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Ultrasonic-assisted extraction of *Curcuma aromatica*: isolation and evaluation of bioactive components with anti-inflammatory activity

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ABSTRACT: *Curcuma aromatica* (Zingiberaceae) has long been used as a promising herb in Thai traditional medicine. Previous chemical constituents of this plant revealed different types of natural compounds, such as terpenoids and curcuminoids. This work evaluated the efficiency of biologically crude extracts from two extraction procedures: ultrasonic-assisted extraction and reflux methods. All extracts investigated the bioactive compounds analyzed by Gas chromatography–mass spectrometry and preliminary cell-based nitric oxide inhibitory screening, and the results implied that 75% ethanol rhizome extract assisted by ultrasonic irradiation exhibited sufficient constituents and biological activity. To explore the major bioactive compounds from this plant, a phytochemical investigation of *Curcuma aromatica* rhizome extract assisted by ultrasonic technique led to the isolation of six known compounds: (3*S*)-1,7-diphenylheptan-3-ol (**1**), germacrone (**2**), zederone (**3**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**). Their structures were determined on the basis of the spectroscopic data. In addition, (3*S*)-1,7-diphenylheptan-3-ol (**1**) was isolated for the first time from a natural source, and the complete spectroscopic data were given. All isolated compounds were submitted to test the anti-inflammatory activity using nitric oxide production in murine macrophage RAW 264.7 cell lines. Except for compounds **5** and **6**, all isolated compounds showed moderate nitric oxide inhibition with IC_{50} ranging from 7.36 ± 0.33 to 70.13 ± 11.14 μg/mL.

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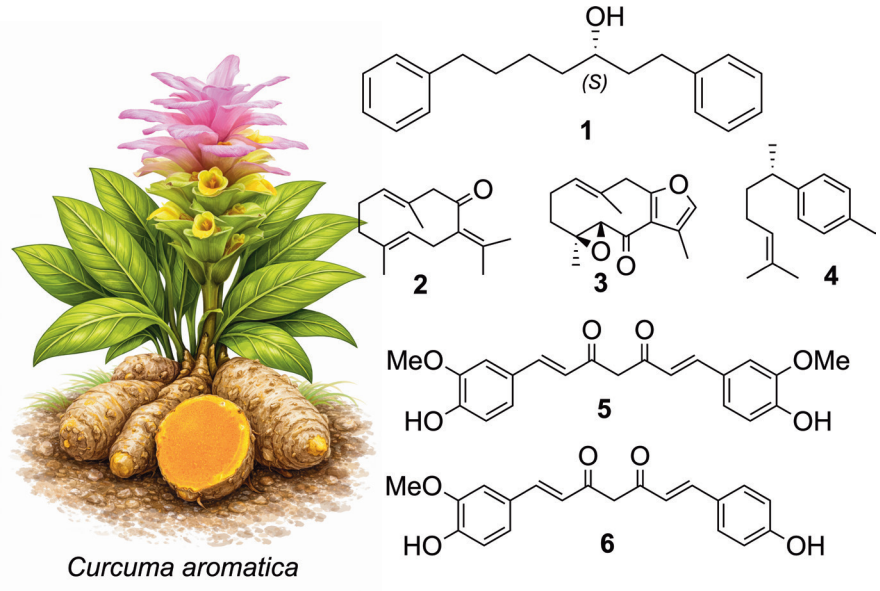
GRAPHICAL ABSTRACT



1. INTRODUCTION

Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells, and toxic compounds. These factors may induce acute and/or chronic inflammatory responses in the heart, pancreas, liver, kidney, lung, brain, intestinal tract, and reproductive system, potentially leading to tissue damage or disease (Chen et al., 2017). Nowadays, plant-derived anti-inflammatory agents continuously pay attention from many researchers not only more pharmacological activities but also low toxicity properties (Chaudhary et al., 2025). For example, curcumin showed potent anti-inflammatory agent found in turmeric, known for its ability to block the activation of NF-κB, a molecule that promotes inflammation (Razavi et al., 2021).

Plants in Curcuma genus belonging to family Zingiberaceae represent a group of perennial herbs native to tropical and subtropical regions of Asia, Australia, and South America. Approximately 100 *Curcuma* species were reported (Saensouk et al., 2025). Among them, *Curcuma aromatica* Salisb. is well known as wild turmeric and is called in Thai as "Wan Ngang Kham" (Pabuprapap et al., 2024). This plant is an essential herb and has been used in folk medicine. In India, aqueous rhizome extract of *C. aromatica* has been used for many treatments of diseases such as rheumatism, wound healing, and dysentery (Umar et al., 2020). In Thailand, the rhizomes and roots of *C. aromatica* have been traditionally used in cosmetics and spas for skincare (Choochote et al., 2005). Essential oil from leaves and rhizomes of this plant was investigated by using different extraction procedures, which provide different chemical compositions (Bordoloi et al., 1999). They were also reported to have potent antioxidant, anticancer, antimicrobial, and anti-inflammatory. For example, the hydro-distilled essential oil from leaves of *C. aromatica* represented 23 compounds detected by Gas chromatography–mass spectrometry (GC–MS) technique exhibited potent DPPH radical-scavenging activities (Al-Reza et al., 2010). Steam distillation of the plant collected from different habitats in China showed different compounds, which was only four compounds; *ar*-curcumene, β-sesquiphyllandrene, *p*-cymene, and *ar*-turmerone were coexisted in all the 12 essential oils (Xiang et al., 2017).

A number of up-to-date modern extraction techniques including ultrasonic method have been proposed to extract the bioactive compounds from various sources. Ultrasound-assisted extraction (UAE) is a technique that uses ultrasonic waves (typically 20–40 kHz) to enhance the extraction of compounds from various materials (Esclapez et al., 2011; Cauduro et al., 2025). It has become an efficient technology that requires much lower solvent consumption, lower energy input, lower temperature, and has a faster extraction rate, with increased stability for plant active compounds compared with conventional extraction methods such as maceration, shaking, or soxhlet extraction (Chemat et al., 2008). This method is widely used in chemistry, food science, pharmaceuticals, and natural product research, including herbal extraction (Chemat et al., 2017). In the published literature, the extraction of bioactive compounds from *C. aromatica* assisted by ultrasonic

method has been approached. For example, curcumin was extracted from *C. aromatica* with a high yield (approximately 73%) under optimized ultrasonic extraction conditions (Shirsath et al., 2021). In addition, phenolic compounds were also obtained from the rhizomes of *C. aromatica* using ultrasonic-assisted extraction (Siramon and Waeonukul, 2020). Therefore, UAE has attracted considerable attention from researchers due to its efficiency and simplicity. In the present study, we compared the extraction efficiency of a traditional method (reflux extraction) with that of a modern technique (ultrasonic-assisted extraction), along with a preliminary evaluation of their anti-inflammatory activity through nitric oxide (NO) inhibition in murine macrophage RAW 264.7 cells. Furthermore, to identify the bioactive constituents from the active UAE of *C. aromatica*, we report herein the isolation of six known compounds: (3*S*)-1,7-diphenylheptan-3-ol (**1**), germacrone (**2**), zederone (**3**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**). Their structures were confirmed by spectroscopic methods and comparison with literature data. Notably, this is the first report of (3*S*)-1,7-diphenylheptan-3-ol (**1**) being isolated from a natural source. In addition, its complete structural characterization and spectroscopic data are provided herein. This compound was also evaluated for its anti-inflammatory activity via NO inhibition, and the results are described in this study.

2. EXPERIMENTAL

2.1. General experimental procedures

IR spectra were obtained on a Perkin Elmer EX FT-IR system spectrometer or a Bruker FT-IR spectrometer, model ALPHA; major bands (ν_{max}) were recorded in wave number (cm^{-1}). UV absorption spectra were performed on a JASCO V-530 spectrometer; principle bands (λ_{max}) were recorded as wavelengths (nm) and log ε in methanol. Optical rotations were measured on a JASCO DIP-370 digital polarimeter by using 50 mm microcell. EIMS spectrometer measures at 70 eV (probe). High-resolution mass spectra were recorded on a Thermo Finnigan Polaris Q, and mass were recorded on a Bruker Micromass model VQ-TOF spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ascend™ 400 MHz NMR spectrometer using tetramethylsilane as an internal standard. Chromatographic methods were performed by using silica gel 60 (Merck, 70–230 mesh) for column chromatography, silica gel plates (Merck, Silica gel 60 PF_{254}) for preparative TLC, and sephadex LH-20 (Merck) for gel filtration. For ultrasonic extractor, UAE was conducted using an Ultrasonic cleaner device (GT SONIC-D3 China). The extraction was carried out at 40 kHz frequency using 100 W output power. The reflux extraction was performed using a reflux apparatus.

2.2. Plant material

The rhizomes of *Curcuma aromatica* Salisb. were collected in March 2022 from Sanamchaikhet District, Chachoengsao Province, Thailand (13°52'N, 101°88'E). The plant was identified by Mr.Pitphiboon Thanphuthon, Department of Thai Traditional Medicine, Faculty of Science, Ramkhamhaeng University, and a voucher specimen (RRU-SH-013) was deposited at the Faculty of Science and Technology, Rajabhat Rajanagarindra University, Thailand. The plant materials were washed, thinly sliced, and dried in an oven at 50 °C for 2 days. The dried rhizomes were then ground using a 2500G electric grain mill (Cereal Spice model) and stored at room temperature until further use.

2.3. Extraction and isolation

2.3.1. For extraction screening

Reflux extraction: the dried, powered rhizome (10 g) of *C. aromatica* was subjected to a round-bottom flask equipped with a reflux apparatus using 500 mL of different solvents (hexane, methanol, 75% ethanol, and 95% ethanol) for an hour. Then, the solvents were filtered and dried using an evaporator and kept at room temperature until analysis.

Ultrasonic-assisted extraction: the dried, powered rhizomes (10 g) of *C. aromatica* was subjected to a conical flask with 500 mL of different solvents (hexane, methanol, 75% ethanol, and 95% ethanol). The extraction was performed in an ultrasonic bath operating at a frequency of 40 kHz and a power output of 100 W for 30 minutes at room temperature. The procedure was carried out similarly to the procedure developed by Siramon & Waeonukul (2020). To prevent overheating, the temperature was carefully monitored throughout the process. The resulting extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator. The crude extract was stored at 4 °C until further analysis.

2.3.2. For extraction and isolation

A dried, powdered rhizomes of *C. aromatica* (1.5 kg) was extracted using ultrasonic cleaner for an hour with 75% ethanol (2 L). The extraction was performed in an ultrasonic bath operating at a frequency of 40 kHz and a power output of 100 W for 30 minutes at room temperature. After filtration using Whatman No. 1 and removal of the solvents by rotary evaporator, followed by vacuum freeze-drying, the crude extract

(328.8 g) was obtained. A portion of the extract (300 g) was separated by vacuum column chromatography (silica gel, 2 kg), eluting with hexane-acetone-MeOH gradient to yield four fractions (A1–A4). In brief, using column chromatography, preparative thin layer chromatography along with crystallization techniques, compounds **1** (84.8 mg), **2** (129.0 mg), **3** (107.9 mg), **4** (55.3 mg), **5** (246.8 g), and **6** (154.0 mg) were isolated from fractions A1–A3 (the details of the isolation procedure are presented in Supplementary data).

*(3S)-1,7-diphenylheptan-3-ol (**1**):* white needles; mp 48–50 °C; $[\alpha]_D^{23}$ +9.0 (*c* 0.41, $CHCl_3$), $[\alpha]_D^{23}$ +8.3 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 210 (4.18) and 260 (3.12) nm; IR (KBr) ν_{max} 3324, 3022, 2924, 2854, 1600, 1493, 1450, 1098, 1047, 1031, 915, 866, 747, 692 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 4; HR-ESI-MS *m/z* 291.1669 $[M+Na]^+$ (calcd for $C_{19}H_{24}ONa$, 291.1719).

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

The GC-MS analysis of the crude extract was performed using an Agilent 5977B GC/MSD with an HP5MS column (30 m × 0.25 mm × 0.25 mm) under Helium as carrier gas with a flow rate of 1.3 mL/min. Samples were analyzed in the column held at an initial temperature of 50 °C for 3 min after injection. Then, increasing temperature was carried out to 280 °C at a program rate of 10 °C/min and then held for 20 min. The injections were performed at 250 °C in splitless mode. The pressure was 10 psi, the run time was 37.5 min, and the temperatures of the injector, and detector were 250°C. Before submission to GC-MS analysis, each crude extract (2.0 mg) was dissolved in 1 mL of methanol and subjected to exhaustive filtration. The isolated constituents were examined to compare with the authentic samples by referring to their retention times and mass weights available in GC-MS NIST library.

2.5. The inflammatory with nitric oxide (NO) inhibitory effect

The assay was performed as previously described by Suthiphasilp et al. (2021). The MTT (3-[4,5-dimethylthizol-2-yl]-2,5-diphenyl tetrazolium bromide; Milipore Sigma) assay was carried out in cell viability tests, as previously described in literature (Ahmed et al., 1991), and cell viability was expressed as a percentage relative to the untreated control. Concentrations exhibiting more than 80% cell viability were considered noncytotoxic. For nitric oxide inhibitory effect test, RAW 264.7 cells were seeded at a density of 4 × 10^4 cells per well in 96-well plates and incubated overnight at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were then stimulated with lipopolysaccharide (LPS, 1 µg/mL) for 1 h, followed by treatment with various concentrations of the isolated compounds (6.25, 12.5, 25, 50, 100, 200, and 400 µg/mL) for 24 h. After incubation, 100 µL of Griess reagent was added to each well and allowed to react for 10 min. Nitric oxide production was quantified by measuring absorbance at 570 nm using a Biochrom EZ Read 400 ELISA microplate reader (Biochrom Ltd., Cambridge, UK). The inhibitory activity was expressed as IC_{50} values, which were calculated using GraphPad Prism version 6.0 software. The data presented in µg/mL was conducted in triplicates. Indomethacin was used as a positive control, exhibiting an IC_{50} value of 14.0 µg/mL.

2.6. Statistical analysis

Statistical analysis was performed utilizing the GrahPad Prism software (version 6), and the results are represented as mean ± SD derived from three experiments. The differences between treatments were evaluated using Duncan's Multiple Range Test (DMRT) implemented through the R package agricolae (de Mendiburu, 2023).

3. RESULTS AND DISCUSSION

This study aimed to investigate the efficiency of two different extraction methods, phytochemical compositions, and anti-inflammatory activity on NO production by murine macrophage-like RAW 264.7 cells of the rhizomes of *C. aromatica,* and the workflow diagram was also provided, as shown in Figure 1. The results were described as follows.

3.1. Efficiency of extraction methods

After extraction of dried, powdered rhizomes of *C. aromatica* using two different methods which are ultrasonic-assisted extraction and reflux method, and the extraction yields were summarized as shown in Table 1. The anti-inflammatory activity on NO production of all extracts was presented as depicted in Table 2. Meanwhile, the refluxing method with ranking from 4.37 ± 0.57 to 12.06 ± 2.68 % yield gave the extraction yield greater than ultrasonic-assisted extraction with ranking from 2.64 ± 0.77 to 9.70 ± 0.40 % yield in all solvent systems. Only 75% ethanol extract from UAE exhibited moderate anti-inflammatory activity with 98.65 ± 1.61 cell viability and 62.15 ± 0.13 % NO inhibition. Thus, to determination of chemical compositions of the active extract,

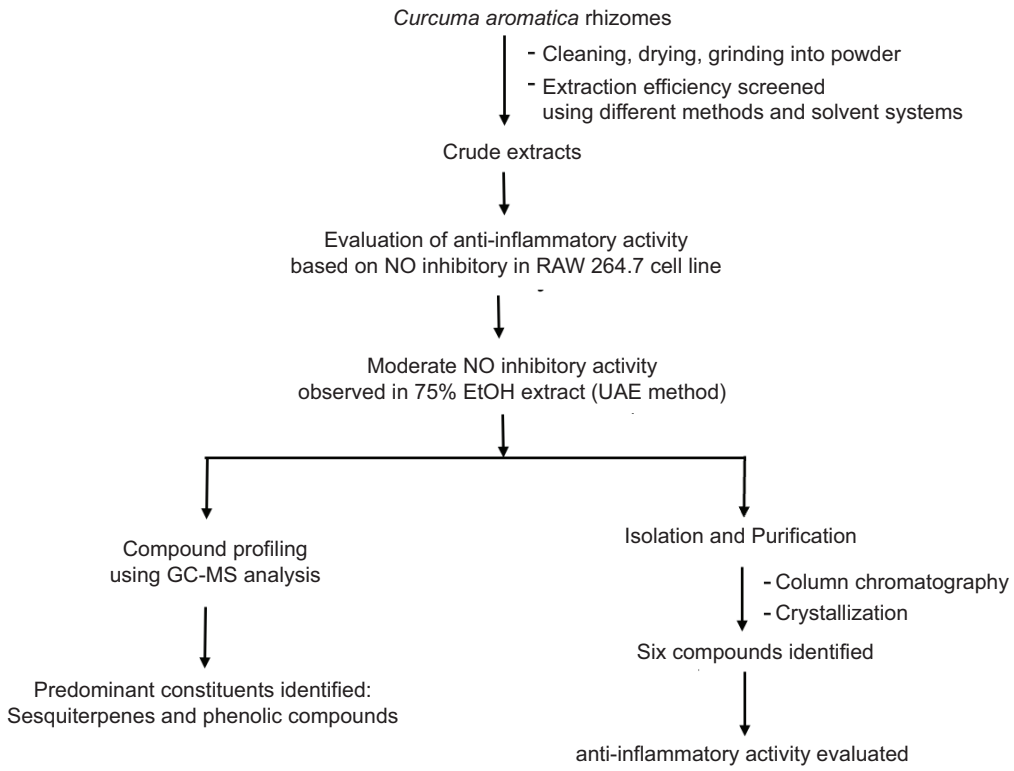


Figure 1. Workflow for the extraction, isolation, and identification of bioactive compounds from *C. aromatica*.

Table 1
Weights and yields of two extraction techniques in different solvents.

Sample	Reflux extraction		Ultrasonic-assisted extraction	
	Weight (g)	Yield (%)[a]	Weight (g)	Yield (%)
95% Ethanol	1.16 ± 0.12	10.87 ± 1.06^{ab}	0.78 ± 0.11	7.65 ± 1.14^{c}
75% Ethanol	1.29 ± 0.29	12.06 ± 2.68^{a}	1.03 ± 0.04	9.70 ± 0.40^{abc}
MeOH	1.12 ± 0.08	10.52 ± 0.72^{ab}	0.93 ± 0.17	8.94 ± 1.41^{bc}
Hexane	0.45 ± 0.06	4.37 ± 0.57^{d}	0.28 ± 0.09	2.64 ± 0.77^{d}

[a]Values are mean ± SD (N = 3). Different superscripts in the yield (%) indicate significant differences (p < 0.05) according to Duncan's multiple range test (DMRT).

the GC-MS technique was employed. The identified chemical constituents were listed in Table 3 by retention time and percentage relative intensity peak area (%RIPA). Most of the detected compounds from GC-MS were volatile compounds such as γ-terpinene, menthadiene, and *o*-cymene. The major constituents from 75% ethanol extract using UAE technique revealed that α-eudesmol (13.12%), and guaiol (12.84%) are the top two bioactive sesquiterpene compound candidates with %RIPA more than 10%. Interestingly, sakuranetin (0.30%) and usnetol (0.64%), two phenolic compounds, were also found within the extract.

3.2. Isolation and identification

As described above, the 75% ethanol extract obtained from UAE exhibited moderate anti-inflammatory activity. GC-MS analysis revealed that sesquiterpene compounds were the predominant constituents, along with phenolic compounds. Consequently, chromatographic isolation of the bioactive components from the extract was carried out. Following purification using chromatographic and crystallization techniques, six compounds were obtained. The known compounds were identified by comparing their spectroscopic data with literature values as germacrone (**2**) (Al-Amin et al., 2021), zederone (**3**) (Al-Amin et al., 2021), (–)-curcumene (**4**) (Ehara et al. 2007), curcumin (**5**) (Araya-Sibaja et al., 2024), and bisdemethoxycurcumin (**6**) (Araya-Sibaja et al., 2024), as shown in Figure 2.

The known compound **1** was identified as 1,7-diphenylheptan-3-ol. The UV maximum absorption bands (λ_{max}) are at 210 and 260 nm. The FT-IR spectrum of **1** revealed the O–H stretching of a hydroxy group at ν_{max} 3324 cm^{-1} and the C–H stretching of aromatic and aliphatic hydrocarbons at ν_{max} 3022 and 2924 cm^{-1}. In addition, the C=C stretching of aromatic hydrocarbons was found at ν_{max} 1600, 1492, and 1450 cm^{-1}. These data indicate that it possesses hydroxyl, aliphatic, and aromatic functional groups. The molecular

Table 2
Anti-inflammatory activity effect on NO inhibition ($N = 3$) of extracts and some isolated compounds.

Sample	% Cell viability	% NO inhibition[a]	IC_{50} (mg/mL)*
Reflux method			
95% Ethanol	9.22 ± 0.37	N/A	-
75% Ethanol	8.01 ± 0.29	N/A	-
MeOH	5.32 ± 0.68	N/A	-
Hexane	4.25 ± 0.53	N/A	-
Ultrasonic-assisted method			
95% Ethanol	7.75 ± 0.21	N/A	-
75% Ethanol	98.65 ± 1.61	62.15 ± 0.13	-
MeOH	8.61 ± 0.56	N/A	-
Hexane	5.80 ± 0.68	N/A	-
Pure compounds			
1	20.66 ± 0.24	102.13 ± 0.81	7.36 ± 0.33[b]
2	25.87 ± 2.14	101.52 ± 0.85	10.57 ± 0.32[b]
3	81.28 ± 0.98	88.84 ± 2.76	70.13 ± 11.14[a]
4	32.21 ± 0.06	99.82 ± 1.55	14.80 ± 0.58[b]
Indomethacin	82.29 ± 0.33	98.63 ± 0.60	14.03 ± 0.23[b]

All data are represented as the mean ± S.D. ($N = 3$); N/A = not assessed due to the %cell viability (<80). [a]%NO inhibition was measured at a concentration of 200 mg/mL. *Different superscripts in the IC_{50} indicate significant differences ($p < 0.05$) according to Duncan's multiple range test (DMRT).

formula as $C_{19}H_{24}O$ using high-resolution electrospray ionization mass spectrometry (HR-ESI-MS), which showed a peak at *m/z* 291.1669 $[M+Na]^+$ (calcd. for $C_{19}H_{24}ONa$, 291.1719). Analysis of double bond equivalent (DBE) data indicated that compound **1** possessed a DBE value of 8, suggesting the presence of two aromatic rings in its structure. The ^{13}C and ^{1}H NMR spectral data of compound **1** are presented in Table 4. The ^{13}C NMR spectrum displayed six methylene carbons at δ_C 39.1, 37.4, 35.9, 32.1, 31.5, and 25.3; four signals corresponding to ten aromatic carbons at δ_C 128.3 (4 × C), 128.4 (4 × C), 128.5, and 125.7; one methine carbon signal at δ_C 71.3; and two quaternary carbon signals at δ_C 142.2 and 142.1. In the ^{1}H NMR spectrum, the signal at δ_H 3.58 (1H, *m*) correlated to a proton at the C-3 position, confirmed by the HMQC correlation with the methine carbon at δ_C 71.3, which was assigned to C-3. Additionally, the signals at δ_H 1.83 (2H, *m*) and 1.67 (2H, *m*) were assigned to protons on C-2 and C-4, respectively, both of which are adjacent to the C-3 methine carbon indicated by ^{1}H-^{1}H COSY correlation (Figure 3A). The signals at δ_H 2.81 (1H, *m*) and δ_H 2.68 (1H, *m*) were assigned to protons on C-1, while the signal at δ_H 1.50 (1H, *m*) and 1.36 (1H, *m*) was attributed to C-5 of the diphenylheptanoid derivatives. These positions were confirmed by using ^{1}H-^{1}H COSY

Table 3
Compositions with retention time and percentage relative intensity peak area (%RIPA) of the rhizomes of *C. aromatica* extracted with 75% ethanol using UAE technique.

Peak	Name	RT	%RIPA	Peak	Name	RT	%RIPA
1	α-Thujene	4.53	1.12	21	Humulene	18.23	3.46
2	α-Pinene	4.71	1.15	22	Muurola-4,9-diene	18.80	2.95
3	α-sabinene	5.45	0.36	23	Selina-4(15),7(11)-diene	19.09	2.41
4	β-Pinene	5.59	0.48	24	α-Selinene	19.26	2.56
5	β-Myrcene	5.76	0.53	25	β-Cadinene	19.68	1.13
6	α-Phellandrene	6.22	1.24	26	Menth-8-ene-4-methanol	20.52	4.89
7	(+)-4-Carene	6.47	1.37	27	Caryophyllene oxide	21.27	1.94
8	*o*-Cymene	6.80	3.13	28	Guaiol	22.03	12.84
9	Limonene	6.88	1.24	29	γ-Eudesmol	22.76	8.11
10	Eucalyptol	6.94	0.79	30	α-Eudesmol	23.47	13.12
11	γ-Terpinene	7.66	4.35	31	*trans*-Geranylgeraniol	24.29	1.06
12	Menthadiene	8.44	5.83	32	Spatulenol	24.62	0.55
13	Linalool	8.66	1.47	33	β-Ionene	24.84	0.50
14	1-Formyl-3-cyclohexene	9.74	0.45	34	Unknown	25.37	0.31
15	p-Cymen-8-ol	11.01	2.58	35	2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol	25.55	0.23
16	α-Terpineol	11.23	1.42	36	6,7-Dimethoxy-4-methylcoumarin	29.74	0.31
17	δ-Elemene	14.99	1.47	37	Epiglobulol	30.34	0.23
18	Copaene	16.03	0.74	38	6,7-Dimethoxy-4-methylcoumarin	29.74	0.30
19	β-Elemene	16.53	3.75	39	Sakuranetin	30.35	0.30
20	Caryophyllene	17.45	7.57	40	Usnetol	30.80	0.64

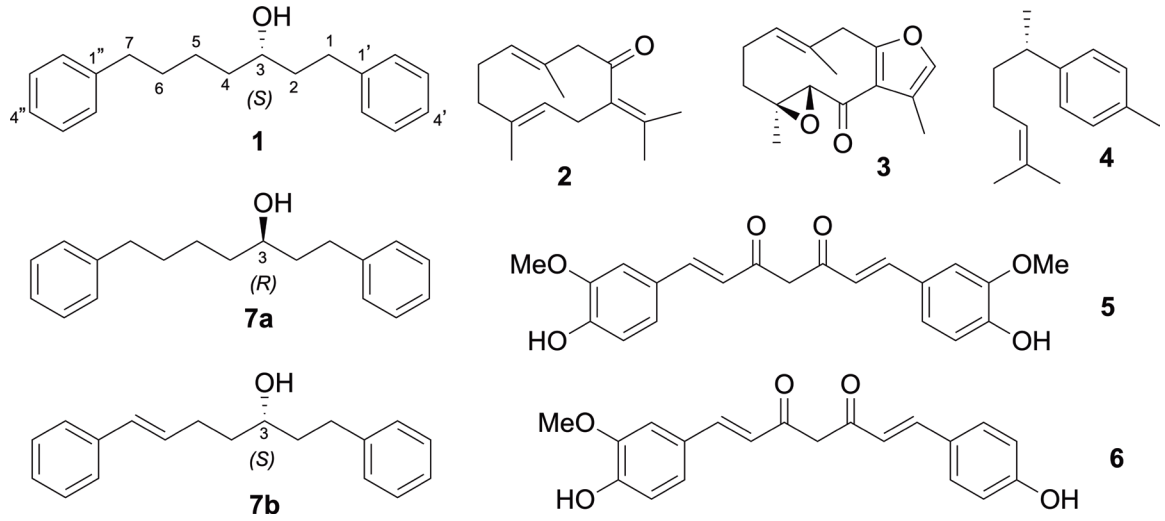


Figure 2. Structures of isolated compounds **1**–**6** and the known diphenylheptanoid analogues (**7a** and **7b**).

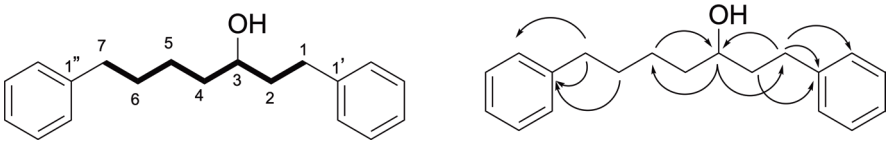


Figure 3. Key ^{1}H-^{1}H COSY (Bolded line; A) and HMBC (arrowed line; B) correlations of **1**.

correlations with C-2/C-3 and C-4/C-5 positions, respectively. The ^{1}H NMR signals corresponding to C-6 and C-7 appeared at δ_H 1.52 (2H, *m*) and δ_H 2.60 (2H, *m*), which were further supported by ^{1}H–^{1}H COSY correlations between C-5 and C-6, as well as between C-6 and C-7. To confirm the complete correlations of the backbone structure, HMBC correlations (Figure 3B) from H-3 to C-1 and from H-3 to C-5 were observed, supporting that C-3 was substituted with a hydroxy group in the diphenylheptanoid derivative. The complete correlations of ^{1}H–^{1}H COSY and HMBC correlations were provided as shown in Figure 3. The EI-MS of this compound was obtained a pseudomolecular ion $[M\text{-}H]^+$ at *m/z* 267, compatible with the molecular formula $C_{19}H_{24}O$. In addition, the EI-MS fragmentation pattern of **1** was also proposed, as shown in Figure 4.

Due to the presence of one asymmetric carbon in diphenylheptanoid derivatives, the configuration can significantly influence their biological activities. For example, centrolobol with an *R* configuration at C-3 exhibited cytotoxicity against the human oral epidermoid carcinoma (KB) cell line, with an IC_{50} value of 4.76 µM, whereas its derivative showed weaker inhibitory activity with an IC_{50} value of 10.09 µM (Chokchaisiri et al., 2014). Therefore, confirming the configuration at the C-3 position of compound **1** was essential.

The core structure, 1,7-diphenylheptan-3-ol, was reported for the first time by Suga et al. (1985), who proposed it as (3*R*)-1,7-diphenylheptan-3-ol (**7a**) for the study of optical rotation dispersion measurements. Subsequently, Claeson et al. (1996) elucidated the backbone structure through the alkene hydrogenation reduction of *E*-1,7-diphenylhepten-5-ol (**7b**). In their reports, the optical rotation (OR) value of the reduction product was recorded as $[\alpha]_D$ –6.5 (*c* 1.2, $CHCl_3$), while the parent compound exhibited an OR value of $[\alpha]_D$ +7 (*c* 1.2, $CHCl_3$) (Claeson et al., 1993). Therefore, the chemical configuration of this compound remains ambiguous. The compound *E*-1,7-diphenylhepten-5-ol (**7b**) was isolated from *Curcuma comosa* (Suksamrarn et al., 2008) as a single enantiomer. Its absolute configuration at C-3 was determined to be *S* using the modified Mosher's method. Furthermore, hydrogenation of the double bond yielded compound **1**, for which an OR value of $[\alpha]_D^{27}$ +8.0 (*c* 0.27, EtOH) was reported, confirming its absolute configuration as 3*S*. From our comprehensive literature review, it is noteworthy that this compound had previously only been obtained via synthetic modification, and this study represents the first report of its isolation from a natural source. To validate the structure and confirm the absolute configuration as previously described, we employed NMR spectroscopy (^{1}D and

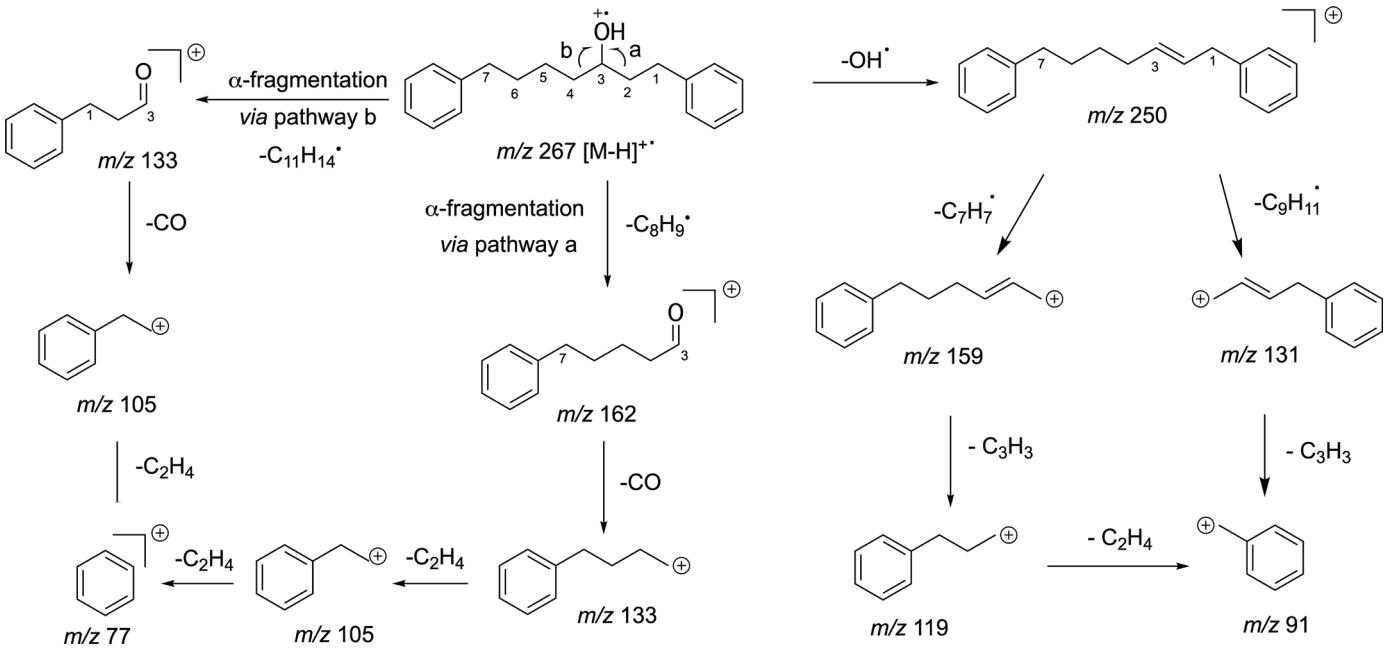


Figure 4. Proposed EI-MS fragmentation pattern of **1**.

^{2}D NMR), HR-ESI-MS, and EI-MS analyses, along with OR measurements. The isolated compound exhibited OR values of $[\alpha]_D^{23}$ +9.0 (*c* 0.41, $CHCl_3$) and $[\alpha]_D^{23}$ +8.2 (*c* 0.41, MeOH). These values are consistent in both sign and magnitude with the data reported by Suksamrarn et al. (2008), $[\alpha]_D^{27}$ +8.0 (*c* 0.27, EtOH). Based on these findings, compound **1** is proposed to be (3*S*)-1,7-diphenylheptan-3-ol.

3.3. Anti-inflammatory of pure compounds

The isolated compounds except for compounds **5** and **6** were evaluated for their anti-inflammatory effects on nitric oxide (NO) production in murine macrophage-like RAW 264.7 cells, and the results are presented in Table 2. All tested compounds exhibited moderate NO inhibitory activity with IC_{50} values ranging from 7.36 ± 0.33 to 70.13 ± 11.14 mg/mL.

Most compounds isolated from *C. aromatica* have been investigated for various biological activities, such as anticancer effects (Thach et al., 2024) and antioxidant properties (Dong et al., 2018). Recently, 22 compounds were isolated from the rhizomes of *C. aromatica* extracted via maceration (Pabuprapap et al., 2024). Among these, germacrone (**2**), zederone (**3**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**) were also identified in our study. These compounds were previously evaluated for nitric oxide (NO) inhibitory activity in LPS-activated RAW 264.7 cells. Based on the reported results, germacrone (**2**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**) exhibited IC_{50} values of 16.0 ± 2.9, 28.9 ± 3.9, 6.5 ± 2.0, and 4.4 ± 0.3 μM, respectively, while zederone (**3**) was not evaluated in that study. In another investigation, the ethyl acetate extract obtained via maceration of *C. aromatica* rhizomes was studied by Pintatum et al. (2020), and zederone (**3**) was also reported. This compound demonstrated moderate anti-inflammatory effects by suppressing NF-κB activation in a reporter gene assay using the HaCaT cell line. By comparison, these data suggested that our observation data agreed well with previously reported (Table 2).

For compound **1**, it was previously examined for estrogenic-like transcriptional activity using RT-PCR analysis of *Bcl-xL* and *ERβ* gene expression in HeLa cells (Suksamrarn et al., 2008). The results indicated weak activity, with inhibition rates of 40.00 ± 9.90% for *Bcl-xL* and 37.03±3.58% for *ERβ*. Furthermore, this compound, with an unassigned absolute configuration at the C-3 position, was tested for anti-inflammatory activity in an ethyl phenylpropiolate-induced ear edema model in rats (Claeson et al., 1996), where it produced less than 25% inhibition of edema formation and failed to establish a clear dose–response relationship. In addition, the synthesized compound **1** was investigated for the inhibitory of LPS-induced NO production in RAW 267.7 cells (Lin et al., 2022). The result revealed that % inhibitory NO production

Table 4
^{1}H (100 MHz) and ^{13}C (400 MHz) NMR data of **1** in $CDCl_3$.[a]

Position	^{13}C	^{1}H
1	32.1	2.81 (m), 2.68 (m)
2	39.1	1.83 (m)
3	71.3	3.58 (m)
4	31.5	1.67 (m)
5	25.3	1.50 (m), 1.36 (m)
6	37.4	1.52 (m)
7	35.9	2.60 (m)
1′	142.1	–
1″	142.5	–
2′, 6′, 2″, 6″	128.3	7.17 (m)
3′, 5′, 3″, 5″	128.4	7.17 (m)
4′	128.5	7.27 (m)
4′′	125.7	7.27 (m)

[a]The chemical shift (δ) reported in ppm in both ^{1}H and ^{13}C NMR data; For ^{13}C NMR data, the carbon types were deduced from the DEPT-135 technique.; For ^{1}H NMR data, the spitting pattern was given in parenthesis.

was 38.92 ± 6.58 at 5 μM concentration. The literature data agreed well with our investigation (% inhibitory NO production as 38.66 ± 4.17 at concentration 6.25 μg/mL).

From our present investigation, compound **1** demonstrated moderate anti-inflammatory effects by inhibiting NO production in murine macrophage-like RAW 264.7 cells, with an IC_{50} value of 7.36 ± 0.33 μg/mL (Table 2).

The NO inhibitory activity observed for the isolated compounds may be attributed to their ability to modulate key inflammatory pathways, particularly through suppression of iNOS expression via inhibition of NF-κB activation. Structurally, diphenylheptanoids and related phenolic compounds have been reported to interfere with inflammatory signaling cascades including the phosphorylation of Janus kinase 2/signal transducer and activator the transcription 3 (JAK2/STAT3) signals and suppression of NF-κB pathway (Lin et al, 2022), which might be consistent with the activity observed in this study. In this study, it was found that a 3*S*-acetoxy diphenylheptanoid derivative inhibits NO production with an IC_{50} of 0.32 μM. In addition, it also provides mechanistic importance in α7 nAchR-JAK2-STAT3 pathway. Furthermore, comparison with previously reported analogs from *Curcuma* species suggests that 3*S*-hydroxydiphenylheptanoids played a significant role in enhancing anti-inflammatory activity, indicating a potential structure–activity relationship.

Based on the aforementioned evidence, this study provides scientific support for the anti-inflammatory potential of *Curcuma aromatica*, in alignment with its long-standing use in traditional medicine.

4. CONCLUSION

This study compared two extraction methods, ultrasonic-assisted extraction (UAE) and reflux extraction, for isolating bioactive compounds from the rhizomes of *C. aromatica*. Extraction yields were higher with refluxing (4.37–12.06%) than with UAE (2.64–9.70%) across all solvent systems. However, only the 75% ethanol extract obtained via UAE exhibited moderate anti-inflammatory activity, with 98.65 ± 1.61% cell viability and 62.15 ± 0.13% nitric oxide (NO) inhibition in LPS-stimulated RAW 264.7 macrophage-like cells. In chemical profiling and isolation, GC–MS analysis of the active UAE extract revealed that sesquiterpenes were the predominant constituents, with α-eudesmol (13.12%) and guaiol (12.84%) as major components, alongside phenolic compounds such as sakuranetin (0.30%) and usnetol (0.64%). Chromatographic isolation and crystallization yielded six known compounds and identified via spectroscopic comparison with literature: (3*S*)-1,7-diphenylheptan-3-ol (**1**), germacrone (**2**), zederone (**3**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**). For structural elucidation of compound **1**, it was characterized as (3*S*)-1,7-diphenylheptan-3-ol using UV, FT-IR, NMR (1D and 2D), HR-ESI-MS, and EI-MS. NMR analysis confirmed the hydroxyl substitution at C-3 in the diphenylheptanoid backbone. OR measurements, $[\alpha]_D^{23}$ +9.0 (*c* 0.41, $CHCl_3$) and $[\alpha]_D^{23}$ +8.2 (*c* 0.41, MeOH), matched reported values for the 3*S* enantiomer, marking the first isolation of this compound from a natural source, as it was previously only obtained synthetically.

Except for compounds **5** and **6**, all tested compounds showed moderate NO inhibitory activity in RAW 264.7 cells, with IC_{50} values in the low micromolar range. Literature comparisons revealed that germacrone (**2**), (–)-curcumene (**4**), curcumin (**5**), and bisdemethoxycurcumin (**6**) have previously demonstrated potent NO inhibition, while zederone (**3**) has been reported to suppress NF-κB activation in HaCaT cells. Compound **1** had previously shown weak estrogenic-like activity and low anti-inflammatory effects in an edema model, but in this study, it demonstrated moderate NO inhibition. In conclusion, the results highlight 75% ethanol UAE as an effective method for obtaining anti-inflammatory constituents from *C. aromatica*, with sesquiterpenes and phenolic compounds as key bioactives. The findings also provide the first report of natural isolation of (3*S*)-1,7-diphenylhepten-3-ol and reinforce the traditional medicinal use of *C. aromatica* for inflammation-related conditions. This discovery identified natural compounds with remarkable potential for further development as anti-inflammatory therapeutics and further development in the cosmetic industry.

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MANDATORY DISCLOSURE ON USE OF ARTIFICIAL INTELLIGENCE

During the preparation of this work, the authors used ChatGPT 5.5 to improve the readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

AUTHOR CONTRIBUTIONS

Rossukhon Senchanthichai (R.S.) and Sakchai Hongthong (S.H.) conceived, designed, and performed the experiments. Chakkree Lekklar (C.L.) and S.H. analyzed the results. Chanchai Sukkum (C.S.) and Pitthaya Hinbuddee (P.H.) performed and commented on the bioassay experiments. Sataporn Deeying (S.D.) and S.H. gave constructive comments. The GC-MS data were measured and analyzed by Suwadee Chokchaisiri (S.C.). R.S. and S.H. drafted the manuscript and revised and finalized the manuscript, which was reviewed and approved by all authors. The authors agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.

ETHICAL APPROVAL

The author declares that this study does not involve any human participants, their data, biological material, or animals.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

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