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Analysis of chemical components from "white turmeric" (*Curcuma* sp.) fraction with antioxidant activity by ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF/MS)

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ABSTRACT

Curcuma (Zingiberaceae) is a large rhizomatous herb genus found in tropical and subtropical areas. The biological activities of *Curcuma* species have been extensively studied. This research concentrated on rapidly determining the phytochemicals with antioxidant activity from *Curcuma* sp. with the aid of high-resolution mass spectrometry (HRMS). In this study, the antioxidant activity of *Curcuma* sp. extract and fractions were determined by DPPH assay. Overall, fraction F3 had the highest antioxidant property (IC_{50} = 20.91 μg/mL), which was then analyzed by UHPLC-Q-TOF/MS in negative electrospray ionization (ESI) mode. LC analysis was performed on an ExionLC™ UHPLC system, and mass spectrometric analysis was conducted on an X500$_R$ QTOF mass spectrometer (AB SCIEX, USA). The data were analyzed using SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA). The characterization of three types of curcuminoids from *Curcuma* sp. was discussed and distinguished by ESI-MS/MS analyses. As a result, 13 curcuminoid compounds from the best bioactive *Curcuma* sp. fraction were successfully identified. This study laid a foundation for isolating specific antioxidant phytochemicals from *Curcuma* sp.

Key words: Curcuma sp., antioxidant, UHPLC-Q-TOF/MS

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INTRODUCTION

The Curcuma genus (120 species) is a rhizomatous annual or perennial herb in the Zingiberaceae family.[1] Most *Curcuma* species are found in tropical evergreen environments. *Curcuma* species grow best in Malaysia, Thailand, India, Indochina, northern Australia, and other parts of Asia.[2–5] *Curcuma* is an economically valuable genus with several applications. Because of its preservation, therapeutic, and flavoring characteristics, *Curcuma* species can contribute economically.[6,7] Moreover, the underground rhizome of *Curcuma* is a significant source of yellow dye.[8]

Many species in this genus have significant medicinal effects, allowing them to treat a variety of health disorders, including stomach ulcers, hepatic disorders, skin diseases, chest pain, diabetes, and rheumatism.[9] *C. longa* has anti-inflammatory properties.[10] *C. amada* has been tested for cytotoxic action in a leukemic cell line, hemolysis, and antioxidant activity.[11] In addition, in vitro testing of volatile oils extracted from *C. caesia* leaves revealed that they have potent anti-inflammatory, antioxidant, and antibiotic properties.[12,13] Bioactive components found in *Curcuma* species include flavonoids, monoterpenes, sesquiterpenes, phenolic compounds, and antioxidants.[14–16]

UHPLC-Q-TOF/MS has proven to be an increasingly useful, powerful, and significant technique for determining chemical components and metabolites in biological materials[17–19] because of its great speed, efficiency, and resolution.[20] Hence, UHPLC-Q-TOF/MS was used to analyze the phytochemicals from *Curcuma* sp. fraction with the best bioactivity result.

The DPPH assay is a typical antioxidant test based on electron transfer, which produces a violet solution in ethanol. In the presence of an antioxidant, the free radical, which is stable at room temperature, is diminished, resulting in a colorless solution. The DPPH assay is a simple and quick approach to evaluate antioxidant activity using spectrophotometry.[21] In this study, the antioxidant activity of *Curcuma* sp. extract, and fractions were determined by DPPH assay.

The aim of this study was to rapidly determine the antioxidant components of *Curcuma* sp. rhizomes with the support of UHPLC-Q-TOF/MS technique.

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MATERIALS AND METHODS

Chemicals and reagents

Deionized water for HPLC; HPLC grade acetonitrile (ACN), methanol (MeOH), n-hexane, ethyl acetate (EtOAc), analytical grade formic acid, dimethyl sulfoxide (DMSO) (Scharlau, Spain); 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma–Aldrich, USA); ascorbic acid (Merck, USA); ethanol absolute (Chemsol, Vietnam).

Plant material

Curcuma sp. rhizomes were collected from Tra Vinh Province, Vietnam. The plant was identified at the Research Institute for Biotechnology and Environment (Nong Lam University Ho Chi Minh City). A voucher specimen (NP0220) is deposited in the Center for Research and Technology Transfer, Vietnam Academy of Science and Technology (VAST).

Sample extraction

Curcuma sp. rhizomes were rinsed thoroughly to remove the sand and soil, naturally dried overnight, and ground into small pieces. 3.5 (kg) of the ground rhizomes were extracted in approximately 20 L of methanol for 24 hours at room temperature. The macerate was filtered successively on Whatman filter paper. The extract was concentrated using a rotavapor (BÜCHI R-300, Switzerland) at 60°C. After three maceration cycles, 70.0 g of the crude extract (CE) was collected. The crude extract was partitioned with n-hexane and ethyl acetate to yield 8.0 (g) of n-hexane extract (HE), 36.0 (g) of ethyl acetate extract (EE), and 26.0 (g) of methanol rest extract (ME).

Fractionation of ethyl acetate extract

The ethyl acetate extract (EE) of *Curcuma* sp. was subjected to fractionation on a PLC 2250 system (Gilson Inc., France). The instrumentation was controlled by Gilson Glider CPC Software V5.1d.01 (Gilson Inc., France). Approximately 1.0 (g) of the EE sample was fractionated with a 50 grams RediSep Rf Gold® C18 reversed-phase column (Teledyne ISCO, USA). Water (A) and methanol (B) were used as mobile phase solvents. The gradient was as follows: 0–25 min, 15-50% B; 25-35 min, 50-100% B; 35-50 min, 100% B. The flow rate was 20.0 mL/min, and UV detection was performed at 254 nm. The solution was collected in a 32 mL tube (25 mL for each tube). As a result, the EE sample was separated into 5 fractions (F1-F5).

Antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging capacity of *Curcuma* sp. extract and fractions were measured based on the method reported by Kwang et al.[22] with some specific modifications. Briefly, 250 μM DPPH reagent in absolute ethanol was prepared. The samples were dissolved in DMSO at a concentration of 400 ppm and then diluted to different concentrations of 20, 100, 200, and 300 ppm. To evaluate antioxidant activity, 150 μL of DPPH reagent was mixed with 50 μL of sample in each well of a 96-well plate. The experiment was carried out three times for each sample. The reaction mixtures were incubated for 30 minutes at room temperature. A PowerWave HT Microplate Spectrophotometer was used to detect the absorbance of the incubated products at 520 nm (BioTek, USA). The positive control was ascorbic acid. The IC_{50} value (the concentration of the sample required for 50% DPPH radical inhibition) was determined for the sample based on the regression equation.

The following formula was used to calculate the DPPH scavenging effect:

Radical scavenging (%) = $\left(\frac{A_{blank}-A_{sample}}{A_{blank}}\right) \times 100\%$

where

A_{blank}: the absorbance at 520 nm of DPPH without sample.

A_{sample}: the absorbance at 520 nm of the mixture of DPPH and sample.

Statistical Analysis

Data were processed using Excel 2016 and Rstudio (version 1.4.1717) software. The results were compared by one-way ANOVA and Tukey's HSD post hoc test. If $p \leq 0.05$, a difference was considered statistically significant.

UHPLC-Q-TOF analysis

All UHPLC-Q-TOF experiments were performed on an instrument consisting of an ExionLC™ UHPLC system and an $X500_R$ QTOF mass spectrometer (AB SCIEX, USA). LC separations were carried out on a Hypersil GOLD C18 column (150 x 2.1 mm, 3 μ) (Thermo Fisher Scientific, USA). The mobile phase solvents were water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The gradient elution profile was as follows: 0–1 min, 2% B; 1-25 min, 2-98% B; 25-29 min, 98% B. The flow rate was 0.4 mL/min, and 2 μL of sample was injected for analysis.

The Q-TOF analysis was acquired in negative electrospray ionization (ESI) mode. The parameters were as

follows: ion source temperature, 500°C; curtain gas, 30 psi; nebulizer gas (GS 1), 45 psi; heater gas (GS 2), 45 psi. For the TOF MS scan, the mass range was set at m/z 70–2000. For the TOF MS/MS scan, the mass range was set at m/z 50–1500. For the negative mode, the ion spray voltage was set at −4.5 kV, the declustering potential (DP) was −70 V, the collision energy (CE) was −20 eV, and the collision energy spread (CES) was 10 eV. All the obtained data were processed by SCIEX OS software version 1.2.0.4122 (AB SCIEX, USA).

In this study, only the fraction with the best antioxidant activity was analyzed by UHPLC-Q-TOF to identify the bioactive compounds of *Curcuma* sp. rhizomes.

RESULTS

Antioxidant activity from Curcuma sp. extracts and fractions

In this experiment, the antioxidant activity of *Curcuma* sp. extract and fractions were evaluated by DPPH assay. Table 1 and Table 2 present the antioxidant results of the extracts and fractions of *Curcuma* sp., respectively. The lowest antioxidant activity was observed in methanol rest extract (ME) (7.76 ± 0.65%), while the best DPPH inhibition activity was observed in ethyl acetate extract (EE) (74.48 ± 0.80%) with an IC_{50} of 29.40 μg/mL. As shown in Table 2, fraction F1 had the lowest antioxidant result (0.82 ± 0.23%), and fraction F3 had the most effective antioxidant activity (84.27 ± 2.53%) with an IC_{50} of 20.91 μg/mL. Ascorbic acid was used as the positive control, of which the IC_{50} value was calculated to be 17.43 μg/mL.

The regression equations and IC_{50} values of the ethyl acetate extract, fraction F3, and vitamin C are presented in Table 3.

UHPLC-Q-TOF analysis of fraction F3

According to the antioxidant activity screening result, fraction F3 showed the best DPPH inhibition, which was then analyzed by UHPLC-Q-TOF. MS and fragment data of phytochemicals contained in fraction F3 are presented in Table 4. The total ion chromatogram (TIC) of fraction F3 is shown in Figure 1. Figure 2 shows the chemical structures of the phytochemicals that were discovered. The fragmentation pathway of compound 11 at T_R = 10.52 is shown in Figure 3.

DISCUSSION

Although the phytochemical composition of *Curcuma* species has been identified and their antioxidant activity has been demonstrated, the actual compounds that contribute to antioxidant activity have yet to be identified.[23] To rapidly determine the antioxidant constituents of *Curcuma* sp., fraction F3 with the best DPPH inhibition activity was analyzed by UHPLC-Q-TOF/MS.

Identification of curcuminoids by MS/MS

Curcuminoid structures can be divided into two parts: a skeleton of a heptane or pentane system and two aryl groups in the side chain.[24] Based on the results of UHPLC-Q-TOF analysis, all 13 compounds identified in fraction F3 were curcuminoids with heptane skeletons. The skeletons (A, B, and C) and aryl groups (Ar_1 – Ar_3) were confirmed to exist in fraction F3 of *Curcuma* sp. (Figure 2). Compared with type A curcuminoids, type B curcuminoids contained one keto group at C-3 or C-5, while type C curcuminoids had a double bond at the position of C-6,7.

In the negative mode, the product ions of aryl groups Ar_1, Ar_2, and Ar_3 were 123, 109, and 93 (Da), respectively. The product ion of 59 (Da) referred to the acetate group at the C-3 or C-5 position of the skeleton. In addition, the $[M\text{-}H\text{-}CH_3]^-$ ion could be observed via loss of a methyl radical from the aryl group. Compounds 5 and 9 were identified as type B, compound 8 was detected as type C, and the remaining 10 compounds were type A curcuminoids.

Correlation of phytochemicals and antioxidant activity of Curcuma sp.

Ibrahim et al. showed the antioxidant activity of three isolated curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) from *Curcuma domestica* and *Curcuma xanthorrhiza* with IC_{50} values of 0.15, 0.16, and 0.36 μmol/L, respectively.[23] Waras et al. stated that the main bioactive substances in the rhizomes of *C. xanthorrhiza* and *C. domestica* that have efficacy as antioxidant and anti-inflammatory activities are curcuminoids.[25] Jesmin et al. reported that nine purified compounds isolated from the fresh rhizome of turmeric Ryudai gold had antioxidant activity, of which six compounds were curcuminoids.[26]

The antioxidant activity and chemical constituents of *Curcuma* sp. showed that the presence of curcuminoids in the fraction was responsible for the considerable antioxidant activity. Our findings suggested that *Curcuma* sp., a new species of the *Curcuma* genus, is a promising source of natural antioxidants. Future studies should aim to isolate compounds in fraction F3 to accurately identify the compound(s) responsible for antioxidant activity from *Curcuma* sp.

Table 1: Antioxidant activity screening of *Curcuma* sp. extracts at a concentration of 100 ppm.

Sample	DPPH inhibition (%)			
	1st	2nd	3rd	Mean ± SD*
CE	27.37	26.61	25.69	26.56 ± 0.84
HE	17.98	20.43	19.65	19.35 ± 1.25
EE	73.85	75.37	74.21	74.48 ± 0.80
ME	7.66	8.46	7.16	7.76 ± 0.65

* Standard deviation.
Means are significantly different at $p \leq 0.05$ according to Tukey's HSD test.

Table 2: Antioxidant activity screening of *Curcuma* sp. fractions at a concentration of 100 ppm.

Sample	DPPH inhibition (%)			
	1st	2nd	3rd	Mean ± SD*
F1	1.07	0.62	0.78	0.82 ± 0.23
F2	13.61	13.45	14.12	13.73 ± 0.35[a]
F3	84.72	81.54	86.54	84.27 ± 2.53
F4	24.97	24.29	25.53	24.93 ± 0.62
F5	13.23	13.14	12.45	12.94 ± 0.42[a]

* Standard deviation.
Mean values followed by the same uppercase letter are not significantly different from each other at $p \leq 0.05$ according to Tukey's HSD test.

Table 3: Regression equations and IC_{50} values of *Curcuma* sp. EtOAc extract, fraction F3, and positive control (vitamin C).

Sample	Regression equation	R^2	IC_{50} (μg/mL)
EE	$y = -0.0147x^2 + 2.4615x - 9.6637$	0.9923	29.40
F3	$y = -0.0152x^2 + 2.2394x + 9.8106$	0.9740	20.91
Vitamin C	$y = -0.0157x^2 + 2.3991x + 12.947$	0.9780	17.43

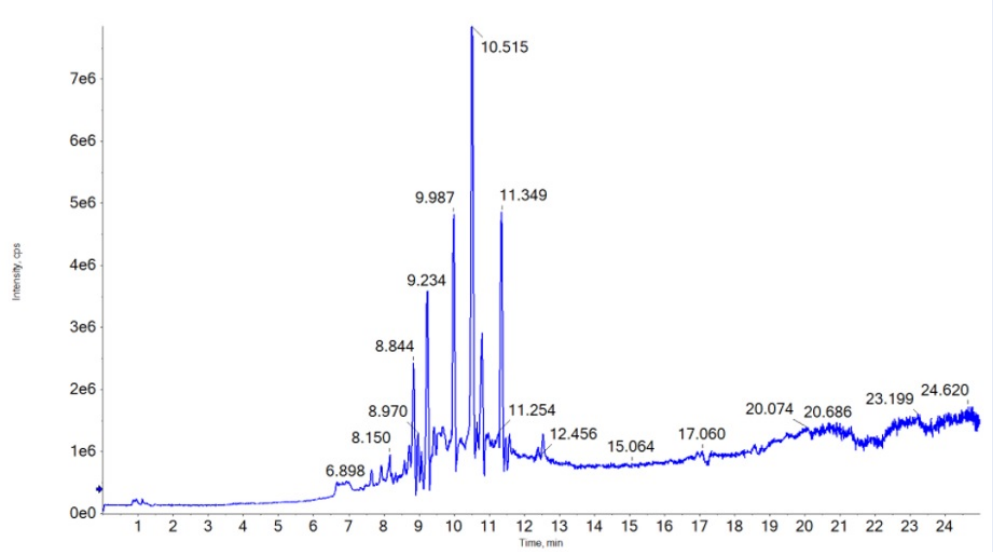

Figure 1: Total ion chromatogram (TIC) of fraction F3.

Table 4: Chemical constituents of fraction F3 characterized by UHPLC-Q-TOF in negative mode.

Peak No.	T_R (min)	Formula	Chemical Name	Found at mass	Exact mass	Error (ppm)	MS/MS
1	8.36	$C_{20}H_{26}O_6$	1,7-Bis(3,4-dihydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3'-Me ether	361.1662	361.1651	3.00	109, 123, 137, 151, 165, 179, 209, 223, 329, 345
2	8.85	$C_{19}H_{24}O_4$	1,7-Bis(4-hydroxyphenyl)-3,5-heptanediol; (3R,5R)-form	315.1600	315.1596	1.16	93, 107, 121, 149, 163, 175, 191, 209, 279, 297
3	9.07	$C_{20}H_{26}O_5$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3R*,5S*)-form, 3'-Me ether	345.1714	345.1702	3.48	93, 109, 123, 137, 151, 165, 179, 209, 223, 313
4	9.17	$C_{21}H_{28}O_6$	1,7-Bis(3,4-dihydroxyphenyl)-3,5-heptanediol; (3S,5S)-form, 3',3"-Di-Me ether	375.1816	375.1807	2.23	109, 123, 137, 151, 165, 179, 193, 209, 223, 329
5	9.24	$C_{19}H_{22}O_5$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3S,5S)-form, 5-Ketone	329.1397	329.1389	2.43	109, 121, 135, 147, 163, 191, 207
6	9.24	$C_{21}H_{26}O_7$	1,7-Bis(3,4-dihydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3-Ac	389.1605	389.1600	1.21	59, 93, 109, 123, 137, 145, 165, 191, 207, 209, 251, 301, 329, 347, 371
7	9.42	$C_{19}H_{24}O_5$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3R*,5S*)-form	331.1562	331.1545	4.98	109, 123, 137, 193, 207, 313
8	9.46	$C_{19}H_{22}O_4$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-6-hepten-3-ol; (3R,6E)-form	313.1447	313.1439	2.28	93, 119, 147, 149, 163, 189, 207
9	9.56	$C_{20}H_{24}O_5$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3S,5S)-form, 3-Ketone, 3'-Me ether	343.1560	343.1545	4.23	93, 107, 121, 123, 149, 165, 179, 193, 207, 219, 325
10	9.99	$C_{21}H_{26}O_6$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3-Ac	373.1661	373.1651	2.64	59, 93, 109, 121, 137, 149, 163, 165, 173, 191, 207, 313, 331
11	10.52	$C_{23}H_{28}O_8$	1,7-Bis(3,4-dihydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3,5-Di-Ac	431.1699	431.1706	-1.61	59, 93, 121, 135, 149, 165, 191, 207, 249, 293, 311, 329, 347, 371, 389
12	10.80	$C_{21}H_{26}O_5$	1,7-Bis(4-hydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3-Ac	357.1709	357.1702	1.96	59, 149, 163, 191, 297, 315
13	11.35	$C_{23}H_{28}O_7$	1-(3,4-Dihydroxyphenyl)-7-(4-hydroxyphenyl)-3,5-heptanediol; (3R,5R)-form, 3,5-Di-Ac	415.1754	415.1756	-0.67	59, 109, 121, 147, 295, 313, 331, 355, 373

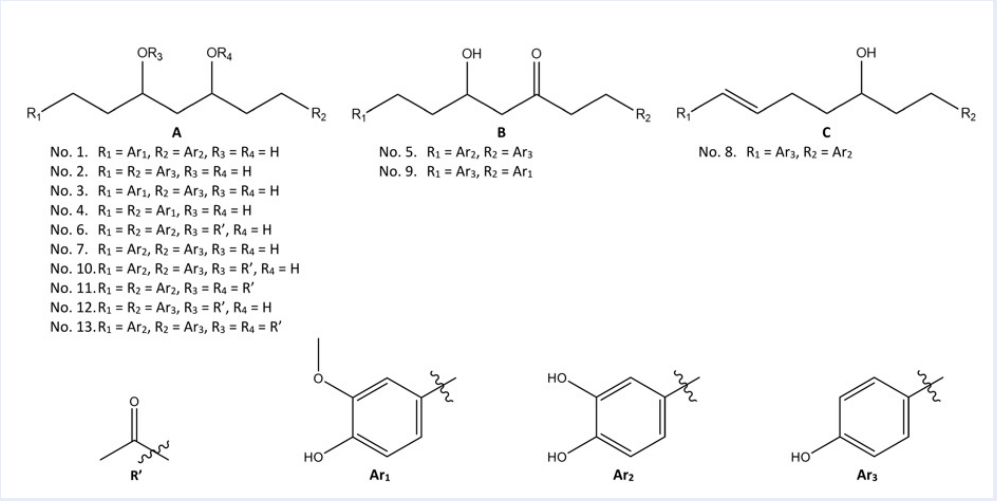

Figure 2: 13 curcuminoids from fraction F3.

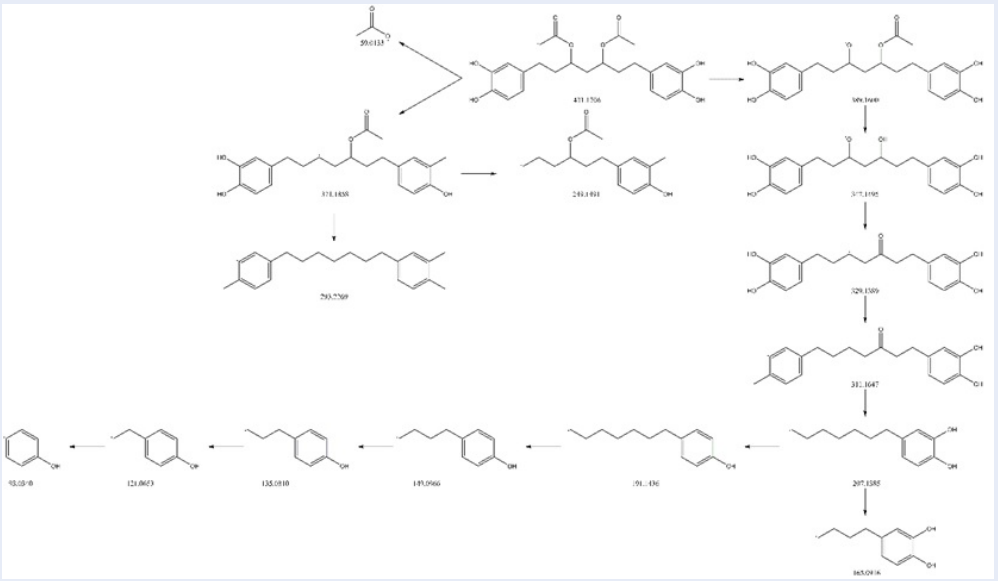

Figure 3: Fragmentation pathway of compound 11.

CONCLUSION

The goal of this study was to use UHPLC-Q-TOF/MS technology to quickly assess the antioxidant components of *Curcuma* sp. rhizomes. In this research, the DPPH radical scavenging activity of *Curcuma* sp. extracts and fractions was determined. Consequently, 13 curcuminoids in fraction F3 with the strongest antioxidant activity were identified by UHPLC-Q-TOF/MS. This research could build a basis for the isolation of targeted antioxidant compounds from *Curcuma* sp.

LIST OF ABBREVIATIONS

ACN: Acetonitrile
CE: Crude extract
DMSO: dimethyl sulfoxide
DPPH: 2,2-Diphenyl-1-picrylhydrazyl
EE: Ethyl acetate extract
ESI: Electrospray Ionization
EtOAc: Ethyl acetate
HE: n-hexane extract
HPLC: High-performance liquid chromatography
HRMS: High-Resolution Mass Spectrometry

IC_{50}: Half-maximal inhibitory concentration
ME: Methanol rest extract
MeOH: Methanol
TIC: Total ion chromatogram
UHPLC-Q-TOF/MS: Ultra-High-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry

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AUTHOR CONTRIBUTION

Tran Chieu An: performed the analytic calculations, analyzed the data, and contributed to the writing of the manuscript.
Pham Hong Ngoc: performed UHPLC-Q-TOF analysis, processed the experimental data, analyzed the data, and contributed to the writing of the manuscript.
Tran Phan Huynh Nhu: prepared the sample and carried out antioxidant experiment.
Ung Nguyen Hoang Kiem: contributed to sample extraction and fractionation.
Le Ngoc Hung: devised the project and the main conceptual ideas.
Phung Van Trung: devised the project, the main conceptual ideas, and developed the theoretical framework.

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

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