Table 1. The ^{1}H NMR (600 MHz) and ^{13}C NMR data (150 MHz) of compound **1** in methanol-d_4

Position	$^1\delta_C$	1	
		δ_C	δ_H (mult., J in Hz)
1, 1'	138.6	138.5	-
2, 2'	129.3	129.2	6.94 (d, 9.0)
3, 3'	116.1	116.0	6.67 (d, 9.0)
4, 4'	156.3	156.3	-
5, 5'	116.1	116.0	6.67 (d, 9.0)
6, 6'	129.3	129.2	6.94 (d, 9.0)
7, 7'	54.9	54.7	4.49 s
8, 8'	61.1	61.0	3.75 s
9, 9'	150.9	150.8	-
10, 10'	123.9	123.8	-
11, 11'	155.7	155.5	-
12, 12'	102.6	102.6	6.13 (d, 1.8)
13, 13'	159.5	159.3	-
14, 14'	103.1	103.4	6.54 (d, 1.8)

$^1\delta_C$ of pallidol measured in methanol-d_4 [6]

4. Conclusion

A chemical study of the methanol extract of *C. cheniana* stems and leaves obtained seven compounds, including pallidol (**1**), *trans*-resveratrol (**2**), piceid (**3**), apigenin (**4**), acacetin (**5**), apigenin 7-*O*-*β*-D-glucopyranoside (**6**), and luteolin 7-*O*-*β*-D-glucopyranoside (**7**). This is the first report on the presence of stilbene and flavonoid derivatives from *C. cheniana*. Our results contribute to understanding secondary metabolites produced by *C. cheniana*.

AcknowledgmentsL *This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2019.310.*

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Journal of Medicinal Materials, 2024, Vol. 29, No. 2 (pp.70 - 76)

CHEMICAL CONSTITUENTS AND CYTOTOXIC ACTIVITY OF THE *n*-HEXANE EXTRACT FROM THE AERIAL PARTS OF *CURCUMA ZEDOAROIDES* CHAVEER. & TANEE

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(Received March 12th, 2024)

Summary

Chemical Constituents and Cytotoxic Activity of the *n*-Hexane Extract from the Aerial Parts of *Curcuma zedoaroides* Chaveer. & Tanee

Curcuma zedoaroides Chaveer. & Tanee, belonging to the gennus *Curcuma* (Zingiberaceae), is a newly discovered species in Vietnam. In the present study, the chemical constituents and the *in vitro* cytotoxic activity of the *n*-hexane extract from the *C. zedoaroides* aerial parts were reported for the first time. The *n*-hexane extract showed the most potent cytotoxic effect among all tested extracts on seven human cancer cell lines (A549, MCF-7, HepG2, HT-29, HL-60, K562, and MDA-MB-231), with IC_{50} values ranging from 49.76 – 86.30 μg/mL using sulforhodamine B and MTT methods. Gas

chromatography-mass spectrometry (GC-MS) analysis revealed that a total of 10 components were identified from the *n*-hexane extract. Among these, curdione (a major compound, accounting for 41.12% of total content) was isolated from *C. zedoaroides* for the first time. Its structure was determined by 1D-, 2D-NMR, and MS spectroscopic techniques and compared with the previous publication. This study provides significant data on the chemical constituents and cytotoxic effects of the *n*-hexane extract of *C. zedoaroides* aerial parts. These findings suggest that *C. zedoaroides* extract can be exploited for its potential cytotoxic effect.

Keywords: *Curcuma zedoaroides, GC-MS, Cytotoxic activity, Curdione.*

1. Introduction

Curcuma L. is a large genus in the family Zingiberaceae with about 130 recognized species [1], predominantly distributed in the tropical and subtropical regions of South and Southeast Asia [2]. This genus has long been used in traditional medicine to address stomach issues, promote digestion, and safeguard digestive organs, including the intestines, stomach, and liver [2]. Many previous studies have revealed that *Curcuma* species contains various compounds such as diphenylalkanoids, phenylpropene derivatives, terpenoids, flavonoids, steroids, alkaloids, and others [2]. Moreover, the extracts and isolated compounds from this genus have been found to possess anti-cancer, anti-inflammatory, antioxidant, anti-diabetic, antiviral, hepatoprotective, anti-allergic properties, etc [2].

Currently, there are 27 recognized species of *Curcuma* found throughout Vietnam [3],[4]. Among these, *C. zedoaroides* was recently discovered in Thai Nguyen Province, Vietnam [5]. Traditionally, the villagers in Ban Khok Sa Nga village, Sai Moon Sub-district, KhonKaen Province, Thailand have used this plant as an antidote for snake bites [6]. In a previous study, seven compounds were identified from the bioactive chloroform extract of *C. zedoaroides* rhizomes. Furthermore, their anti-inflammatory activity, specifically focusing on the production of NO and TNF-α using RAW264.7 cells, was also reported [7].

In our ongoing research project, "Study on anti-cancer and immune-modulation of some Vietnamese medicinal plants", the rhizomes of *C. zedoaroides* were investigated to find potential anti-cancer agents [8]. This study resulted led to the isolation of 10 sesquiterpenes and 2 diterpenes for the first time. Additionally, the fractionated extracts of *C. zedoaroides* rhizomes (*n*-hexane, ethyl acetate, and water) displayed considerable activity on eight cancer cell lines (A549, MCF-7, HT-29, MB49, HepG2, MDA-MB-231, JB6-C141, and K562), with IC_{50} values ranging from 5.43 to 11.96 μg/mL. These components exhibited significant effects against five cancer cell lines (A549, MCF-7, MDA-MB-231, HL-60, and HepG2), with IC_{50} values ranging from 3.13 μM to 30.10 μM. Besides, the chemical constituents and the *in vitro* cytotoxic activity of the essential oil from *C. zedoaroides* rhizomes were studied [9]. The result revealed the essential oil of *C. zedoaroides* rhizomes was oxygenated sesquiterpenes (61.54%) with curdione (27.45%) being the most major component, followed by widdrol (8.03%), and *trans*-β-elemenone (5.47%). Notably, the essential oil of *C. zedoaroides* rhizomes exhibited cytotoxic activities against seven cancer cell lines (A549, MCF-7, HepG2, HT29, HL-60, K562, and MDA-MB-231), with IC_{50} values of 23.14 – 83.67 μg/mL.

Up to now, there have been no reports about the constituents and bioactivities of the aerial parts of *C. zedoaroides*. In the present study, we focus on providing the scientific report for the *in vitro* cytotoxic activities and the chemical constituents of *C. zedoaroides* aerial parts for the first time.

2. Materials and methods

2.1. Plant materials

Curcuma zedoaroides Chaveer. & Tanee was collected from Dong Hy District, Thai Nguyen Province, Vietnam, in August 2020. The plant was identified by botanists, MSc. Nguyen Quynh Nga and MSc. Nguyen Van Hieu (the Medicinal Material Resources Center at NIMM). A voucher specimen (DL-120820) was deposited at the Herbarium of NIMM.

2.2. General experimental procedures

NMR spectra were recorded on Bruker AM 500 and 600 FT-NMR spectrometers (Karlsruhe, Germany) with TMS as the internal standard, *J* in Hz. MS data were analyzed with an LC-MS/MS and detector DAD (Shimadzu, Japan). The optical rotation values were recorded with an MCP-100 polarimeter (Anton Paar, Austria). *Silica gel* (Merck, 0.040 - 0.063 mm particle size) was used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ (Merck, *silica gel*, 0.25 mm) and RP-$18F_{254}$ (Merck, 0.25 mm) plates. Spots were detected under UV 254 and 366 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dried sample (4.0 kg of the aerial parts) was well crushed and extracted with 70% ethanol at room temperature (10 L × 3 times). The resulting extracts were combined, filtered, and evaporated under reduced pressure, yielding an ethanol residue (360.0 g). This residue was then suspended in water and partitioned using organic solvents of increasing polarity, resulting in obtaining *n*-hexane (80.6 g), ethyl acetate (100.1 g), and aqueous (150.0 g) extracts.

The *n*-hexane extract (80.0 g) was fractionated using *silica gel* column chromatography (CC) and eluted with *n*-hexane – acetone solvent system (100:0 – 1:1, v/v) to yield 6 fractions (H1 – H6). Fraction H6 (2.0 g) was subsequently purified *via silica gel* CC and eluted with *n*-hexane – acetone (50:1 – 20:1, v/v) to give compound **I** (120 mg).

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical constituents of the *n*-hexane extract of *C. zedoaroides* aerial parts were analyzed using a Gas Chromatograph (7890B GC) coupled with a Mass Selective Detector (5977B MSD), as reported in previous study [10]. The GC column was an HP-5MS UI (30 m × 0.25 mm i.d. × 0.25 µm film thickness, Agilent Technologies). Helium was used as the carrier gas at a flow rate of 1.0 mL/min, with an injection volume of 1.0 µL (split ratio of 25:1). The Inlet-F temperature was maintained at 300°C, while the MS Quad temperature was set at 50°C and the MS source at 230°C. The GC oven temperature began at 60°C for 1 min, then increased at a rate of 4°C/min to 240°C, and held for 4 min. Mass spectrometry was conducted across the range of 50 – 550 amu (2 scans/s) with an ionization energy of 70 eV. By comparing the mass spectra to the literature (NIST17 and Adams's book) [11], the constituents were identified and the retention index was calculated using a standard solution of C7 - C30 alkanes (Merck). The GC peak area was used to calculate the relative quantities of each component, without correction.

2.5. In vitro cytotoxic activity assay

Seven human cancer cell lines, including A549 (human lung carcinoma), MCF-7 (human breast carcinoma), HepG2 (human hepatocellular carcinoma), HT-29 (human colon adenocarcinoma), HL-60 (human leukemia), K562 (human chronic myelogenous leukemia), and MDA-MB-231 (human breast carcinoma), were used for cytotoxic evaluation. The cancer cell lines were cultured following the standard guidelines of the American Type Culture Collection.

The cytotoxic activity of extracts against two cancer cell lines (HL-60 and K562) was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, following established protocols [12]. In summary, cells were seeded at a density of 1×10^4 cells/well in 96-well plates and then cultured at 37°C with 5% CO_2 for 24 hours. Following this, the cells were treated to varying concentrations of extracts for 72 hours under the same conditions. After the incubation period, MTT solution (10 µL, final concentration 5 mg/mL) was added to each well, and the cells were incubated for an additional 4 hours at 37°C with 5% CO_2. Subsequently, formazan crystals formed were dissolved in dimethyl sulfoxide (DMSO), and the optical density (OD) values were measured at a wavelength of 540 nm using an ELISA Plate Reader (BioTek, Winooski, VT, USA). Ellipticine was used as a positive control. The inhibition percentage of cell growth in the presence of the treated sample was determined by the following formula: (%) inhibition = 100% - $[(OD_{sample} - OD_{blank})/ (OD_{DMSO} - OD_{blank})] \times 100$.

The cytotoxic activity of extracts against five cancer cell lines (A549, MCF-7, HepG2, HT-29, and MDA-MB-231) was assessed using the sulforhodamine B (SRB) assay, following a previously established procedure [13]. This assay aimed to determine cell density by quantifying the total cellular protein content, which was stained with SRB. In brief, trypsinized cells were seeded in a 96-well plate and treated with the samples for 72 hours. Negative control wells contained cells treated with a diluted solution. After the incubation period, cells were fixed with trichloroacetic acid (TCA) for 1 hour, followed by staining with SRB for 30 minutes at 37°C. Subsequently, the plates were washed three times with acetic acid to remove any nonstaining dye before being air-dried at room temperature. The SRB-stained protein within the cells was then dissolved in 10 mM unbuffered Tris base, and the plate was gently shaken for 10 minutes at room temperature. The OD at 540 nm was measured using an ELISA Plate Reader (BioTek, Winooski, VT, USA). Ellipticine was also used as a positive control. The percentage of inhibition in cell growth in the presence of the treated sample was calculated using the following formula: (%) inhibition = 100% - $[(OD_{sample} - OD_{day\ 0})/ (OD_{DMSO} - OD_{day\ 0})] \times 100$.

2.6. Data analysis

The experimental data was analyzed and recorded. Each experiment was conducted in triplicates, with findings presented as the mean value ± standard deviation (SD), and calculated using Microsoft Excel 2023 software. The IC_{50} value was determined using TableCurve 2Dv4 software.

3. Results and discussion

3.1. Cytotoxic activity of the extracts

The results revealed that the *n*-hexane extract exhibits the strongest cytotoxic activity among all tested cell lines, with IC_{50} values ranging from 49.76 to 86.30 µg/mL, corresponding to IC_{50} for HT-29 (49.76 ± 2.35 µg/mL), K562 (53.42 ± 2.28 µg/mL), MCF-7 (54.71 ± 4.45 µg/mL), MDA-MB-231 (60.12 ± 5.21 µg/mL), HepG2 (65.09 ± 4.01 µg/mL), HL-60 (65.14 ± 2.68 µg/mL), and A549 (86.30 ± 5.49 µg/mL) (**Table 1**). The ethyl acetate extract showed weak activity against the HT-29 and HepG2 cell lines, with IC_{50} values of 76.75 ± 4.95 and 78.94 ± 4.13 µg/mL, respectively. Moreover, the total extract displayed weak activity against the MCF-7 (IC_{50} 68.41 ± 5.24 µg/mL), HT-29 (IC_{50} 79.02 ± 3.11 µg/mL), and MDA-MB-231 (IC_{50} 84.89 ± 4.57 µg/mL) cell lines. In contrast, the aqueous extract showed no significant activity against the tested cell lines (IC_{50} > 100 µg/mL). Consequently, the *n*-hexane extract was selected for further investigation into its chemical constituents.

Table 1. Cytotoxicity of the extracts from the aerial parts of *C. zedoaroides*

Samples	IC_{50} values (µg/mL)						
	A549	MCF-7	HepG2	HT-29	HL-60	K562	MDA-MB-231
Ethanol extract	> 100	68.41 ± 5.24	> 100	79.02 ± 3.11	> 100	> 100	84.89 ± 4.57
***n*-Hexane extract**	**86.30 ± 5.49**	**54.71 ± 4.45**	**65.09 ± 4.01**	**49.76 ± 2.35**	**65.14 ± 2.68**	**53.42 ± 2.28**	**60.12 ± 5.21**
Ethyl acetate extract	> 100	> 100	78.94 ± 4.13	76.75 ± 4.95	> 100	> 100	> 100
Water extract	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Ellipticine	0.37 ± 0.03	0.44 ± 0.02	0.34 ± 0.03	0.45 ± 0.02	0.37 ± 0.02	0.40 ± 0.02	0.36 ± 0.02

3.2. Chemical constituents of the n-hexane extract

The GC-MS analysis revealed the presence of 10 components in the *n*-hexane extract of the *C. zedoaroides* aerial parts (**Fig. 1**, **Table 2**), of which curdione (41.12%) and curcumenone (9.93%) were identified as major volatile compounds. Other components were detected at lower level, including phytol (4.15%), isoprocurcumenol (2.89%), cedr-8-en-13-ol (2.67%), endo-borneol (2.29%), humulene epoxide II (2.00%), neophytadiene (1.91%), isoborneol (1.71%), and neocurdione (1.13%). Notably, there were six unknown components found in the *n*-hexane extract of the *C. zedoaroides* aerial parts *via* GC-MS analysis.

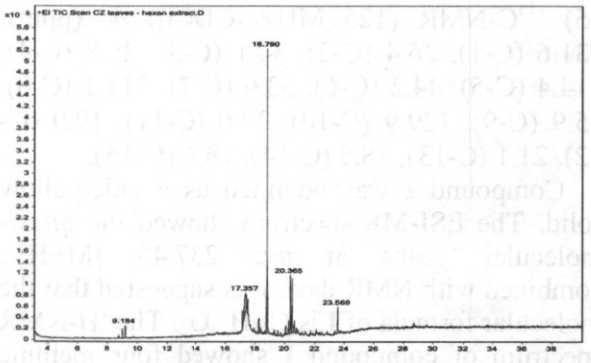

Fig.1. GC chromatogram of the *n*-hexane extract of the *C. zedoaroides* aerial parts

Table 2. Chemical constituents of the *n*-hexane extract of the *C. zedoaroides* aerial parts

No.	RT (min)	Compounds	Formula	RI (cal.)	RI (lit.)	Area Sum %	Identification method
1	8.987	Isoborneol	$C_{10}H_{18}O$	1164	1157	1.71	MS, RI
2	9.184	endo-Borneol	$C_{10}H_{18}O$	1172	1167	2.29	MS, RI
3	17.133	Humulene epoxide II	$C_{15}H_{24}O$	1614	1608	2.00	MS, RI
4	17.357	Unknown	-	1630	-	14.59	
5	17.493	Cedr-8-en-13-ol	$C_{15}H_{24}O$	1693	1688	2.67	MS, RI
6	17.900	Unknown	-	1668	-	1.58	
7	18.246	Unknown	-	1691	-	1.79	
8	18.369	Unknown	-	1700	-	0.84	
9	18.79	Curdione	$C_{15}H_{24}O_2$	1731	1729	41.12	MS, RI, Co
10	19.278	Neocurdione	$C_{15}H_{24}O_2$	1768	1762	1.13	MS, RI
11	20.052	Unknown	-	1826	-	1.29	
12	20.242	Neophytadiene	$C_{20}H_{38}$	1841	1837	1.91	MS, RI
13	20.365	Curcumenone	$C_{15}H_{22}O_2$	1851	1844	9.93	MS, RI

No.	RT (min)	Compounds	Formula	RI (cal.)	RI (lit.)	Area Sum %	Identification method
14	20.487	Unknown	-	1860	-	10.09	
15	20.636	Isoprocurcumenol	$C_{15}H_{22}O_2$	1872		2.89	MS, Co
16	23.569	Phytol	$C_{20}H_{40}O$	2115	2114	4.15	MS, RI
		Total				**99.98**	

Abbreviations: RT: Retention time, MS: Mass spectrum; Co: Co-injection with authentic compounds; RI (cal.): Retention indices obtained in HP-5MS UI column; RI (lit.): Retention indices obtained from the literature.

3.3. Isolation of major compound from the n-hexane extract

The findings in Section 3.2 indicated that curdione (compound **I**) is the primary constituent in the *n*-hexane extract. As a result, compound **I** was isolated using the CC method. The spectral data for compound **I** is as follows:

Curdione (compound **I**): pale-yellow solid; $[\alpha]_D^{20}$ +25.5 (*c* 2.0, $CHCl_3$); ESI-MS: *m/z* = 237.45 $[M+H]^+$; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): 5.10 (1H, br s, H-1), 2.08-2.16 (3H, m, H-2, H-3), 1.57 (1H, m, H-3), 2.34 (1H, m, H-4), 2.39 (1H, dd, *J* = 2.4, 16.8 Hz, H-6), 2.70 (1H, m, H-6), 2.85 (1H, ddd, *J* = 1.8, 9.0, 9.0 Hz, H-7), 3.06 (1H, d, *J* = 10.8 Hz, H-9), 2.93 (1H, d, *J* = 10.8 Hz, H-9), 1.88 (1H, m, H-11), 0.95 (3H, d, *J* = 6.6 Hz, H-12), 0.88 (3H, d, *J* = 6.6 Hz, H-13), 0.98 (3H, d, *J* = 7.2 Hz, H-14), 1.66 (3H, s, H-15); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C (ppm): 131.6 (C-1), 26.4 (C-2), 34.1 (C-3), 46.8 (C-4), 214.4 (C-5), 44.2 (C-6), 53.6 (C-7), 211.1 (C-8), 55.9 (C-9), 129.9 (C-10), 30.0 (C-11), 19.9 (C-12), 21.1 (C-13), 18.5 (C-14), 16.6 (C-15).

Compound **I** was obtained as a pale-yellow solid. The ESI-MS spectrum showed the *quasi*-molecular peaks at *m/z* 237.45 $[M+H]^+$; combined with NMR data, it is suggested that the molecular formula of **I** is $C_{15}H_{24}O_2$. The ^{1}H-NMR spectrum of compound **I** showed four methine (CH) groups with a vinylic methine signal at δ_H 5.10 (1H, br s, H-1) and three proton signals at δ_H 2.85 (1H, ddd, *J* = 1.8, 9.0, 9.0 Hz, H-7), 2.34 (1H, m, H-4), and 1.88 (1H, m, H-11). Additionally, eight proton signals of four methylene (CH_2) group were observed at δ_H 3.06 (1H, d, *J* = 10.8 Hz, H-9), 2.93 (1H, d, *J* = 10.8 Hz, H-9), 2.70 (1H, m, H-6), 2.39 (1H, dd, *J* = 2.4, 16.8 Hz, H-6), 2.08-2.16 (3H, m, H-2, H-3), and 1.57 (1H, m, H-3). Furthermore, four methyl (CH_3) groups were identified with signals at δ_H 1.66 (3H, s, H-15), 0.98 (3H, d, *J* = 7.2 Hz, H-14), 0.95 (3H, d, *J* = 6.6 Hz, H-12), and 0.88 (3H, d, *J* = 6.6 Hz, H-13). The ^{13}C-NMR and HSQC spectra analysis of compound **I** revealed two quaternary carbon signals attributed to two ketone groups (C=O) at δ_C 214.4 (C-5) and 211.1 (C-8), one quaternary carbon signal from a vinylic group (C=C) at 129.9 (C-10), four methine carbon signals (CH) at δ_C 131.6 (C-1), 53.6 (C-7), 46.8 (C-4), and 30.0 (C-11). Additionally, four carbon signals corresponding to the methylene group were observed at δ_C 55.9 (C-9), 44.2 (C-6), 34.1 (C-3), and 26.4 (C-2), along with four methyl groups at δ_C 16.6 (C-15), 18.5 (C-14), 19.9 (C-12), and 21.1 (C-13). The presence of 15 carbon signals indicates the sesquiterpenoid class [14]. The ^{1}H-^{1}H COSY spectrum showed a set of cross-peaks between protons H-6 (δ_H 2.70) /H-7 (δ_H 2.85)/H-11 (δ_H 1.88)/H-12 (δ_H 0.95) and H-11/H-13 (δ_H 0.88), indicating the position of the isopropyl fragment. The HMBC correlation between H-15 (δ_H 1.66) and C-1 (δ_C 131.6), C-9 (δ_C 55.9), and C-10 (δ_C 129.9), along with a set of ^{1}H-^{1}H COSY cross-peaks H-1 (δ_H 5.10) /H-2, H-3 (δ_H 2.08-2.16)/H-4 (δ_H 2.34)/H-14 (δ_H 0.98), identified the position of the alkene group (**Fig. 2**). Additionally, the HMBC correlations between C-5 (δ_C 214.4) and H-3 (δ_H 1.57), H-7 (δ_H 2.85), H-14 (δ_H 0.98), as well as between C-8 (δ_C 211.1) and H-7, H-9 (δ_H 2.93 and 3.06), H-11 (δ_H 1.88), determined the location of the ketone groups. According to Zhang et al. [15], compound **I** also has a C-C conformation with a *trans* relationship (*E*) between the C-4 methyl and C-7 isopropyl side chains. Furthermore, this conformation is typical of the syn-arrangement between the C-5 carbonyl and C-1 methine [16]. Based on the ESI-MS, NMR data, and comparison of the literature data, compound **I** was identified as curdione [14].

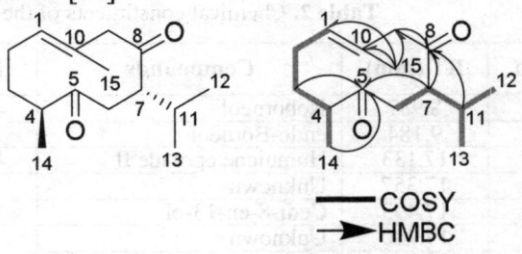

Fig.2. The structure and key HMBC, COSY correlations of curdione

Discussion

Curcuma zedoaroides is a newly discovered species by our research group in Vietnam [5]. To date, minimal research has been conducted on

this species. However, previous studies have suggested that *Curcuma* species may possess potential anti-cancer properties [17]. Therefore, this species was screened for cytotoxic effects against cancer cell lines, and chemical constituents were analyzed using GC-MS. The results indicated that the *n*-hexane extract exhibited greater activity compared to other extracts on all cell lines, consistent with the pattern observed with the rhizome [8]. Consequently, we identified the volatile components of the *n*-hexane extract.

The findings revealed that this extract from the aerial parts contains a smaller number of ingredients in comparison to the rhizomes. This disparity in composition may account for the notably weaker bioactivity of the *n*-hexane extract derived from the aerial parts compared to that from the rhizome.

Several components identified in the *n*-hexane extract from the aerial parts were similar to those present in the rhizomes, such as curdione, neocurdione, curcumenone, and isoprocurcumenol [8], which are also present in other species of the genus *Curcuma* L. [9]. Of these, curdione was the main component, consistent with its primary presence in the essential oil from *C. zedoaroides* rhizomes [9]. Curdione inhibited the proliferation of MDA-MB-231 cells, potentially through cell cycle arrest and apoptosis induction [18]. Additionally, curdione has been found to inhibit the migration and invasion of human breast cancer HCC1937 cells. This effect may be attributed to the down-regulation of phosphorylation levels of key proteins such as ERK, JNK, and Akt on MAPK and Akt signaling pathways, leading to decreased expressions of MMP2 and MMP-9 [19]. Moreover, curdione has shown effectiveness in suppressing the proliferation of MCF-7 cells by inducing apoptosis [20]. Besides, phytol [21] and isoprocurcumenol [8] have shown significant anti-cancer activity, suggesting their potential as active components within the *n*-hexane extract.

4. Conclusion

This study presents the first report on the cytotoxic activity and chemical constituents of the aerial parts of *C. zedoaroides*. Among the tested extracts, the *n*-hexane extract exhibited the most potent cytotoxic activity against all cell lines (A549, MCF-7, HepG2, HT-29, HL-60, K562, and MDA-MB-231), with IC_{50} values ranging from 49.76 to 86.30 µg/mL. Analysis of the volatile components of this extract revealed the presence of 10 identified compounds, with curdione represented as the major component, constituting the highest concentration (41.12%). Curdione was isolated and structurally determined using ESI-MS and NMR data. Based on our findings, this component is a potential candidate for the active principle in the aerial parts of *C. zedoaroides*.

Acknowledgment: *This research was a part of the research project "Study on anticancer and immune-modulation of some Vietnamese medicinal plants", code NĐT.85.KR/20. Nguyen Thi Thu was funded by the Master, PhD Scholarship Programme of Vingroup Innovation Foundation (VINIF), code VINIF.2023.TS.123.*

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Journal of Medicinal Materials, 2024, Vol. 29, No. 2 (pp. 76 - 80)

CHEMICAL CONSTITUENTS FROM THE LEAVES OF *ARDISIA GIGANTIFOLIA*

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(Received March 03rd, 2024)

Summary

Chemical Constituents from the Leaves of *Ardisia gigantifolia*

Phytochemical investigation of *Ardisia gigantifolia* Stapf leaves led to the isolation of five compounds, including nicotiflorin (**1**), rutin (**2**), 4'-*O*-methyl-myricetin 3-*O*-rutinoside (**3**), (*R*)-methyl mandelate (**4**), and (2*S*)-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-3-*O*-*β*-D-galactopyranosyl glycerol (**5**). Their chemical structures were elucidated by the analysis of NMR and MS data, compared with the previously reported data. This is the first time to report the existence of compounds **1 - 5** from the genus *Ardisia*.

Keywords: *Ardisia gigantifolia* Stapf *leaves, Flavonoids, Phenolic, Glycoglycerolipid.*

1. Introduction

The *Ardisia*, one of the largest genera in the family Myrsinaceae with approximately 732 species of evergreen shrubs and trees are distributed throughout the tropical and subtropical regions of the world, such as East and Southeast Asia, America, and Australia [1]. Many *Ardisia* species: *A. cornudetata*, *A. crenata*, *A. crispa*, *A. crassa*, *A. japonica*,... have been used for the treatment of liver disease, cough, diarrhea, anti-inflammatory/analgesic diseases, fracture coalescence, rheumatism, cancer, among others...[2]. The phytochemical investigations of *Ardisia* species indicate the discovery of flavonoids, triterpenoids [3],[4],[5],[6],[7],[8], quinones, coumarins, and phenolic compounds [8]. Pharmacological studies reported that the extracts and pure compounds display a wide range of bioactive effects: anti-inflammatory, antitumor, antioxidant, anticancer, antibacterial, and antidiabetic activities [8]...

In the ongoing research to discover new naturally occurring compounds for treating peptic ulcer diseases from medicinal plants in Vietnam, we are interested in *A. gigantifolia*, known as Khoi tia or Khoi nhung (in Vietnam). *A. gigantifolia* is used in traditional Vietnam medicine for colic and stomachache, however, as far as we know, most of the studies have focused on the chemical constituents of the rhizomes or roots and only a limited number of phytochemical studies about the leaves of this plant have been investigated or described in detail in the literature [9],[10],[11],[12],[13],[14]. Therefore, in this study, the chemical constituents from the leaves of *A. gigantifolia* were conducted and resulted in the isolation and structural elucidation of five compounds, including three flavonol glycosides (**1 - 3**), a phenolic compound (**4**), and a glycoglycerolipid (**5**).

2. Materials and methods

2.1. Plant material

A. gigantifolia leaves were collected in Yen Bai province and identified by Dr. Phuong Thien