d, J = 12.3 Hz, H-1), 4.64 (1H, d, J = 11.6 Hz, H-5), 4.28 (1H, d, J = 12.2 Hz, H-13a), 4.16 (1H, d, J = 12.2 Hz, H-13b), 3.41 (1H, d, J = 10.5 Hz, H-9a), 2.95 (1H, d, J = 10.5 Hz, H-9b), 2.93 (2H, m, CH_2-6), 1.79 (3H, s, CH_3-12), 1.61 (3H, s, CH_3-15), 1.41 (3H, s, CH_3-14); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 133.3 (C-1), 24.3 (C-2), 38.3 (C-3), 136.0 (C-4), 125.2 (C-5), 28.8 (C-6), 131.5 (C-7), 207.3 (C-8), 55.8 (C-9), 126.6 (C-10), 140.1 (C-11), 18.0 (C-12), 63.1 (C-13), 15.8 (C-14), 16.9 (C-15).

Zederone (5) – Colourless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 7.05 (3H, brs, CH_3-12), 5.44 (1H, dd, J = 11.5, 2.6 Hz, H-1), 3.72 (1H, d, J = 16.5 Hz, H-9a), 3.65 (1H, d, J = 16.5 Hz, H-9b), 2.07 (3H, s, H-13), 1.56 (3H, s, H-15), 1.30 (3H, s, H-14); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 131.4 (C-1), 24.8 (C-2), 38.1 (C-3), 64.2 (C-4), 66.7 (C-5), 192.4 (C-6), 123.4 (C-7), 157.3 (C-8), 42.0 (C-9), 131.2 (C-10), 122.3 (C-11), 138.2 (C-12), 10.5 (C-13), 15.3 (C-14), 15.9 (C-15).

Curcumalactone (6) – Colourless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 4.99 (1H, s, H-9a), 4.91 (1H, s, H-9b), 1.80 (3H, s, CH_3-15), 0.99 (3H, d, J = 6.8 Hz, 13-OCH_3), 0.94 (3H, d, J = 6.9 Hz, CH_3-14), 0.90 (3H, d, J = 6.6 Hz, CH_3-12); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 53.7(C-1), 24.0 (C-2), 27.0 (C-3), 41.9 (C-4), 92.7 (C-5), 22.6 (C-6), 46.7 (C-7), 178.7 (C-8), 113.6 (C-9), 143.5 (C-10), 28.4 (C-11), 18.2 (C-12), 20.7 (C-13), 13.9 (C-14), 24.2 (C-15).

Anti-α-glucosidase assay – The α-glucosidase inhibitory activity was measured according to the method previously described by Kim et al.[11] The test sample was diluted with DMSO and deionized water into a series of concentrations. Acarbose was used as a positive control. The reaction mixture includes 25 μL *p*-nitrophenyl *α*-D-glucopyranoside 2.5 mM in 40 μL phosphate buffer 100 mM pH 6.8, 25 μL *α*-glucosidase 0.4 U/mL, and 10 μL test sample. The mixture was incubated at 37°C for 30 minutes and stopped with 100 μL 0.1 M Na_2CO_3. The absorbance of the reaction mixture was determined by a BIOTEK microplate absorbance reader at 405 nm. The IC_{50} value is defined as the concentration of sample that inhibits 50% of *α*-glucosidase activity.

Results and Discussion

From the *n*-hexane fraction of *C. zedoaria* rhizomes, six compounds (**1**–**6**) were isolated, and their structures were determined through spectroscopic analysis and comparison with previously reported (Fig. 1). These compounds were identified as curcumenol (**1**),[2] procurcumenol (**2**),[12] neo-procurcumenol (**3**),[13] 13-hydroxygermacrone (**4**),[14] zederone (**5**),[2] curcumalactone (**6**).[15] The isolated compounds are sesquiterpenes, including three guaiane, two germacrene, and one fused tricyclic β-lactones sesquiterpenes. Among these, compounds **1**,[16] **2**,[5] **4**,[17] and **5**[18] were previously reported from *C. zedoaria*. Notably, this study marked the first report of neoprocurcumenol (**3**), curcumalactone (**6**) from *C. zedoaria*. Specifically, compounds **3** was reported in *C. aromatic*,[12] and compound **6** was isolated from both *C. aromatica* and *C. wenyujin*.[13] These results show the close taxonomic relationship among species in the genus *Curcuma* from a phytochemical perspective. Sesquiterpene is the largest class of molecular found in *Curcuma* species with diverse structures. Sesquiterpenes display various bioactivities, including antibacterial and antifungal,[19] anticancer,[20] anti-inflammatory activities.[21] These findings have the potential for research and development of health products derived from this medicinal plant.

To assess the inhibitory potential of isolated compounds on α-glucosidase activity, the enzymatic hydrolysis of the substrate *p*-nitrophenyl α-D-glucopyranoside was quantified, measuring the production of glucose and *p*-nitrophenol. The obtained results (Table 1) revealed that out of the six examined compounds, only **5** displayed a noteworthy inhibitory effect, characterized by an IC_{50} value of 99.45 ± 0.50 μg/mL, whereas the remaining compounds exhibited either weak activity (compounds **2** and **6**) or negligible activity (compounds **1**, **3**, and **4**). Sesquiterpenes is a substantial and diverse category within natural chemistry with varied structural characteristics. They are prevalent in specific plant families, such as Asteraceae, Apiaceae, Illiciaceae, Magnoliaceae, Solanaceae, and Zingiberaceae. Numerous sesquiterpenes have demonstrated anti-diabetic effects, particularly those classified as sesquiterpene lactones.[22] Although compound **5** in this study does not possess a sesquiterpene lactone structure, its inhibitory activity may be attributed to its gemacrane structure. Certain gemacrane sesquiterpenes have been previously reported for their anti-hyperglycemic properties.[23] Several compounds derived from various *Curcuma* species have been reported to possess α-glucosidase inhibitory activity. Curcumin, a principal bioactive compound in *C. longa* and other *Curcuma* species, exhibited α-glucosidase inhibitory

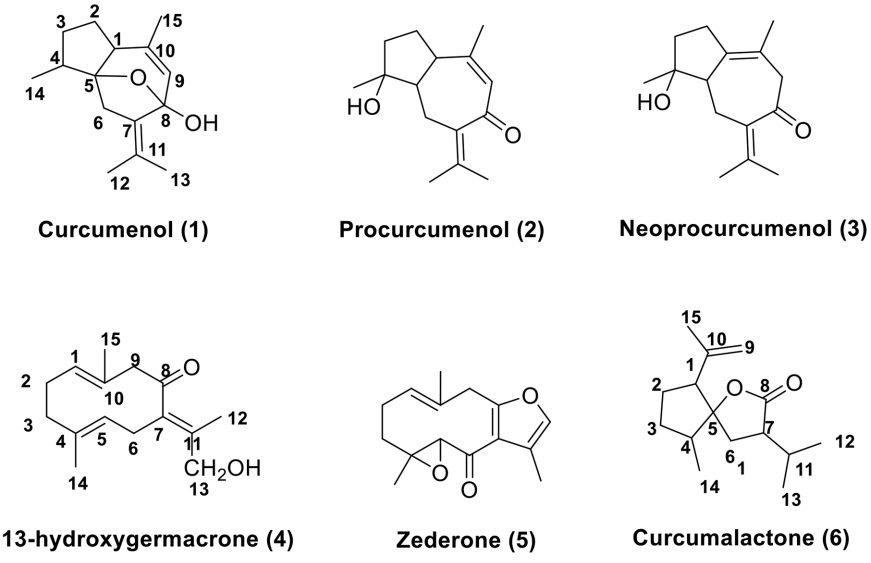

Fig. 1. Structures of sesquiterpenes from *C. zedoaria.*

Table 1. Anti-α-glucosidase activity of compounds (**1**–**6**)

Comps	**1**	**2**	**3**	**4**	**5**	**6**	Acarbose*
IC_{50} (μg/mL)	> 256	224.00 ± 6.55	> 256	> 256	99.45 ± 0.50	249.14 ± 8.03	156.16 ± 5.43

*Positive control.

effects in molecular docking studies as well as *in vitro* and *in vivo* assays.[9] Additionally, ar-turmerone, one of the three turmerones found in *Curcuma* species, has also shown significant alpha-glucosidase inhibitory activity, with an IC_{50} value of 0.28 μg/mL.[8]

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

(1) Đỗ, Tất Lợi; *Những cây thuốc và vị thuốc Việt Nam*; Nhà xuất bản Y Học. Vietnam, **2004**, pp 377–378.
(2) Hamdi, O. A. A.; Ye, L. J.; Kamarudin, M. N. A.; Hazni, H.; Paydar, M.; Looi, C. Y.; Shilpi, J. A.; Kadir, H. A.; Awang, K. *Rec. Nat. Prod.* **2015**, *9*, 349–355.
(3) Cui, H.; Zhang, B.; Li, G.; Li, L.; Chen, H.; Qi, J.; Liu, W.; Chen, J.; Wang, P.; Lei, H. *Molecules* **2019**, *24*, 2073.
(4) Lee, T. K.; Lee, D.; Lee, S. R.; Ko, Y.-J.; Kang, K. S.; Chung, S. J.; Kim, K. H. *Bioorg. Chem.* **2019**, *87*, 117–122.
(5) Ahmed Hamdi, O. A.; Syed Abdul Rahman, S. N.; Awang, K.; Abdul Wahab, N.; Looi, C. Y.; Thomas, N. F.; Malek, S. N. A.; *ScientificWorldJournal* **2014**, *2014*, 321943.
(6) Hakamata, W.; Kurihara, M.; Okuda, H.; Nishio, T.; Oku, T. *Curr. Top. Med. Chem.* **2009**, *9*, 3–12.
(7) Lekshmi, P. C.; Arimboor, R.; Nisha, V. M.; Menon, A. N.; Raghu, K. G. *J. Food Sci. Technol.* **2014**, *51*, 3910–3917.
(8) Lekshmi, P. C.; Arimboor, R.; Indulekha, P. S.; Nirmala Menon, A. *Int. J. Food Sci. Nut.* **2012**, *63*, 832–834.
(9) Cao, W.; Chen, X.; Chin, Y.; Zheng, J.; Lim, P. E.; Xue, C.; Tang, Q. *J. Food Biochem.* **2022**, *46*, e13686.
(10) Awin, T.; Mediani, A.; Mohd Faudzi, S. M.; Maulidiani; Leong, S. W.; Shaari, K.; Abas, F. *Int. J. Food Prop.* **2020**, *23*, 154–166.
(11) Kim, Y.-M.; Wang, M.-H.; Rhee, H.-I. *Carbohydr. Res.* **2004**, *339*, 715–717.
(12) Kuroyanagi, M.; Ueno, A.; Ujiie, K.; Sato, S. *Chem. Pharm. Bull.* **1987**, *35*, 53–59.
(13) Gao, S.; Xia, G.; Wang, L.; Zhou, L.; Zhao, F.; Huang, J.; Chen, L. *Nat. Prod. Res.* **2017**, *31*, 548–554.
(14) Park, J.-H.; Mohamed, M. A. A.; Jung, Y.-J.; Shrestha, S.; Lee, T. H.; Lee, C.-H.; Han, D.; Kim, J.; Baek, N.-I. *Arch. Pharm. Res.* **2015**, *38*, 1752–1760.
(15) Qin, B.; Li, Y.; Meng, L.; Ouyang, J.; Jin, D.; Wu, L.; Zhang, X.; Jia, Z.; You, S. *J. Nat. Prod.* **2015**, *78*, 272–278.
(16) Lo, J. Y.; Kamarudin, M. N. A.; Hamdi, O. A. A.; Awang, K.; Kadir, H. A. *Food Funct.* **2015**, *6*, 3550–3559.
(17) Shiobara, Y.; Asakawa, Y.; Kodama, M.; Takemoto, T. *Phytochemistry* **1986**, *25*, 1351–1353.
(18) Makabe, H.; Maru, N.; Kuwabara, A.; Kamo, T.; Hirota, M. *Nat. Prod. Res.* **2006**, *20*, 680–685.
(19) Huang, H.-F.; Zheng, C.-J.; Chen, G.-Y.; Yin, W.-Q.; Huang, X.; Mo, Z.-R. *Chin. Chem. Lett.* **2016**, *27*, 1612–1616.
(20) Wu, P.; Dong, X.-M.; Song, G.-Q.; Wei, M.-M.; Fang, C.; Zheng, F.-B.; Zhao, Y.-J.; Lu, H.-Q.; Cheng, L.-H. Zhou, J.-L.; Xie, T. *Phytomedicine* **2021**, *86*, 153559.
(21) Dong, J.; Shao, W.; Yan, P.; Cai, X.; Fang, L.; Zhao, X.; Lin, W.; Cai, Y. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 198–202.
(22) Chen, L.; Lu, X.; El-Seedi, H.; Teng, H. *Trends Food Sci. Technol.* **2019**, *88*, 46–56.
(23) Zhao, G.; Li, X.; Chen, W.; Xi, Z.; Sun, L. *Fitoterapia* **2012**, *83*, 1590–1597.

Received August 27, 2024
Revised December 12, 2024
Accepted December 18, 2024