

Chemical Composition and Product Quality Control of Turmeric (*Curcuma longa* L.)

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Abstract: Chemical constituents of various tissues of turmeric (*Curcuma longa* L.) have been extensively investigated. To date, at least 235 compounds, primarily phenolic compounds and terpenoids have been identified from the species, including 22 diarylheptanoids and diarylpentanoids, eight phenylpropene and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, five diterpenes, three triterpenoids, four sterols, two alkaloids, and 14 other compounds. Curcuminoids (diarylheptanoids) and essential oils are major bioactive ingredients showing various bioactivities in *in vitro* and *in vivo* bioassays. Curcuminoids in turmeric are primarily accumulated in rhizomes. The essential oils from leaves and flowers are usually dominated by monoterpenes while those from roots and rhizomes primarily contained sesquiterpenes. The contents of curcuminoids in turmeric rhizomes vary often with varieties, locations, sources, and cultivation conditions, while there are significant variations in composition of essential oils of turmeric rhizomes with varieties and geographical locations. Further, both curcuminoids and essential oils vary in contents with different extraction methods and are unstable with extraction and storage processes. As a result, the quality of commercial turmeric products can be markedly varied. While curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**5**) have been used as marker compounds for the quality control of rhizomes, powders, and extract ("curcumin") products, *Ar*-turmerone (**99**), α -turmerone (**100**), and β -turmerone (**101**) may be used to control the product quality of turmeric oil and oleoresin products. Authentication of turmeric products can be achieved by chromatographic and NMR techniques, DNA markers, with morphological and anatomic data as well as GAP and other information available.

Keywords: Turmeric, *Curcuma longa* L., rhizomes, ground turmeric, turmeric oils, turmeric oleoresin, curcuminoids, curcumin, sesquiterpenes, marker compounds, adulteration, standardization.

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia but is now widely cultivated in the tropical and subtropical regions of the world. The deep orange-yellow powder known as turmeric is prepared from boiled and dried rhizomes of the plant. It has been commonly used as spice and medicine (*Rhizome Curcumae Longae*), particularly in Asia. In Ayurveda medicine, turmeric is primarily used as a treatment for inflammatory conditions and in traditional Chinese medicine, it is used as stimulant, aspirant, carminative, cordial, emenagogue, astringent, detergent, diuretic and martinet [1-3]. In India and China, wild turmeric (*C. aromatica* Salisb., commonly called as Kasthuri manjal or yujin) is sometimes used as turmeric production [4]. This species is known as *C. wenyujin* Y.H. Chen et C. Ling in China. It was also occasionally used to substitute *Rhizome Curcumae*

Longae but recently it has been separated as *Rhizoma Wenyujin Concisum* in the 2005 version of the Pharmacopoeia of People's Republic of China [5]. In Thailand and some other countries, *C. domestica* Val. is also used as the scientific name of turmeric [6-8] although it is recognized as a synonym of *C. longa* [9].

There are extensive *in vitro* and *in vivo* investigations on turmeric extracts (ethanol, methanol, water, and ethyl acetate extracts) or "pure" active "curcumin" (actually it was a mixture of three major curcuminoids in many cases) powder over the last half century. The role of curcumin (**1**), one of the most studied chemopreventive agents, on anti-inflammatory and cancer activity has been well appreciated [3, 10-19]. Data from cell culture, animal research, and clinical trials indicate that curcumin may have potential as a therapeutic agent in diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis [3, 20]. The anti-cancer effect has been reported in a few clinical trials, mainly as a chemoprevention agent in colon and pancreatic cancer, cervical neoplasia and Barrets metaplasia [16]. The compound modulates several molecular targets and inhibits transcription factors (NF-kB, AP-1), enzymes (COX-1, COX-2, LOX), cytokines (TNF, IL-1, IL-6) and

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antiapoptotic genes (BCL2, BCL2L1) [21-24]. As a result, curcumin (**1**) is able to induce apoptosis and has antiangiogenic activity [25, 26].

Turmeric extracts or the active curcuminoids have also shown hepato- and cardioprotective [27, 28], hypoglycemic [29, 30], anti-amyloidogenic [31], antifungal [32], parasiticidal [33, 34], antioxidant [35, 36], and chemo-resistance and radio-resistance activities [16]. Recent *in vitro* and *in vivo* studies and clinical trials in China and USA suggest that curcumin might be one of the most promising compounds for the development of Alzheimer's disease therapies [37]. Accumulating evidences suggest that curcumin (**1**) may regulate lipid metabolism, which plays a central role in the development of obesity and its complications [38]. Recently, it was found that curcumin (**1**) and demethoxycurcumin (**2**) can reduce lead-induced memory deficits in rats [39]. Turmeric oils/oleoresin or a major compound *ar*-turmerone (**99**) have shown antimicrobial [40-43], larvicidal [44], and antioxidant activities [45]. Essential oils of *Curcuma* also exerts triglyceride-lowering activity on serum as well as liver triglycerides [46].

Curcuma longa is often cultivated to harvest rhizomes (Fig. 1) for ground turmeric powder as a spice and food coloring agent (used alone or in mustard paste or curry powder). The plant has also been recognized as a pharmaceutical crop for production of standardized therapeutic extracts (STEs) or small therapeutic molecules (STMs) [47]. India is the largest producer of turmeric supplying over 90% of the world's demand [48]. The country produced about 716,900 Mt of turmeric from approximately 161,300 hectares of crops during 2004-2005 [49]. China also has cultivated turmeric for both domestic use and export. There are about 70 cultivars or varieties of *C. longa* cultivated in India, some important regional trade varieties of turmeric are 'Rajapuri', 'Duggirala', 'Cuddappah', 'Berhampur', 'Erode', 'Nizamabad', 'Koraput', 'Kasturi', 'Chaya', 'Kodur', 'Salem', 'Waigon', 'Alleppey', 'Karur', 'Tekurpeta' [2]. Turmeric is valued primarily for curcumin (**1**). Thus, curcumin (**1**) content has been an important factor in developing and selecting cultivar or variety for turmeric production and in determining the price of turmeric [50]. For example, PTS-10 and PTS-24, two clones were selected for rhizome yield and high dry recovery and both can yield 9.3% curcumin (**1**) [51]. Curcumin (**1**) can also be produced by chemical synthesis but the synthetic curcumin (**1**) is not used as a food additive. The main pharmaceutical products from turmeric are dried whole rhizomes, ground turmeric, turmeric oils, turmeric oleoresin, and curcumin (maybe actually mixture of three curcuminoids) [49, 52] (Table 1).

CHEMICAL CONSTITUENTS

Of 110 species of the genus *Curcuma* L., only about 20 species have been studied phytochemically [53]. *Curcuma longa* is the most chemically investigated species of *Curcuma*. To date, at least 235 compounds, primarily phenolic compounds and terpenoids have been identified, including diarylheptanoids (including commonly known as curcuminoids), diarylpentanoids, monoterpenes, sesquiterpenes, diterpenes, triterpenoids, alkaloid, and sterols, etc.



Fig. (1). *Curcuma longa* is primarily cultivated for turmeric rhizomes and their products (The upper picture shows the plants cultivated at the SFA Mast Arboretum, Stephen F. Austin State University in Nacogdoches, Texas, USA and the lower picture shows rhizomes and ground turmeric as well as curry powder. Photos by S.Y. Li).

Phenolic Compounds

Diarylheptanoids and Diarylpentanoids

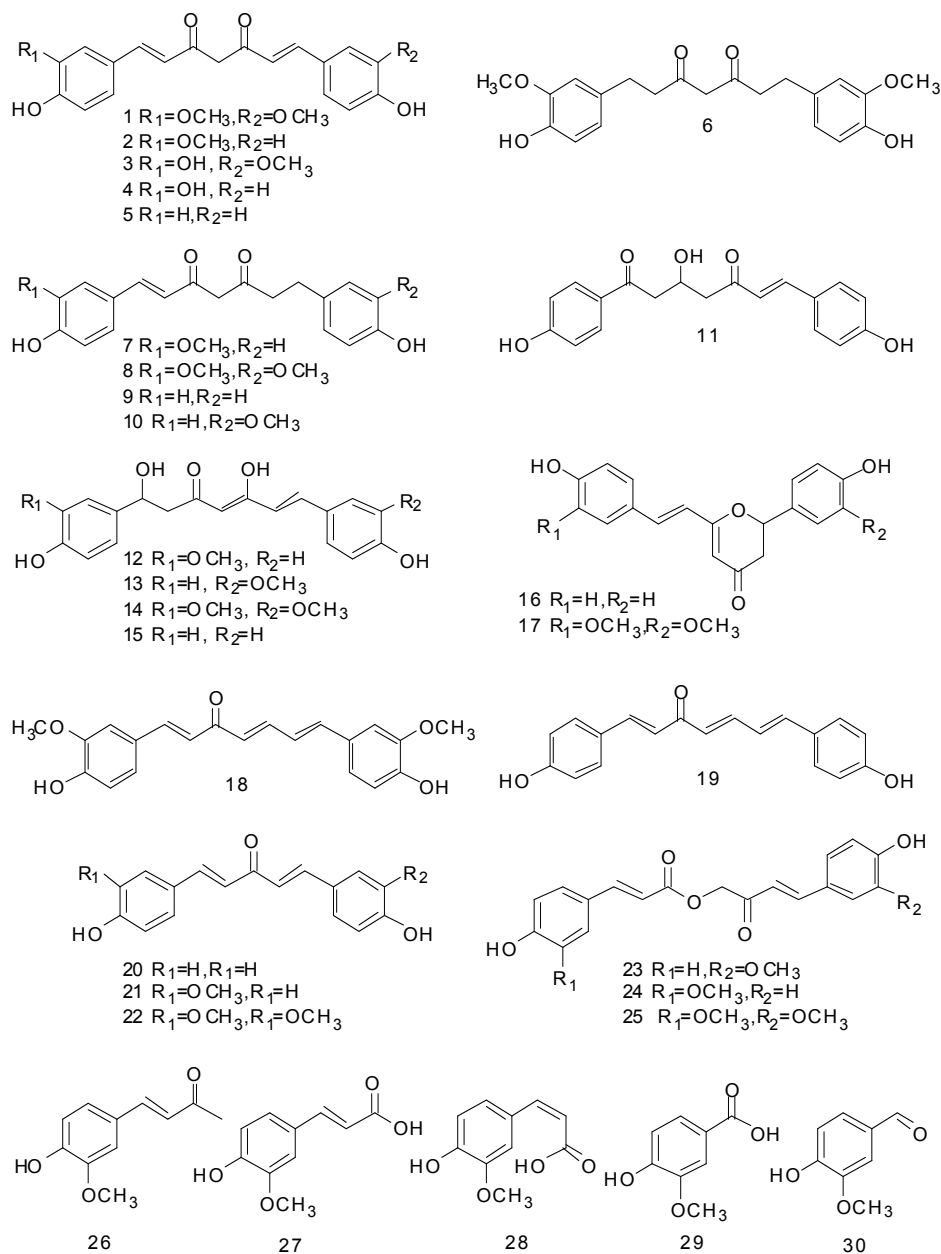
Over 300 diarylheptanoids have been reported in the family Zingiberaceae and some non-closely related families [54]. Curcuminoids belong to the group of diarylheptanoids (or diphenylheptanoids) having an aryl-C7-aryl skeleton (1-19). These yellow pigments are usually used as food coloring agents and they are the main active compounds in turmeric. Usually, these polyphenols are present in 3-15% of turmeric rhizomes with curcumin (**1**) as the principal compound. Curcumin ($C_{21}H_{20}O_5$) (**1**), also known as diferuloyl methane or 1,6-heptadiene-3,5-dione-1,7-bis(4-hydroxy-3-methoxyphenyl)-(1E,6E), was isolated in 1815 [49] and its chemical structure was determined in 1910 [55]. The compound is a yellow-orange powder with a molecular weight of 368.37. It is water insoluble but can be dissolved well in ethanol, methanol, acetone, and dimethylsulfoxide. Commercial "curcumin" is usually a mixture of three curcuminoids. For example, the composition of a commercial "curcumin" is about 71.5% curcumin (curcumin I) (**1**), 19.4% demethoxycurcumin (curcumin II) (**2**), and 9.1% bisdemethoxycurcumin (curcumin III) (**5**) [56]. These three major curcuminoids are also found in some other species of *Curcuma* but have lower concentrations, e.g., *C. amada* Roxb. [57], *C. aeruginosa*

Table 1. Main products of turmeric (*Curcuma longa*)

Product	Description	Uses
Dried Whole Rhizome	<p><i>Preparation:</i> mother rhizomes (egg-shipped primary rhizomes) and finger rhizomes (cylindrical and multibranched secondary rhizomes) are usually boiled separately for about 40-60 min under slightly alkaline conditions in copper, galvanized iron or earth vessels and then sun-dried on bamboo mats for 10-15 days to reduce the moisture to 10-11%</p> <p><i>Harvest:</i> usually 7-9 months after planting during January-March</p> <p><i>Appearance:</i> orange-brown, pale yellow or red-yellow</p> <p><i>Chemical Composition:</i> may contain 3-15% cucuminoids and 1.5 to 5% essential oils</p>	Medicine (<i>Rhizoma Curcumae Longae</i>) and process of other turmeric products
Ground Turmeric	<p><i>Preparation:</i> Powder is prepared from dried finger rhizomes (60-80 mesh)</p> <p><i>Appearance:</i> yellow or red-yellow powder</p> <p><i>Chemical Composition:</i> The contents of active ingredients curcuminoids and essential oils may decrease during the process and exposure to light, it is appropriate to pack the powder in a UV protective container (e.g., fiber hard drums, glass packs, etc.)</p>	<p>Spice: as alone or in curry powder and pastes</p> <p>dye: for food, textile, cosmetic</p> <p>Medicine: e.g., in Ayurveda, Chinese medicine</p> <p>Dietary supplement</p>
Tumeric Oils	<p><i>Preparation:</i> Extract from dried rhizomes (ground turmeric) or leaves by steam distillation or supercritical CO₂ extraction</p> <p><i>Chemical Composition:</i> essential oils from leaves is usually dominated by monoterpenes while the oil from rhizomes mainly contains sesquiterpenes</p> <p><i>Appearance:</i> yellow to brown viscous liquid</p> <p><i>Refractive Index:</i> 1.4850-1.5250</p> <p><i>Flash Point:</i> 78°C</p> <p><i>Solubility in Water:</i> insoluble</p>	Spice, medicine, and dietary supplement
Tumeric Oleoresin	<p><i>Preparation:</i> Extract from dried rhizomes by solvent extraction with aceone, dichloromethane, 1,2-dichloroethane, methanol, ethanol, isopropanol and light petroleum (hexanes) or supercritical CO₂ extraction. Graded by the content of curcuminoids or color value</p> <p><i>Chemical Composition:</i> 37-55% curcuminoids and up to 25% essential oil</p> <p><i>Appearance:</i> yellow-dark reddish brown oily fluids</p> <p><i>Refractive Index:</i> 1.4850-1.5250</p> <p><i>Flash Point:</i> 78°C</p> <p><i>Solubility in Water:</i> insoluble</p>	Food coloring, medicine, and dietary supplement
Curcumin (turmeric yellow, kurkum)	<p><i>Preparation:</i> obtained by solvent extraction from ground turmeric rhizomes and purification of the extract by crystallization. The suitable solvents include aceone, carbon dioxide, ethanol, ethyl acetate, hexane, methanol, isopropanol</p> <p><i>Chemical Composition:</i> the product is often the mixture of curcumin and its demethoxy- and bisdemethoxy- derivatives in turmeric in varying proportions. The three major curcuminoids may account no less than 90%. Minor compounds may include oils and resins naturally occurring in turmeric rhizomes</p> <p><i>Appearance:</i> yellowish to orange red crystalline powder</p> <p><i>Molecular Formula:</i> C₂₁H₂₀O₆</p> <p><i>Molecular Weight:</i> 368.38</p> <p><i>Solubility in Water:</i> insoluble</p>	Medicine and dietary supplement

Roxb. [58, 59], *C. aromatica* [53], *C. chuanyujin* Roxb. [60], *C. heyneana* Val. & Zijp. [59], *C. mangga* Val. & Zijp. [59], *C. soloensis* Val. [59], *C. xanthorrhiza* Roxb. [58, 61], and *Curcuma zedoaria* (Berg.) Rosc. [62]. It is expected that these common curcuminoids may occur in some other species of *Curcuma* although there are no chemical investigations conducted on most of the 110 species of the genus. Some minor

and rare curcuminoids of *C. longa* or their analogs may be identified in other species. For example, cyclocurcumin (**17**) with cyclization of the seven-carbon unit as a pyrone ring, was only found in *C. longa* [53, 63]. Recently, 3'-demethoxycyclocurcumin was isolated from *C. xanthorrhiza* [64].



No.	Compound Name	Compound Type	Ref.
1	curcumin (curcumin I)	Diarylheptanoid	[65, 66]
2	demethoxycurcumin (curcumin II)	Diarylheptanoid	[65, 66]
3	1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione	Diarylheptanoid	[67]
4	1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione	Diarylheptanoid	[67]
5	bisdemethoxycurcumin (curcumin III)	Diarylheptanoid	[65]
6	tetrahydroxycurcumin	Diarylheptanoid	[65]
7	5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[68]
8	5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[68, 69]
9	1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione	Diarylheptanoid	[68]

No.	Compound Name	Compound Type	Ref.
10	5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[68]
11	3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	Diarylheptanoid	[67]
12	1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[67]
13	1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[67]
14	1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[67]
15	1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one	Diarylheptanoid	[67]
16	1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene	Diarylheptanoid	[7]
17	cyclocurcumin	Diarylheptanoid	[70]
18	1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one	Diarylheptanoid	[69]
19	1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one	Diarylheptanoid	[71]
20	1,5-bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one	Diarylpentanoid	[71]
21	1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1,4-pentadiene-3-one	Diarylpentanoid	[67]
22	1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one	Diarylpentanoid	[72]
23	4''-(4'''-hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate	Phenylpropene	[73]
24	4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate	Phenylpropene	[73]
25	calebin-A	Phenylpropene	[65]
26	(E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one	Phenylpropene	[72]
27	(E)-ferulic acid	Phenylpropene	[72]
28	(Z)-ferulic acid	Phenylpropene	[72]
29	vanillic acid*	Phenolic	[72]
30	vanillin	Phenolic	[72]

There are three diarylpentanoids (or diphenylpentanoids) with a five-carbon chain between two phenyl groups (**20-22**).

Curcuminoids have shown different activities. A recent study suggested that curcumin (**1**) had the relative higher potency for suppression of tumor necrosis factor (TNF)-induced nuclear factor- κ B (NF- κ B) activation than that of demethoxycurcumin (**2**) and bisdemethoxycurcumin (**5**), while tetrahydrocurcumin (**6**) without the conjugated bonds in the central seven-carbon chain was completely inactive [21]. The results suggest that the methoxy groups on the phenyl ring has critical role but conjugated bonds in the central seven-carbon chain also important for curcuminoids' NF- κ B activity [21]. However, the suppression of proliferation of various tumor cell lines by curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**5**) was found to be comparable; indicating the methoxy groups play minimum role in the anti-proliferative effects of curcuminoids [21]. A further investigation of structure-activity relationship is needed by using pure curcuminoids.

It was interesting to mention that synergistic effect of mixture of compounds in turmeric had been observed. For example, it was found that methanolic and chloroformic extracts of turmeric demonstrated nematocidal activity against *Toxocara canis* [63]. All the substances including cyclocur-

cumin (**17**) did not show activity when applied independently, but the activity was observed when they were mixed, suggesting a synergistic action between them.

Because the difficulty in separation of three curcuminoids each other, the commercially pure compounds of curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**5**) available as authentic samples are limited. According to our analysis, the commercial "pure" curcumin (**1**) (labeled as 94%) actually has only purity of about 70%. Therefore, at least some existing studies and discoveries on "curcumin" actually used the mixture of three curcuminoids. However, a pure (>95%) curcumin (**1**) becomes important for bioassays and mechanism investigations as well as clinical trials. Research on bioactivity of curcuminoids are primarily focused on the above three major curcuminoids (**1**, **2**, and **5**), and bioactivities of minor curcuminoids remain elusive.

Phenylpropenes and Other Phenolic Compounds

Six monomeric phenylpropenes (**23-28**), vanillic acid (**29**), and vanillin (**30**) were identified in *C. longa*.

Terpenes

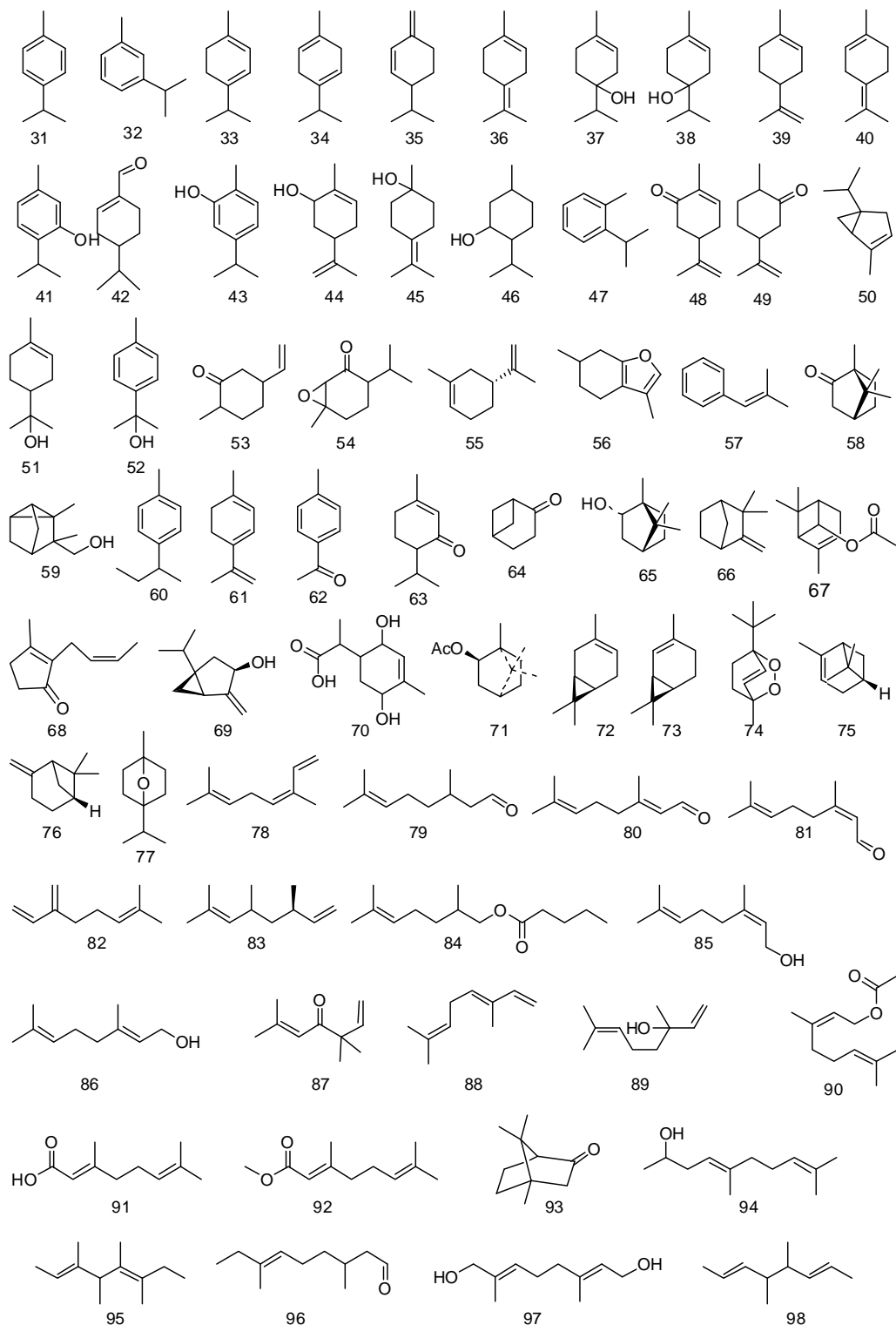
To date, at least 185 compounds of terpenes have been isolated or detected from leaves, flowers, roots and rhizomes

of *C. longa*, including 68 monoterpenes (31-98), 109 sesquiterpenes (99-207), five diterpenes (208-212), and three triterpenoids (213-215).

Monoterpenes

The volatile oils from leaves and flowers of *C. longa* were usually dominated by monoterpenes, particularly *p*-cymene (31), β -phellandrene (β -felandrene) (35), terpinolene

(terpenoline) (40), *p*-cymen-8-ol (55), cineole (77), and myrcene (82) while the major part of the oil from roots and rhizomes contained sesquiterpenes [49, 74, 75]. This chemical characteristic can be useful in identification of leaves or flowers of *C. longa* used to substitute its rhizome for turmeric oil production. In total, 68 monoterpenes (31-98) have been identified from various tissues of *C. longa* [75-82].



No.	Compound Name	Compound Type	Ref.
31	<i>p</i> -cymene*	Monoterpenoid	[77]
32	<i>m</i> -cymene *	Monoterpenoid	[78]
33	α -terpinene*	Monoterpenoid	[78]
34	γ -terpinene*	Monoterpenoid	[78]
35	β -phellandrene*	Monoterpenoid	[78]
36	<i>p</i> -mentha-1,4(8)-diene*	Monoterpenoid	[79]
37	terpinen-4-ol*	Monoterpenoid	[79]
38	4-terpinol*	Monoterpenoid	[80]
39	limonene*	Monoterpenoid	[81]
40	terpinolene*	Monoterpenoid	[81]
41	thymol*	Monoterpenoid	[78]
42	phellandrol*	Monoterpenoid	[77]
43	carvacrol*	Monoterpenoid	[81]
44	(<i>E</i>)-carveol*	Monoterpenoid	[81]
45	γ -terpineol*	Monoterpenoid	[79]
46	menthol	Monoterpenoid	[76]
47	1,3,8-paramenthatriene	Monoterpenoid	[75]
48	<i>p</i> -methylacetophenone	Monoterpenoid	[75]
49	piperitone	Monoterpenoid	[76]
50	<i>o</i> -cymene*	Monoterpenoid	[79]
51	carvone*	Monoterpenoid	[77]
52	<i>p</i> -menth-8-en-2-one*	Monoterpenoid	[81]
53	α -thujene*	Monoterpenoid	[78]
54	α -terpineol*	Monoterpenoid	[77]
55	<i>p</i> -cymen-8-ol*	Monoterpenoid	[79]
56	<i>p</i> -meth-8-en-2-one*	Monoterpenoid	[79]
57	piperitone epoxide*	Monoterpenoid	[79]
58	sylvestrene*	Monoterpenoid	[79]
59	menthofuran*	Monoterpenoid	[77]
60	β , β -dimethylstyrene	Monoterpenoid	[76]
61	camphor	Monoterpenoid	[76]
62	teresantalol	Monoterpenoid	[76]
63	benzene, 1-methyl-4-(1-methylpropyl)	Monoterpenoid	[76]
64	2-norpinanone*	Monoterpenoid	[81]
65	borneol*	Monoterpenoid	[78]
66	bornyl acetate*	Monoterpenoid	[81]
67	(<i>E</i>)-chrysanthenyl acetate*	Monoterpenoid	[81]
68	(<i>Z</i>)-cinerone*	Monoterpenoid	[18]

Contd....

No.	Compound Name	Compound Type	Ref.
69	(Z)-sabinol*	Monoterpenoid	[18]
70	2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid	Monoterpenoid	[82]
71	camphene*	Monoterpenoid	[78]
72	3-carene*	Monoterpenoid	[81]
73	2-carene*	Monoterpenoid	[77]
74	ascaridole*	Monoterpenoid	[81]
75	α -pinene*	Monoterpenoid	[77]
76	β -pinene*	Monoterpenoid	[79]
77	cineole*	Monoterpenoid	[79]
78	<i>cis</i> -ocimene*	Monoterpenoid	[78]
79	citronellal*	Monoterpenoid	[77]
80	geranial*	Monoterpenoid	[78]
81	neral*	Monoterpenoid	[78]
82	myrcene*	Monoterpenoid	[78]
83	<i>R</i> -citronellene*	Monoterpenoid	[79]
84	citronellyl pentanoate*	Monoterpenoid	[77]
85	nerol*	Monoterpenoid	[77]
86	geraniol	Monoterpenoid	[76]
87	iso-artemisia ketone*	Monoterpenoid	[78]
88	<i>trans</i> -ocimene*	Monoterpenoid	[78]
89	linalool*	Monoterpenoid	[77]
90	neryl acetate	Monoterpenoid	[76]
91	geranic acid	Monoterpenoid	[76]
92	geranyl acetate	Monoterpenoid	[76]
93	3-bornanone	Monoterpenoid	[76]
94	4,8-dimethyl-3,7-nonadien-2-ol	Monoterpenoid	[76]
95	3,4,5,6-tetramethyl-2,5-octadiene	Monoterpenoid	[76]
96	3,7-dimethyl-6-nonenal	Monoterpenoid	[76]
97	2,6-dimethyl-2,6-octadiene-1,8-diol	Monoterpenoid	[76]
98	4,5-dimethyl-2,6-octadiene	Monoterpenoid	[76]

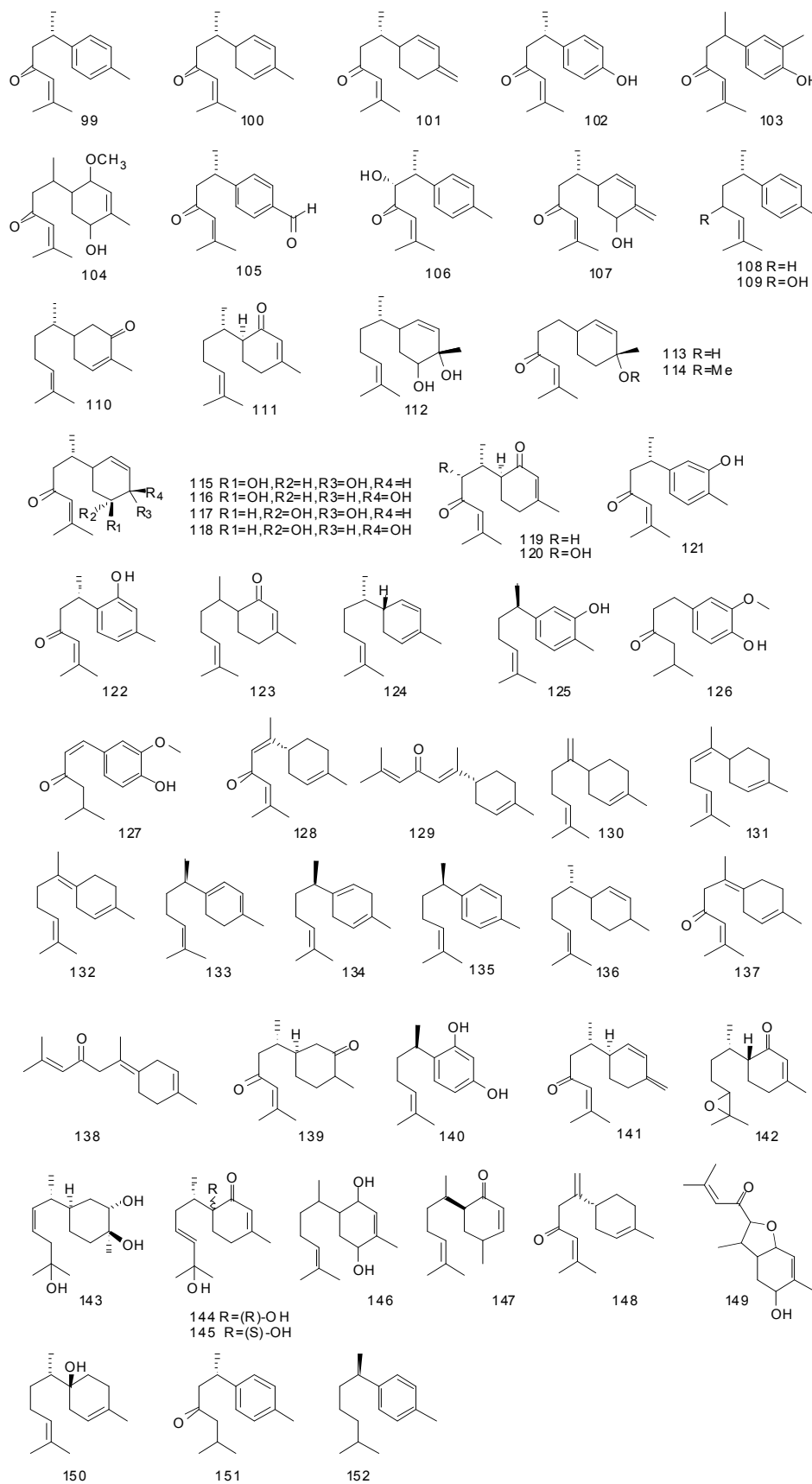
Sesquiterpenes

Dried turmeric rhizomes usually yields 1.5 to 5% essential oils which are dominated by sesquiterpenes and are responsible for its aromatic taste and smell. *Ar*-turmerone (**99**), α -turmerone (**100**) [83], and β -turmerone (**101**) [83] are major ketonic sesquiterpenes of essential oils, and these compounds may account for at least 40% of essential oils of turmeric rhizomes [84-86]. Two sesquiterpene ketoalcohols-turmeronol A (**121**) and turmeronol B (**122**) were isolated

from the dried turmeric rhizome [87]. To date, 109 compounds of sesquiterpenes have been identified, belonging to various types: 54 bisabolanes (**99-152**) [15, 16, 72-80, 82, 83, 87-93], six germacrane (**153-158**) [77, 78, 90], seven guaianes (**159-165**) [72, 90], four selinanes (**166-169**) [76, 79], three santalanes (**170-172**) [76], two caryophyllanes (**173** and **174**) [76, 81], two elemans (**175** and **176**) [76, 79], acorane (**177**) [76], aristolene (**178**) [76], bergamotane (**179**) [81], carabrane (**180**) [90], cedrane (**181**) [76], himachalene

(182) [76], and sesquisabinane (183) [81] as well as 24 other sesquiterpenes (184-203) [72, 75, 76, 78, 79, 81, 92, 94]. Bisabolanes are the most abundant sesquiterpenes in *Cur-*

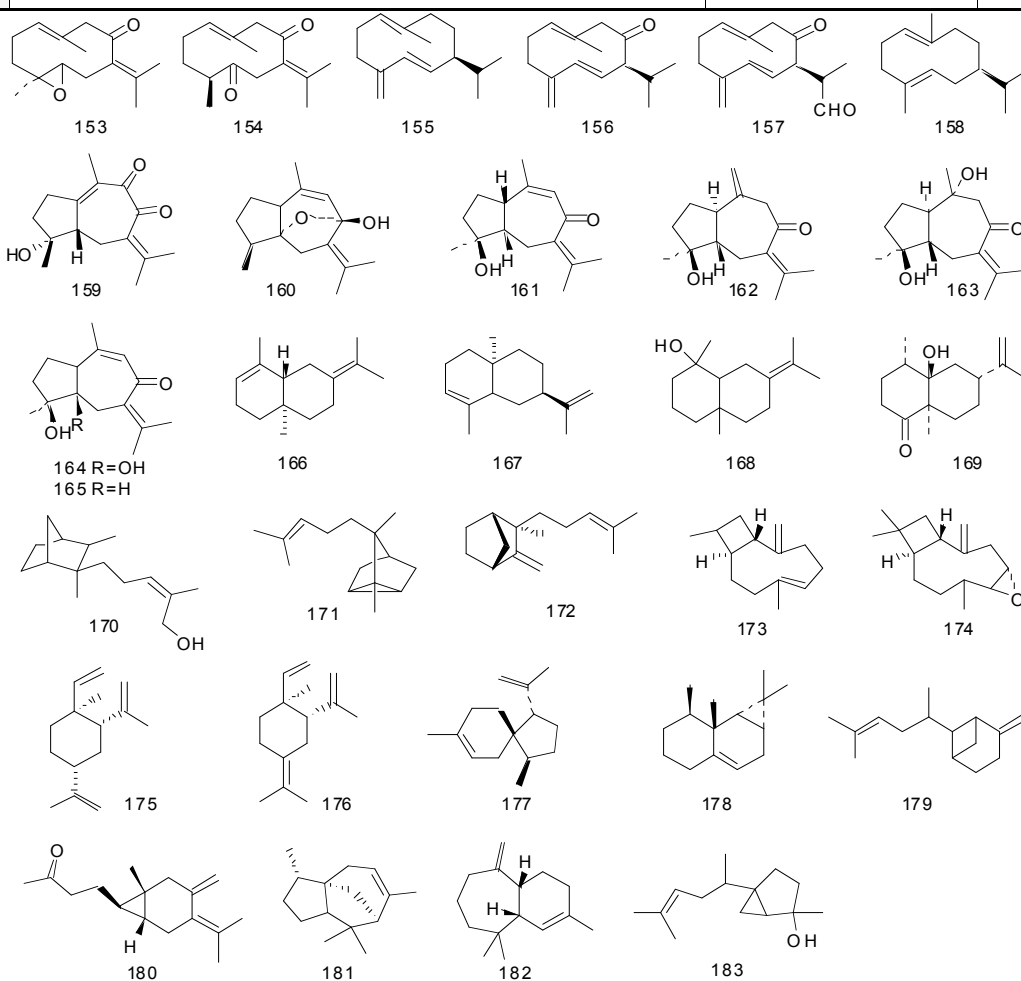
cuma, and *Ar-turmerone* (99) widely occurs in many species of the genus [53].



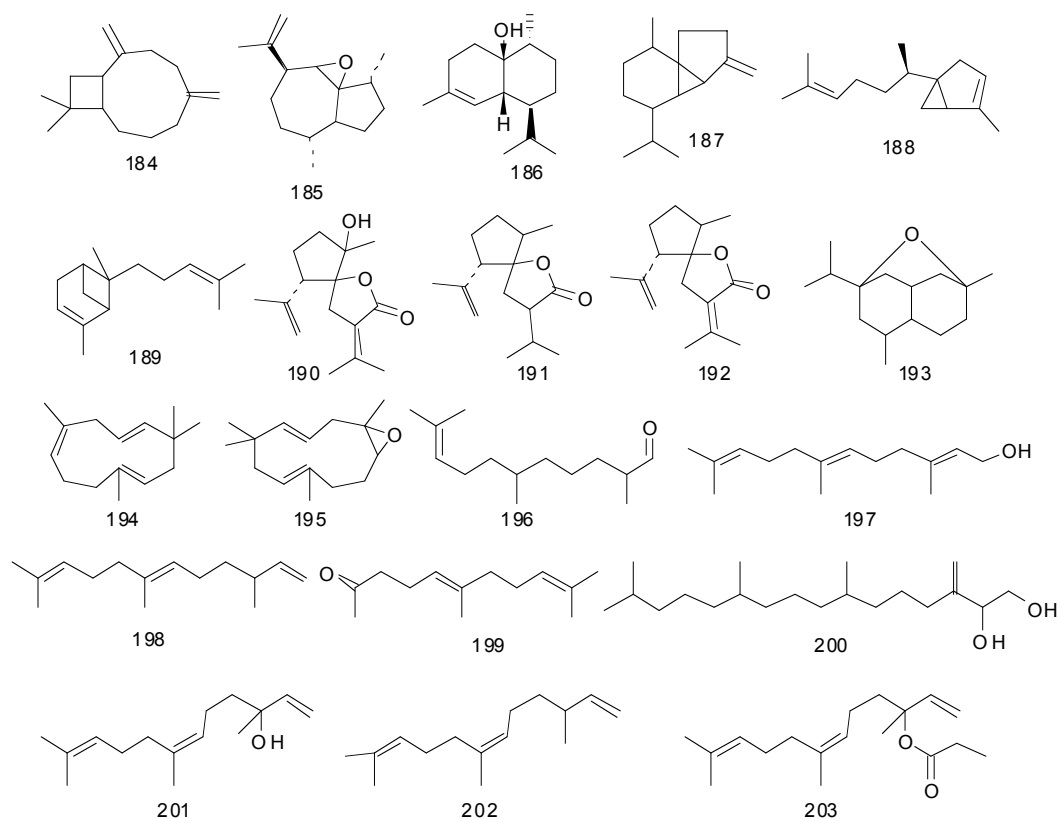
No.	Compound Name	Compound Type	Ref.
99	<i>ar</i> -turmerone*	Bisabolane	[14, 88]
100	α -turmerone*	Bisabolane	[83, 88]
101	β -turmerone*	Bisabolane	[83, 89]
102	2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one	Bisabolane	[73]
103	2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one	Bisabolane	[82]
104	2-methoxy-5-hydroxybisabola-3,10-diene-9-one	Bisabolane	[82]
105	2-methyl-6-(4-formylphenyl)-2-hepten-4-one	Bisabolane	[73]
106	5-hydroxyl- <i>ar</i> -turmerone	Bisabolane	[73]
107	4-methylene-5-hydroxybisabola-2,10-diene-9-one	Bisabolane	[82]
108	<i>ar</i> -curcumene*	Bisabolane	[80]
109	<i>ar</i> -turmerol*	Bisabolane	[80]
110	bisabola-3,10-diene-2-one	Bisabolane	[15]
111	bisabolone	Bisabolane	[73]
112	4, 5-dihydroxybisabola-2,10-diene	Bisabolane	[91]
113	4-hydroxybisabola-2,10-diene-9-one	Bisabolane	[90]
114	4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one	Bisabolane	[90]
115	bisacurone	Bisabolane	[72]
116	bisacurone A	Bisabolane	[82]
117	bisacurone B	Bisabolane	[91]
118	bisacurone C	Bisabolane	[91]
119	bisabolone-9-one	Bisabolane	[80]
120	bisacumol	Bisabolane	[90]
121	turmeronol A	Bisabolane	[82, 87]
122	turmeronol B	Bisabolane	[73, 87]
123	α -oxobisabolene*	Bisabolane	[79]
124	α -zingiberene*	Bisabolane	[78]
125	xanthorrhizol*	Bisabolane	[77]
126	zingerone	Bisabolane	[91]
127	dehydrozingerone	Bisabolane	[91]
128	(<i>Z</i>)- α -atlantone*	Bisabolane	[78, 92]
129	(<i>E</i>)- α -atlantone*	Bisabolane	[92]
130	β -bisabolene*	Bisabolane	[78]
131	(6 <i>S</i> ,7 <i>R</i>)-bisabolene*	Bisabolane	[81]
132	γ -bisabolene*	Bisabolane	[79]
133	γ -curcumene*	Bisabolane	[78]
134	β -curcumene*	Bisabolane	[93]
135	α -curcumene*	Bisabolane	[77]
136	β -sesquiphellandrene*	Bisabolane	[77]

Contd....

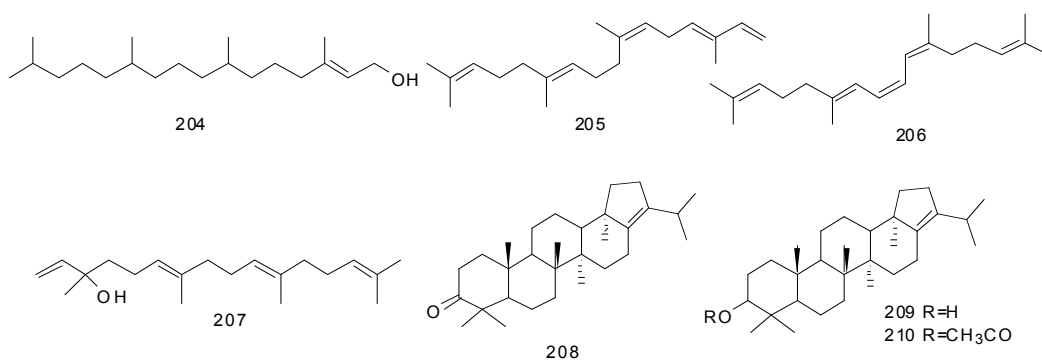
No.	Compound Name	Compound Type	Ref.
137	(Z)- γ -atlantone*	Bisabolane	[78, 92]
138	(E)- γ -atlantone*	Bisabolane	[92]
139	(6S)-2-methyl-6-[(1R,5S)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one	Bisabolane	[80]
140	curcuphenol*	Bisabolane	[79]
141	curlone	Bisabolane	[72]
142	curculonone C	Bisabolane	[72]
143	curculonone D	Bisabolane	[72]
144	curculonone B	Bisabolane	[72]
145	curculonone A	Bisabolane	[72]
146	2, 5-dihydroxybisabola-3, 10-diene	Bisabolane	[91]
147	(6R)-[(1R)-1,5-dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one	Bisabolane	[72]
148	β -atlantone	Bisabolane	[72]
149	2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one	Bisabolane	[82]
150	α -bisabolol	Bisabolane	[76]
151	dihydro-ar-turmerone	Bisabolane	[92]
152	dehydrocurcumene	Bisabolane	[75]



No.	Compound Name	Compound Type	Ref.
153	(4 <i>S</i> ,5 <i>S</i>)-germacrone-4,5-epoxide	Germacrane	[90]
154	dehydrocurdione	Germacrane	[90]
155	germacrene D*	Germacrane	[77]
156	germacrone	Germacrane	[90]
157	germacrone-13-al	Germacrane	[90]
158	β -germacene*	Germacrane	[78]
159	1,10-dehydro-10-deoxy-9-oxozedoarondiol	Guaiane	[72]
160	curcumenol	Guaiane	[90]
161	epiprocurcumenol	Guaiane	[90]
162	isoprocurcumenol	Guaiane	[90]
163	zedoaronediol	Guaiane	[90]
164	procurcumadiol	Guaiane	[90]
165	procurcumenol	Guaiane	[90]
166	naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)	Selinane	[76]
167	α -selinene	Selinane	[76]
168	juniper camphor	Selinane	[76]
169	corymbolone*	Selinane	[79]
170	α -santalol	Santalane	[76]
171	α -santalene	Santalane	[76]
172	β -santalene	Santalane	[76]
173	(<i>E</i>)-caryophyllene*	Caryophyllane	[81]
174	caryophyllene oxide	Caryophyllane	[76]
175	β -elemene*	Elemene	[79]
176	γ -elemene	Elemene	[76]
177	acoradiene	Acorane	[76]
178	aristolene	Aristolene	[76]
179	(<i>Z</i>)- α -bergamotene*	Bergamotane	[81]
180	curcumenone	Carabrane	[90]
181	di-epi-cedrene	Cedrane	[76]
182	himachalene	Himachalene	[76]
183	(<i>E</i>)-sesquisabinene hydrate*	sesquisabinane	[81]



No.	Compound Name	Compound Type	Ref.
184	bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene)	Sesquiterpene	[76]
185	γ -gurjunen epoxide	Sesquiterpene	[76]
186	1-epi-cubenol	Sesquiterpene	[92]
187	cubebene*	Sesquiterpene	[78]
188	7-epi-sesquithujene*	Sesquiterpene	[81]
189	caryophyllene*	Sesquiterpene	[77]
190	6 α -hydroxycurcumanolide A	Sesquiterpene	[72]
191	curcumanolide A	Sesquiterpene	[72]
192	curcumanolide B	Sesquiterpene	[72]
193	curcumin L	Sesquiterpene	[94]
194	α -humulene*	Sesquiterpene	[78]
195	12-oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,	Sesquiterpene	[76]
196	adoxal	Sesquiterpene	[76]
197	2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-	Sesquiterpene	[76]
198	(<i>E,E</i>)- α -farnesene*	Sesquiterpene	[81]
199	5,9-undecadien-2-one, 6,10-dimethyl-, (<i>Z</i>)-	Sesquiterpene	[76]
200	hxadecane-1,2-diol*	Sesquiterpene	[79]
201	nerolidal	Sesquiterpene	[75]
202	(<i>Z</i>)- β -farnesene*	Sesquiterpene	[81]
203	nerolidyl propionate	Sesquiterpene	[76]



No.	Compound Name	Compound Type	Ref.
204	phytol*	Diterpenoid	[79]
205	(<i>E,E,E</i>)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene	Diterpenoid	[76]
206	2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene	Diterpenoid	[76]
207	1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (<i>E,E</i>)-	Diterpenoid	[76]
208	hopenone I	Triterpenoid	[95]
209	hop-17(21)-en-3 β -ol	Triterpenoid	[95]
210	hop-17(21)-en-3 β -yl acetate	Triterpenoid	[95]

Other Terpenes

Four diterpenes (**204-207**) and three triterpenoids (**208-210**) were also identified in turmeric [76, 79, 88, 95].

Steroids

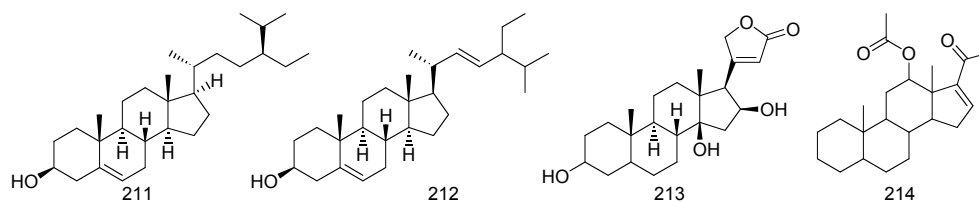
Four steroids (**211-214**) were identified from *C. longa* [72, 76]. But it is questionable that compounds **213** and **214** are present in *C. longa*.

Fatty Acids

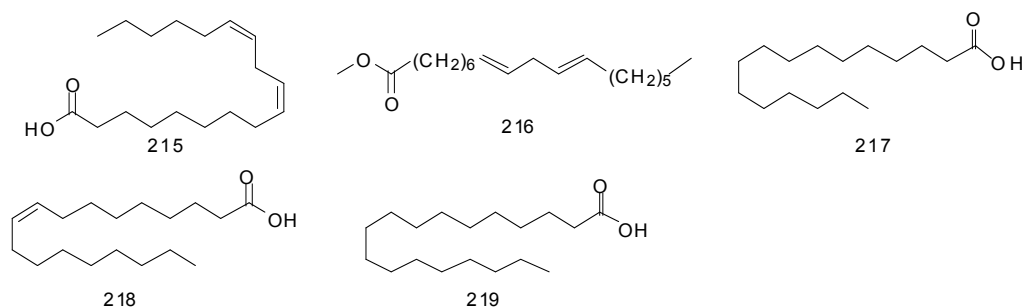
Following are five long chain fatty acids (**215-219**) identified from *C. longa* [72, 76].

Miscellaneous

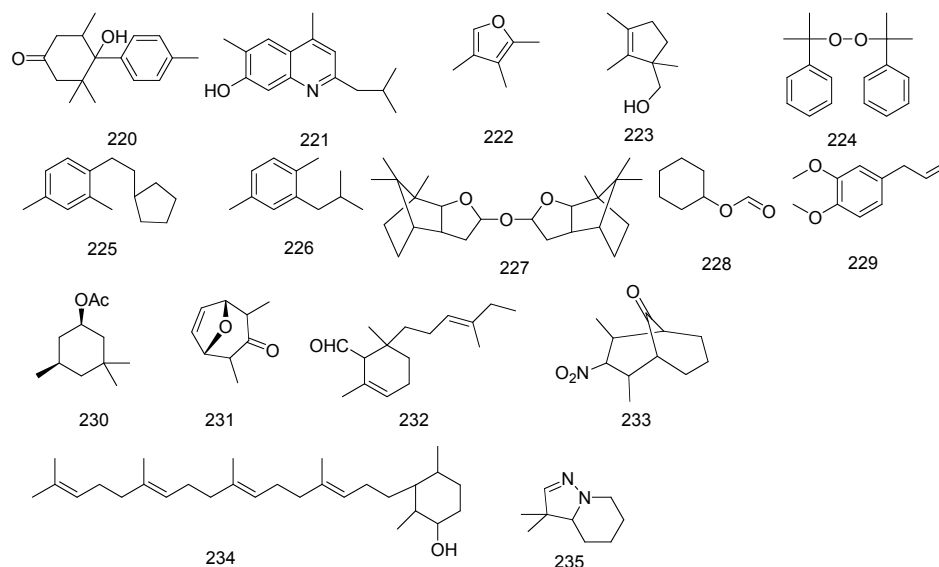
There are 16 other compounds (**220-235**) found in *C. longa* [76, 91, 96].



No.	Compound Name	Compound Type	Ref.
211	β -sitosterol	Steroid	[72]
212	stigmasterol	Steroid	[72]
213	gitoxigenin	Steroid	[76]
214	20-oxopregn-16-en-12-yl acetate	Steroid	[76]



No.	Compound Name	Compound Type	Ref.
215	linoleic acid*	Fatty acid	[79]
216	8,11-Octadecadienoic acid, methyl ester *	Fatty acid	[79]
217	palmitic acid (n-hexadecanoic acid)*	Fatty acid	[79]
218	oleic acid*	Fatty acid	[79]
219	stearic acid*	Fatty acid	[79]



No.	Compound Name	Ref.
220	curcuma-J	[96]
221	2-(2'-methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline	[91]
222	2,3,5-trimethylfuran	[76]
223	(1,2,3-trimethyl-cyclopent-2-enyl)-methanol	[76]
224	dicumyl peroxide	[76]
225	1-(3-cyclopentylpropyl)-2,4-dimethylbenzene,	[76]
226	1,4-dimethyl-2-(2-methylpropyl)-benzene	[76]
227	2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran	[76]
228	cyclohexyl formate	[76]
229	methyleugenol	[76]
230	3,3,5-trimethyl-cyclohexanol acetate	[76]
231	2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one	[76]
232	2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde	[76]
233	bicyclo[3.3.1]nonan-9-one, 2,4-dimethyl-3-nitro- (exo)-	[76]
234	2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol	[76]
235	pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS)	[76]

ACTIVE INGREDIENTS

Variations of Curcuminoids

Curcuminoids in turmeric are primarily accumulated in rhizomes of turmeric [97]. The contents of curcuminoids in

turmeric rhizomes vary often with varieties, locations, sources, and cultivation conditions [2, 8, 97-100] (Table 2). Ratnambak *et al.* reported curcumin (**1**) varied from 2.8% to 10.9% among the 120 cultivars or accessions of *C. longa* from all over India and 0.02% to 8.0% among the 64 culti-

vars or accessions of related species [50]. It was found that there are significant variations in curcumin (**1**) (0.61 to 1.45% on a dry weight basis) in turmeric rhizomes in its native North Indian plains [101]. Curcumin (**1**) contents of turmeric rhizomes in Thailand range from 1.28 to 6.6% (on a dry weight basis) [6]. Curcumin (**1**) contents in commercial turmeric powders vary from 0.58 to 3.14% (on a dry weight basis) [102]. It was also reported the total curcuminoids content of rhizomes from 66 locations in Thailand varies from 0.46 to 10.23% (on a dry weight basis) [103]. Turmeric crop cultivars in India have been classified as either high or low curcumin (**1**) varieties (e.g., 'Alleppey' having 5.5% curcumin (**1**)) as well as short, medium or long duration [2, 50, 104].

Plant maturity has significant impact on chemical constituents of turmeric rhizomes of *Curcuma*. In Sri Lanka, both total curcuminoids and curcumin (**1**) in rhizome reach the highest yield at 5.5 months and maturity results in decline of these pigments but essential oils will not reach maximum yield until 7.5 to 8 months [84]. Similarly, it was found the rhizomes of five-month old plants yield the highest contents of both total curcuminoids and curcumin (**1**) and contents declined from five to ten month-old plants in Thailand [7]. In Japan, it was reported that curcumin (**1**) content of primary rhizome increased from September to October [97]. However, there is no significant change in the content of curcuminoids was observed during the growing season from October to February [105]. In general, curcumin (**1**) content in mother rhizome is higher than in finger rhizomes [98, 106]. The composition of both curcuminoids and essential oils of the turmeric rhizomes from *in vitro* propagated plants had no significant difference from those in traditionally propagated plants [79].

Plants grow in different habitats may also affect curcumin (**1**) yield in rhizomes. It was found that turmeric grown in the South of Thailand with rainfall all year contained higher levels of total and individual curcuminoids [107]. A recent Japanese study showed that curcumin (**1**) content in rhizomes from the plants cultivated in dark-red soil is about 100% higher than in those from gray soil and more than 200% high than those from red soil [108]. It was also reported that potassium in soil positively affect curcumin (**1**) yield in rhizomes [109]. Post harvest processing of turmeric is also an important factor to affect the content of curcuminoids. However, some reports are controversial, for example, one study found that concentration of curcumin (**1**) was reduced by 27-53% from heat processing of turmeric (e.g., curing with boiling water) [110], but another investigation indicated that heat treatment of turmeric prior to dehydration increased curcuminoid levels [111].

There are a few physiological investigations in plant growth and development of turmeric [112] [113-116]. Also, significant variations of curcumin contents were observed in different varieties, locations, growth stages, and environments (Table 2). It is believed that curcumin is produced in leaves and is then translocated to rhizome [117]. However, the mechanism of metabolism and accumulation of curcuminoids remain elusive and induced production of the active ingredients has not been addressed.

Variations of Essential Oils

There are significant variations in both content level and constituents of essential oils of turmeric rhizomes with geographical locations (Table 3). Among the 27 accessions North Indian plains at Lucknow, India, the percentage essential oil content in the fresh rhizomes varied between 0.16% and 1.94% (on a fresh weight basis) [101]. Although usually sesquiterpenes, particularly *Ar*-turmerone (**99**), α -turmerone (**100**) [83], and β -turmerone (**101**) are major compounds in turmeric oils in Asia, presence of other compounds in *C. longa* often vary with various locations. By using GC-MS analysis, Chowdhury and his coworkers identified 54 compounds of essential oils from the "yellow type" of *C. longa* while only 39 compounds were detected from the "red type" growing in Bangladesh [76]. The essential oil in "yellow type" were dominated by *ar*-turmerone (**99**) (27.78%), turmerones (**100** and **101**) (17.16%), curlone (**141**) (13.82%), 2-carene (**73**) (4.78%), zingiberene (**124**) (4.37%) and β -sesquiphellandrene (**136**) (5.57%), but the "red type" oil mainly contained carvacrol (**43**) (21.14%), citral (13.91%), methyleugenol (**229**) (7.31%), geraniol (**86**) (6.99%), menthol (**46**) (5.11%) and caryophyllene oxide (**168**) (4.14%) [76]. However, there is no significant difference was observed in essential oil composition between T3C turmeric and Hawaiian red turmeric [79]. Unlike those from Asia, interestingly, turmeric oils from Brazil contained 50-80% of *ar*-turmerone (**99**), (*Z*)- γ -atlantone (**137**), and (*E*)- γ -atlantone (**138**) [81, 92]. (*Z*)- γ -atlantone (**137**) and (*E*)- γ -atlantone (**138**) are only found in the turmeric from Brazil. Without information of which cultivars or varieties were used in these studies, however, it is impossible to determine the difference caused by geographical or genetic variation. Constituent of essential oil varies with different species of *Curcuma* [119-125]. For example, α -curcumene, a minor compound in *C. longa*, is the major constituent (approx. 65%) of the essential oil of Javanese turmeric (*C. xanthorrhiza*) [46].

Stability of Active Ingredients

In addition to their significant variations with geographic locations and genotypes, both curcuminoids and essential oils are unstable under different extraction and storage conditions.

Curcumin (**1**) is absorbed poorly by the gastrointestinal tract and/or undergoes presystematic transformation [133, 134]. As a natural coloring agent, it is known to be unstable particularly under alkaline conditions, light, and high temperature [135-137]. Curcumin (**1**) rapidly decomposes into trans-6-(4,-hydroxy-3,-methoxyphenyl)-2,4-dioxo-5-hexenal, vanillion, ferulic acid, and feruloy-methane at condition of pH>7.0 [134, 138, 139]. For example, approximately 90% of curcumin (**1**) decomposed when it was incubated in 0.1 M phosphate buffer and serum-free medium, pH 7.2 at 37°C for 30 min [134]. Half-life period $T_{1/2}$ of curcumin (**1**) is about 26.2 h at pH 9.0 in comparison of 501.5 h at pH 6.0 [138]. In another report, more than 90% of curcuminoids (composition of curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**5**)) were decomposed within 12 h when serum was omitted [56]. All curcuminoids are stable when they

Table 2. Content Variations of Curcuminoid Contents in Different Varieties, Locations, Products, or Extracts of Turmeric (*C. longa*)

Sample Type and Description	Extraction	Mean Content of Curcuminoids (%, dry Wt)				Ref.
		C (1)	DMC (2)	BDMC (5)	Total	
Rhizomes in India Kalimpong PTS-43	methanol	2.19 1.21	1.58 0.72	1.60 1.22	5.37 3.15	[118]
Rhizome Powder: Commercial Varieties available in India Rajapurl Krishna Mydukur Salem Tekurpetta Armoor Ambehalada Ranahalada	ethanol	Mother/finger 4.31/4.77 3.23/2.64 5.71/4.18 5.18/5.09 4.94/4.58 4.09/3.64 0.09/0.06 5.16/4.72				[98]
Rhizome Powder: Commercial Varieties available in India Salem Mysore Erode Balasore	hexane/ methanol	4.14 1.06 4.00 5.65	2.88 0.86 3.36 0.83	2.16 0.42 1.75 0.62	9.18 2.34 9.11 7.11	[99]
Rhizomes in various locations of Thailand	Tetrahydro- furan/methanol	1.28-6.02	0.92- 3.80	1.10- 5.18	3.30- 14.99	[6]
Rhizomes in Thailand		8.55-15.88	1.50- 4.16	5.54- 9.33	14.14- 26.76	[107]
Rhizomes in Thailand	methanol				0.46- 10.23	[103]
Rhizome Powder: Commercial Varieties Madras Alleppey Jamaica	ethanol	0.84 3.16 1.56	0.48 1.75 0.92	0.22 0.89 0.62	1.54 5.80 1.56	[8]
Rhizome powder: Ukon (Japan) Ukon (Guangdong, China) Turmeric ganter finger (Gunter, India) Jianghuang (Yunnan, China) Jianghuang (Yunnan, China) Kunir (Indonesia) Kunir (Indonesia)	methanol	0.53 0.76 1.21 1.19 1.05 1.36 1.38	0.17 0.34 0.33 0.41 0.33 0.48 0.43	0.09 0.37 0.19 0.34 0.30 0.54 0.39	0.79 1.47 1.73 1.94 1.68 2.38 2.20	[100]
Rhizome powder: Korea		Mother/finger 0.24/0.1				[106]
Rhizome powder: Commercial Products sold in CA, USA (vari- ous brands)		0.58-3.14				[102]
Extract: from Commercial Rhizome Powder sold in CA, USA	methanol	21.4	7.2	5.1	33.7	[20]
Commercial Extracts		4.4-13.7	2.2- 16.3	1.3- 4.97	10.1-33.7	[15]
Essential Oil-depleted Turmeric Fraction	Hexane/ methanol	25.7	8.7	6.2		[20]
Commercial Curcumin		74.2	14.9	4.5		[20]

Note: C: Curcumin, DMC: Demethoxycurcumin, BDMC: Bidemethoxycurcumin.

Table 3. Contd....

	Monoterpenes											Sesquiterpenes											Ref.									
	p-cymene	α/β -terpinene	α/β -phellandrene	terpinolene	<i>P</i> -cymen-8-ol	camphor	camphene	α/β -pinene	cinole	myrcene	linalool	isoborneol	<i>ar</i> -tumerone	α -tumerone	β -tumerone	<i>ar</i> -curcumene	<i>ar</i> -turmerol	bisabolone	<i>ar</i> -Zingiberene	(<i>Z</i>)- α -atlantone	β -bisabolene	α/β -curcumene		β -sesquiphellandrene	(<i>Z</i>)- γ -atlantone	(<i>E</i>)- γ -atlantone	curlone	germacrone	β -farnesin	curzerone		
Rhizome, Brazil “(SFE)”	1.5		4.1	1.3				4.0				15.5			3.6	1.5		6.4		1.7			7.7	20.3	15.6							[81]
Rhizome, Brazil “(SFE)”												15.0			0.9	0.7		2.2	1.7	0.4			1.9	36.2	17.1							[92]
Rhizome, Brazil (HD)							2.7	1.4				18.0			1.0	1.1		2.4	0.6			1.9	44.0	18.3							[92]	
Rhizome, Japan “(SFE)”	1.2					5.5	1.3			1.0		42.0	16.1								4.2	3.2										[77]
Rhizome, Japan (HD)	0.9					5.1	1.2			1.1		41.1	16.4								4.1	3.0										[77]
Rhizome, India, <i>C. aromatica</i>						25.6	7.4	9.3			8.2																10.6		10.9		[130]	
Rhizome, India, <i>C. aromatica</i>						3.2	2.7	10.6				14.6		33.8				10.7														[131]
Rhizome, India, <i>C. aromatica</i>						32.3		5.5				6.3	6.7																11.0		[132]	

Note: CRTO: Curcumin (1) removed turmeric oleoresin; The data were obtained by GC-MS analysis except those as noted by SFE (supercritical fluid extraction), HD (hydrodistillation), or Soxhlet extraction. tr: trace.

are kept under minimum light condition [140]. In methanol sparged with air, stability to photooxidation was curcumin (1) > demethoxycurcumin (2) > bisdemethoxycurcumin (5) [140]. It was found that the stability of curcumin (1) in aqueous solution is strongly increased by the presence of some antioxidants [141]. But the presence of other curcuminoids (II and III) which are antioxidants seem not prevent degradation of curcumin (1) [56].

Extraction Methods

Extraction rate and composition of curcuminoids and essential oils are significantly affected by various extraction techniques such as hydrodistillation, low pressure solvent extraction, Soxhlet, and supercritical fluid extraction (SFE) using carbon dioxide, with different co-extraction solvents [92]. The largest yield (27%, weight) was obtained in the Soxhlet extraction with ethanol; the lowest yield was detected in the hydrodistillation process (2.1%). For SFE, the best cosolvent was a mixture of

ethanol and isopropyl alcohol [92]. Although SFE and hydrodistillation processes may produce different yields of total oils, but the percentage of their major compounds extracted by these two methods are similar [77, 92].

Isolation of pure curcumin (1) from turmeric is difficult and time-consuming, and thus the commercial “pure” curcumin (1) is in fact a mixture of at least three curcuminoids. Such curcuminoids can decompose rapidly, but curcuminoids in both turmeric powder and extracts are more stable [138]. Among different extraction solvents, ethanol extraction gives the highest yield of curcuminoids [92, 142]. In comparison to hexane extraction which yields sesquiterpenoids only, ethanol extraction yields both curcuminoids and sesquiterpenoids. It was found that curcuminoids and sesquiterpenoids together have synergistic effect on their bioactivities [89]. Our preliminary study showed that 70% ethanol is better than water (boiling or at room temperature), 30%, 50% ethanol in extraction of three curcuminoids. It was reported that ethanol

Table 4. Contents of Active Ingredients in the Plant Samples and Extracts of Different Batches

Sample/Active Ingredients	Rhizome Powder (Mean) (%, Dry wt)	Extracts (Mean \pm s.d.) (%, Dry wt)	Yield of Extracts (%)
Curcumin	2.86	19.51 \pm 2.07	10-13
Demethoxycurcumin	1.47	8.31 \pm 1.13	
Bidemethoxycurcumin	1.36	6.22 \pm 0.88	
Total Curcuminoids	5.69	34.04 \pm 4.08	

Note: authors' unpublished data.

extract, particularly high grade ethanol extract is more stable (e.g., 80% ethanol > acetone > 60% ethanol, > 50% ethanol or ethyl ether > ethyl acetate > 40% ethanol > petroleum ether or water or pure curcumin (**1**)) [138]. Thus, ethanol not only can produce effective extraction of curcuminoids but also the curcuminoids in ethanol extract can be less decomposed than the three curcuminoids combined.

Recently, we have used the following extraction method of curcuminoids in our research projects. Ground plant powders of turmeric (Sami Labs Limited, moisture content 5%, ~60 mesh) were extracted separately with 70% ethanol at room temperature for 18 h (solvent volume/material weight, 10:1) for three times. After vacuum filtration, the combined ethanol extracts of each sample were concentrated under reduced pressure with rotary evaporator. The residues were then transferred to 40 mL vials and further evaporated with speed vacuum at ~45°C for 48 h. Authentic compounds, curcumin (**1**) (94%) were purchased from Sigma-Aldrich and re-purified in our study. These three curcuminoids were analyzed and quantified by HPLC method that was carried out on Agilent 1100 instrument with Agilent Zorbax SB-C18 column (4.6 \times 250 mm, 5 μ m) and linear gradient of 30% acetonitrile to 70% acetonitrile in 45 min then increase to 90% by 65 min. The flow rate was 0.7 mL/min with detection wavelength at 230 and 425 nm. The sample description and analysis result of active ingredients of the plant samples and experimental extracts are summarized in **Table 4**.

QUALITY AND STANDARDIZATION OF TURMERIC PRODUCTS

Marker Compounds

Because of variations of active ingredients with seed sources, habitats, plant age, harvest and dry process, commercial turmeric rhizomes and products have significant variations in curcumin (**1**) contents (0.58 to 6.5% on a dry weight basis) (**Table 2**). Also, curcuminoids could rapidly decompose under certain storage conditions. The chemical variability will result in inconsistent results and uncertain efficacy in experiments and clinical trials. Thus, there is need for standardization of chemical ingredients

in turmeric products.

According to the Indian Pharmacopoeia (1996), dried turmeric rhizomes should contain not less than 1.5% of curcumin (**1**) (w/w). The Pharmacopoeia of People's Republic of China (2005) requires no less than 1.0% of curcumin (**1**) content (w/w) in dried turmeric rhizomes [5]. The Thai Herbal Pharmacopoeia recommended that dried turmeric should contain no less than 6% of turmeric oil (v/w) and 5% of total curcuminoids (w/w) [103]. WHO (World Health Organization) suggests that not less than 4.0% of volatile oil, and not less than 3.0% of curcuminoids in turmeric [9]. In our initial investigations for chemoprevention of colorectal cancer, we required that the rhizomes contain no less than 5% of total contents of three bioactive curcuminoids and the experimental rhizome extracts contain no less than 25% of the three curcuminoids (Aggarwal *et al.*, unpublished data).

As a number of studies have suggested that ethanol extract showed better extraction efficiency and stability of active curcuminoids, the ethanol was selected as a solvent for turmeric extraction. Each turmeric samples were labeled with individual code and extract with 95% ethanol by Dionex ASE 200 Accelerated Solvent Extractor. The chemical profiles of each product were characterized by defining and verifying the curcumin (**1**), demethoxycurcumin (**2**), and bisdemethoxycurcumin (**5**) and determining its concentrations by HPLC (Fig. 2). The HPLC chromatogram with UV/VIS detection at 425 nm provided an important basis for product quality control. The minimum 5% (w/w) of three major curcuminoids (**1**, **2**, and **5**) in the turmeric extract were determined based on the initial activity data with consideration of the requirements for the turmeric rhizomes from above Pharmacopoeias.

Turmeric oils and oleoresins have shown various promising activities and have been marketed globally. The major ketonic sesquiterpenes, particularly, *Ar*-turmerone (**99**), α -turmerone (**100**), and β -turmerone (**101**) have been used to control the product quality, e.g., minimum 40% of these marker compounds in turmeric oils and oleoresins produced by hydrodistillation (see **Table 3**). Turmeric oils and oleoresins produced by SFE (supercritical fluid extraction) usually have lower yield of these compounds, and may use *Ar*-turmerone (**99**), (*Z*)- γ -atlantone (**133**), and (*E*)- γ -atlantone (**134**) to control the quality of these particular products (e.g., >50% of these three compounds).

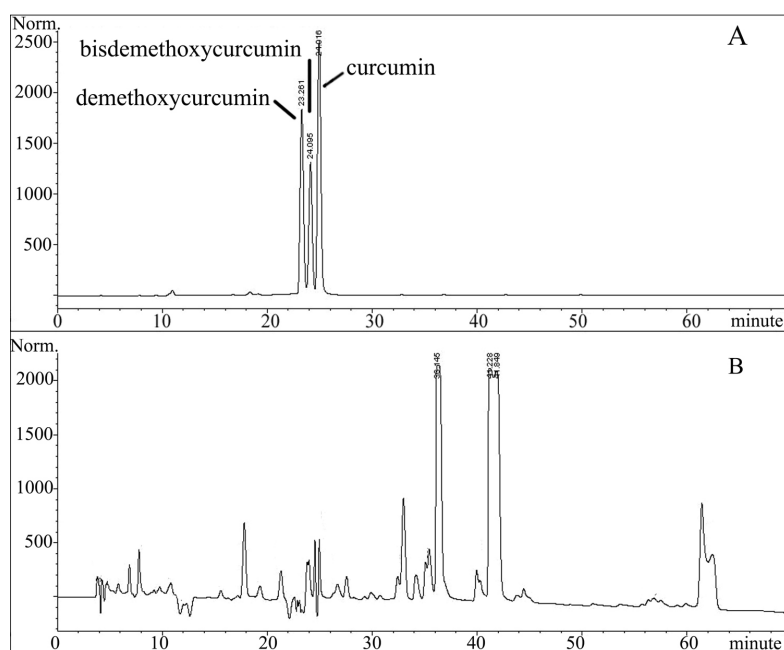


Fig. (2). HPLC chromatograms of ethanol extracts of “Ground Turmeric” (A detected at 425 nm and B detected at 230 nm, respectively). The samples were extracted by ASE-200 and analyzed by HPLC (Agilent 1100) (Column: SB-C18; 30% acetonitrile to 70% acetonitrile in 45 min, increase to 90% by 65min; flow rate, 0.7 mL/min).

Adulteration

Though whole dried or fresh turmeric are usually free from adulteration, turmeric powder can be adulterated with powders of certain other species of *Curcuma* [143]. Usually, adulterated products of ground turmeric have low content of curcuminoids and are even toxic when *C. zedoaria*, a common adulterant is included in turmeric powder [144]. Morphologically, the rhizomes of the two species can be distinguished by nature and color (highly branched and yellow rhizomes of *C. longa* vs. less branched bulbous and orange yellow rhizomes of *C. zedoaria*) or oil cell sizes (small in *C. longa* vs. large in *C. zedoaria*) [145]. Occasionally, other *Curcuma* species such as *C. aromatica* (*C. wenyujin*) may be used to substitute *C. longa* for turmeric production. *C. aromatica* can be identified by its less branched and creamy rhizomes with less primary vascular bundles and few curcumin cells [145]. The identity of questionable samples of turmeric powders can be examined under light or/and scanning electronic microscope in comparison with authentic samples and referencing with literatures. Comparative rhizome anatomy and microscopic characters of *C. longa* and related species are reported [51, 145, 146].

DNA markers can also be useful in identification of some species of *Curcuma*. For example, ISSR (inter simple sequence repeats) and RAPD (random amplified polymorphic DNA) markers were developed for 15 species of *Curcuma* from India [147]. Based on *trnK* gene sequences, a rapid LAMP (loop-mediated isothermal amplification) method was developed to identify *C. longa* and *C. aromatica* [148]. More recently, method for identification of these species and *C. zedoaria* as well as *C. xanthorrhiza* was reported by determining DNA polymorphisms in the *trnS-trnM* intergenic spacer in chloroplast DNA [149]. It was found DNA sequence characterized amplified region (SCAR)

markers can be useful in detection of *Curcuma* hybrids [150].

Only few species of the genus *Curcuma* have ever been phytochemically investigated and the chemical compositions are markedly varied among these species [53, 151-156]. Thus chemical fingerprints of curcuminoids or essential oils of each species became necessary and served as identification markers. Chemical analysis will provide useful information for any questionable samples of dried rhizomes, ground turmeric, turmeric oils or oleoresins, and curcuminoids/curcumin. For turmeric extracts or isolates, chemical fingerprints appear to be the only approach to determine the product quality. Chemical analysis becomes particularly necessary when exotic chemicals (either natural or synthetic compounds) adulterants in turmeric, e.g., dyes such as Sudan dyes [157-159]. Chromatographic techniques (HPLC, HPLC-ESI-MS, LC-MS, GC-MS, CE) [99, 105, 152, 154-156, 160-166], UV spectrophotometry [107, 158, 159], X-ray crystallography [157, 159], or NMR spectroscopic analysis have been used as powerful tools for identification of the quality of product in various studies. In contrast, thin layer chromatography may not be helpful in identifying adulterated products in the marketed samples of turmeric [167]. For turmeric oils or oleoresins from rhizomes of *C. curcuma* adulterated with leaf oils, GS-MS or NMR techniques will be helpful. Because essential oils from leaves or flowers of *C. longa* are usually dominated by monoterpenes while those from roots and rhizomes mainly contain sesquiterpenes, this chemical feature and color difference could be used to distinguish the leaf or flower oils from turmeric oils derived from rhizome or root of *C. longa*.

However, the molecular methods as tools for authentication of species/variety have limitations. There are significant variations in compositions of both curcuminoids and essential oils of *C. longa* with genotypes, environments,

harvest methods and season, dry process, and storage conditions. As a result, chemical analysis may not provide reliable authentication in some cases, particularly when involving multiple species or hybrids. Genetic methods directly detect genotypes of *Curcuma*, however, it may be difficult to isolate or amplify DNA and develop DNA fingerprints and the replication could be problematic under certain circumstances. Like morphological and anatomic methods, molecular methods may be more useful in identification of *C. longa* than characterization of other species in the adulterated samples. Therefore, it would be better to apply chromatographic and NMR techniques as well as DNA markers of the questionable samples with morphological and anatomic data as well as GAP (good agricultural practices) and other information provided by the farmers and manufacturers.

Microbial, Heavy Metal, and Pesticide Analyses

The microbial, heavy metal, and pesticide contents in all turmeric products (including plant matters, extracts, and isolates) should be investigated by authentic methods (e.g., USP <61>, <231>, and <561> methods). For example, authors recently tested ground turmeric and ethanol extracts by using EPA (Environmental Protection Agency, USA) Method 200.2 (Table 5). Levels of Ag, Cd, Se, and Cr were below the detection limit (ppm) by ThermoFisher XSP Intrepid Radial Dedicated Inductively Coupled Plasma and levels of As and Hg were below the detection limit by Atomic Absorption (ppb) by PerkinElmer AA700 Analyst Atomic Absorption with a Graphite Furnace and Mercury Hydride System. Ba and Ni were detectable in all three plant samples but both were at below detectable levels in the ethanolic extract of turmeric. Lead levels in both ground turmeric and ethanol extract were between 104.6 to 328.4 ppb, much lower than the minimum levels of 10 mg/kg (10,000 ppb) for turmeric recommended by WHO (WHO 1999).

For turmeric oleoresins and curcumin (1) (curcuminoids), residual solvents could be a problem. The residual solvent and heavy metal contents are limited to 25 mg/kg for hexane, 30 mg/kg for acetone, dichloromethane, and 1,2-dichloroethane, 50 mg/kg for ethanol, methanol, and isopropanol, 3 mg/kg for As and 2 mg/kg for Pb, according to JECFA specifications (FNP 52 add. 11, 2003) [168].

CONCLUSIONS

Turmeric (*Curcuma longa* L.) is one of the most extensively phytochemically investigated plant species. At least 235 compounds have been isolated or detected from leaves, flowers, roots, and rhizomes of *C. longa*, including 22 diarylheptanoids and diarylpentanoids, eight phenylpropene and other phenolic compounds, 68 monoterpenes, 109 sesquiterpenes, five diterpenes, three triterpenoids, four sterols, two alkaloids, and 14 other compounds. Curcuminoids and essential oils have shown various bioactivities in *in vitro* and *in vivo* bioassays. Curcumin is one of the most studied chemopreventive agents and showed promising results in cell culture, animal research, and clinical trials. Curcuminoids in turmeric are primarily accumulated in rhizomes. The essential oils from leaves and flowers are usually dominated by monoterpenes while the major part of the oil from roots and rhizomes contain sesquiterpenes. The contents of curcuminoids in turmeric rhizomes vary often with varieties, locations, sources, and cultivation conditions, while significant variations were observed in composition of essential oils of turmeric rhizomes with varieties and geographical locations. Furthermore, both curcuminoids and essential oils vary in contents with different extraction methods and are unstable with extraction and storage processes. As a result, commercial turmeric products (whole rhizomes, ground turmeric, turmeric oils, turmeric oleoresin, and "curcumin") have significant variations in composition of bioactive compounds. Ethanol extraction showed advantages in both effective extraction and stability of active curcuminoids. Curcumin (1), demethoxycurcumin (2), and bisdemethoxycurcumin (5) can be used as marker compounds for the quality control of rhizomes, powders, and extract ("curcumin") products with minimum limit of the contents. The major ketonic sesquiterpenes (*Ar*-turmerone (99), α -turmerone (100), and β -turmerone (101)) can be used to control the product quality of turmeric oil and oleoresin products. Authentication of turmeric products can be achieved by using chromatographic and NMR techniques and DNA markers of the questionable samples with morphological and anatomic data as well as GAP and other information provided by the farmers and manufacturers. Other quality aspects such as the microbial, heavy metal, and pesticide contents in all turmeric products (including plant matters, extracts, and isolates) should be investigated by authentic methods as well.

Table 5. Heavy metal analysis of the plant samples (N.D.: Not Detected)

	ThermoFisher XSP Intrepid Radial Dedicated Inductively Coupled Plasma (ppm)						AA700 Analyst Atomic Absorption (ppb)		
	Ag	Ba	Cd	Cr	Se	Ni	As	Hg	Pb
Wavelength (nm)	328.068	230.424	228.802	284.325	196.090	231.604	193.7	253.7	283.3
Detection Limit	0.3963	0.0588	0.135	0.2172	1.627	0.2982	0.429	0.0666	0.2622
Ground Turmeric	N.D.	13.86	N.D.	N.D.	N.D.	1.382	N.D.	N.D.	222.4
Ethanol Extract	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	118.4

Note: authors' unpublished data.

ABBREVIATIONS

AP-1	=	Activator protein 1
BCL2	=	B-cell lymphoma 2
BCL2L1	=	BCL2-like 1
CE	=	Capillary electrophoresis
COX-1	=	Cyclooxygenase-1
COX-2	=	Cyclooxygenase-2,
EPA	=	Environmental Protection Agency, USA
GC-MS	=	Gas chromatography-mass spectrometry
GAP	=	Good agricultural practices
HPLC	=	High performance liquid chromatography
HPLC-ESI-MS	=	high-performance liquid chromatography-electrospray ionization tandem-mass spectrometry
ISSR	=	Inter simple sequence repeats
IL-1	=	Interleukin-1
IL-6	=	Interleukin-6
JECFA	=	Joint FAO/WHO Expert Committee on Food Additives
LAMP	=	Loop-mediated isothermal amplification)
LC-MS	=	Liquid chromatography-mass spectrometry
LOX	=	Lipoxygenase
NF- κ B	=	Tumor necrosis factor (TNF)-induced nuclear factor- κ B
NMR	=	Nuclear magnetic resonance
RAPD	=	Random amplified polymorphic DNA
SFE	=	Supercritical fluid extraction
STEs	=	Standardized therapeutic extracts
STMs	=	Small therapeutic molecules
TNF	=	Tumor necrosis factor
<i>trnS-trnfM</i>	=	Intergenic spacer between <i>trnS</i> and <i>trnfM</i>
USP	=	The United States Pharmacopeia
WHO	=	World Health Organization

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