CHAPTER 1

INTRODUCTION

Curcuma longa Linn., commonly called turmeric (known in Thai as Khamin or Khamin Chan), is a tropical herb of the Zingiberaceae family (Saralamp *et al.*, 2000). It is cultivated in many Southeast Asia and South American countries, as well as in India, China and the Caribbean Islands (Souza *et al.*, 1997). Its rhizome is widely used in foods as a condiment. It has been known for its coloring and flavoring properties, and its digestive properties have been recognised since ancient times. The turmeric color has become widely used in the entire food industry. It has been added to margarine, mayonnaise, salad dressing, oil, mustard, pickles, relishes, sauces, cereals, baked, products, beverages and dairy products such as cheese, butter and ice cream (Souza *et al.*, 1997). It has also been used for centuries as a traditional medicine. The antiinflammatory, antibacterial, anticarcinogenic properties and other activities of turmeric have been well documented (Araujo and Leon, 2001; Ammon and Wahl, 1991). The main active constituents of the rhizome are coloring matters and volatile oil (Evan, 2002). The coloring matters comprise mainly of curcumin, demethoxycurcumin and bisdemethoxycurcumin (Purseglove *et al.*, 1981). The volatile oil contains mainly aromatic principles, i.e. turmerone, ar-turmerone and zingiberene (Su *et al.*, 1982).

The Zingiberaceous plant *Curcuma zedoaria* (Berg.) Roscoe, is commonly called zedoary and known in Thai as Khamin Oi. It is similar to turmeric, but the rhizome and leaf are larger (Saralamp *et al.*, 2000). It has been extensively cultivated as a vegetable, spice and perfume in South and Southeast Asian countries. The rhizome of this plant is used medically as a stimulant, stomachic, carminative, diuretic, antidiarrheal, anti-emetic, antiinflammatory, antipyretic, antimicrobial, antioxidant and also to treat and cure ulcers, wounds and other kinds of skin disorder (Mau *et al.*, 2003; Matsuda *et al.*, 2001b; Syu *et al.*, 1998; Yoshioka *et al.*, 1998). As it contains bioactive principles, the constituents of zedoary have been extensively investigated and it is now recognized to be a rich source of many types of sesquiterpenoids in its volatile oil. Active curcuminoids, curcumin, demethoxycurcumin and bisdemethoxycurcumin, have previously been isolated and characterized (Mau *et al.*, 2003; Evan, 2002; Syu *et al.*, 1998; Shiobara *et al.*, 1985).

Since the quality of turmeric and zedoary are based directly on the content of the curcuminoids and volatile oil, the contents of the curcuminoids (calculated as curcumin) and volatile oil in turmeric have been specified as not less than 5.0 % w/w and 6.0 % v/w, respectively (Ministry of Public Health, 1998). Turmeric and zedoary rhizomes are exposed to a variety of conditions during processing, packaging and storage, and some of these may be detrimental to the stability of their active constituents and biological activities (e.g. antibacterial and antioxidant activities).

Concern over pathogenic bacteria is because of their ability to cause a

variety of diseases, such as, abscess of skin and other organs, urinary tract infection, diarrhea (Lorian, 1996). Currently, there is a growing interest to use natural antibacterial compounds, like plant extracts of herbs and spices for the treatment of bacterial infections. Curcumin and volatile oil, the active constituents of *Curcuma longa* Linn, exhibited antibacterial activity against bacterial pathogens, such as, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus typhosus* (Araujo and Leon, 2001; Tang and Eisenbrand, 1992; Lutomski *et al.*, 1974).

Oxidation is a process that occurs naturally in the body when oxygen combines with reduced carbon-based molecules (carbohydrates or fats) and produced energy. This normal process propagates short-lived intermediates, known as free radicals. Some free radicals escape and initiate further oxidation. Since free radicals are difficult to measure directly, the measurement of damaged molecules caused by free radicals is performed instead. Although free radicals will react with any type of molecule, the most frequently damaged are carbohydrates, lipids, nucleic acids and proteins. This is known as oxidative stress, resulting in degeneration of body tissues, leading to inappropriate cell responses and disease states (Rapport and Lockwood, 2002). Antioxidants, or free radical scavengers, are the molecular defences that prevent free radicals from causing excess cellular damage. Therefore, the ingestion of certain antioxidants may be used to prevent the damage caused by this process. There is a great amount of data suggesting that curcumin and other antioxidant products from the rhizome of *Curcuma longa* Linn. and *Curcuma zedoaria* (Berg.) Roscoe show a strong antioxidant action (Mau *et al.*, 2003; Miquel *et al.*, 2002).

Stability testing represents a crucial part of the testing program for drug substances because the instability of the product modifies the three essential requisites, i.e. quality, efficacy and safety. Stability, defined as the time during which a drug retains its integrity in terms of quality and chemical identity, can be affected by environmental factors such as temperature, pH, light and air, which can have dramatic effects on some constituents (Bilia *et al.*, 2001). Some factors affecting the active constituents of turmeric and zedoary have been investigated. Light is known to be particularly damaging source to most natural colorants and volatile oil. The uniformity and stability of curcuminoids and volatile oil are adversely affected by light. The result is rapid degradation which is a major limitation of turmeric and zedoary usage in medication (Price and Buescher, 1996). Alkaline pH has been reported to affect the curcuminoids content (Price and Buescher, 1997). Climatic and geographical conditions have an influence on the content and composition of curcuminoids and volatile oil (Tewtrakul, 1993). The fresh rhizome of turmeric contained higher curcuminoids and volatile oil than the dried rhizome from old-styled drugstores (Chavalittumrong and Dechatiwongse, 1988). Little information is available on the effect of the growth period, the conditions and duration of storage, on the quality of content and biological activity.

Therefore, it is of interest to study the effect of growth stage, the conditions and duration of storage on the active constituents and antibacterial and antioxidant activities of turmeric and zedoary rhizomes.

The results of this study will be beneficial for developing appropriate industrial production technologies which hopefully result in better maintenance of the active constituents in the commercially available rhizomes.

Literature review

1. Botanical aspect of *Curcuma longa* Linn. and *Curcuma zedoaria* (Berg.) Roscoe.

1. 1 Botanical aspect of *Curcuma longa* Linn.

Curcuma longa Linn. (Figure 1-1) is a plant in the family Zingiberaceae. Its synonym is *Curcuma domestica* Valeton. Its common names in various countries are Turmeric, Curcuma, Yellow root, Indian saffron (English); Kurkumawurzelstock, Gelbwurzel (Germany); Rhizome de curcuma (France) (Bisset, 1994); Haldi (Hindi), Jiang Huang (Chinese) (Horn and Weil, 1996); Kunyit (Indonesia, Malaysia, Singapore); Luyang dilaw (Phillipines); Khamin-Chan (Thailand) (Ministry of Public Health, 1998; Aian countries, 1993)

Turmeric is native to India and Southern Asia. It is cultivated throughout the tropical countries including Southern and Eastern Asia especially Thailand. It is an erect perenial herb with a thick, ellipsoid-ovate rhizome, about 5 cm long, much branched, giving rise to short blunt daughter rhizomes called fingers about 5 – 8 cm long. The rhizome has a distinctive taste and smell. It is brownish and scaly outside and inside is a bright orange color. The young tips are white. The leafy shoots rarely exceed 1 metre in height and are erect, bearing 6-10 leaves with the leaf sheaths forming a pseudostem. The thin petiole is rather abruptly broadened to the shealth. The ligule is a small lobe, about 1 mm long. The lamina is lanceolate, acuminate and thin, dark green above and pale green beneath

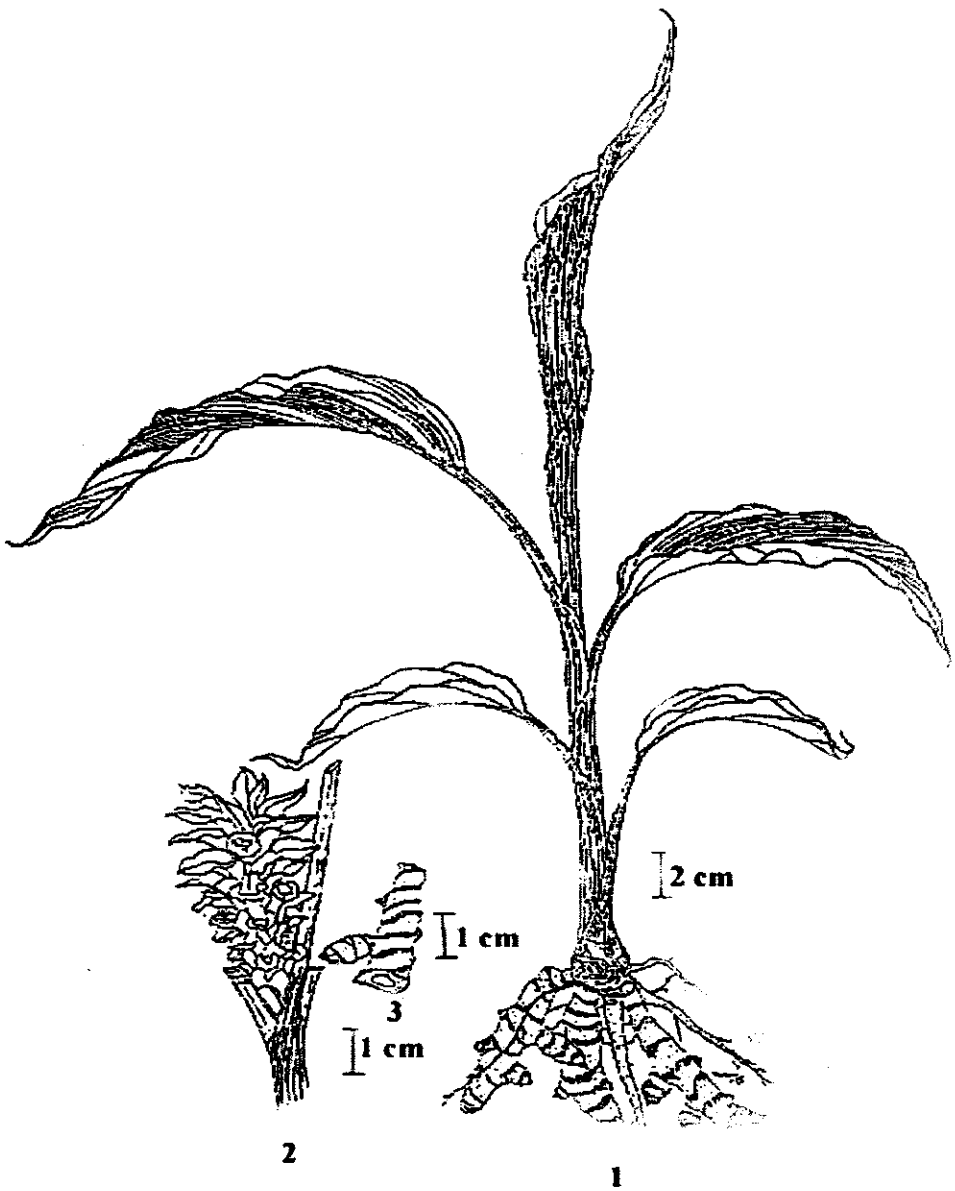


Figure 1-1 *Curcuma longa* Linn. (Ministry of Public Health, 1998).

1. Whole plant
2. Inflorescence
3. Rhizome

with pellucid dots. It is usually up to 30 cm long and 7-8 cm wide, and is rarely over 50 cm long. The shealth near the ligule has ciliate margins. The inflorescence is a cylindrical spike, 10-15 cm long and 5-7 cm wide, which is terminal on the leaf shoot with the scape partly enclosed by the leaf sheaths. The bracts are adnate for less than half their length and are elliptic-lanceolate and acute, 5-6 cm long and about 2.5 cm wide. The upper sterile bracts are white or white streaked with green, pink-tipped, grading to light-green bracts lower down. The bracteoles are thin, elliptic and up to 3.5 cm long. The pale yellow flowers, thin-textured and fugacious are borne in the axils of the bracts, about 5 cm long. The tube-shaped calyx is short, unequally toothed, splitted nearly half-way down one side. The corolla-tube is more or less funnel-shaped, not exserted beyond the bract, with 3-unequal lobes inserted on edge of cup lip. Its color is white. The two lateral staminodes, elliptic-oblong, are creamy white in color, and folded under the dosal petal. The lip or labellum is obovate, with a broad thickened yellow band down the centre and thinner creamy white side-lobes upcurved and overlapping the staminodes. A fertile stamen with short filament, broad and constricted at the apex is found in the floret. The anther is versatile and usually spurred at the base. The ovary consists of 3-locules, each locule contains 2 ovules. Seed are rare (Ministry of Public Health, 1998; Farnsworth and Bunapraphatsara, 1992; Purseglove *et al.*, 1981; Parry, 1969). Turmeric grows very well in rather hot climates with high humidity at night. It grows well on well-drain loam; clayish or sandy soil are unsuitable. Cultivation should start in May. After 7 months, the leaves become yellow, indicating the maturity of the rhizomes. However, rhizomes are left in the

ground until they are 9-10 months old before harvesting (Farnworth and Bunapraphatsara, 1992; Purseglove *et al.*, 1981).

1. 2 Botanical aspect of *Curcuma zedoaria* (Berg.) Roscoe.

Curcuma zedoaria (Berg.) Roscoe (Figure 1-2) is in the family of Zingiberaceae. Its common names in various countries are Zedoary (English); Zedoarwurzel, Zittwer (Germany); Zedoaire, Zedoaire bulbeux (France); Zedoaria (Italian); Kachura, Kalihaladi (Hindi); Temoelawa (Java) (Kirtikar, 1980); Gajusutsu (Japan) (Matsuda, *et al.*, 2001a); Er-chu (China) (Mau *et al.*, 2003); Khamin oi (Thailand) (Saralamp *et al.*, 2000).

Zedoary is considered to be a native of the North-Eastern India and spread in cultivation throughout the Indian subcontinent and Malaysia. It grows mainly in South and Southeast Asian countries including China, Vietnam, India and Japan. This plant is a perennial herb. It is a rhizome, or underground stem, like turmeric and ginger. The rhizome is large and tuberous with many branches. The interior of the rhizome is yellow and when dried, has an agreeable musky odour with a slight smell of camphor and a pungent bitter taste. The main tubers, known as bulb, are ovoid about 8 x 5 cm, with many short, thick branches and tuberous roots, known as finger (Hong *et al.*, 2002; Morikawa *et al.*, 2002; Purseglove *et al.*, 1981). The rhizome contains many types of sesquiterpenoids and yellow pigments called curcuminoids (Evan, 2002). The leafly shoots are up to 1 metre tall with about 4-6 leaves with long green petioles. Its leaves are oblong-

lanceolate, finely acuminate, glabrous on both surfaces, clouded with purple down the middle, 30-60 cm long. The inflorescences, about 22 cm tall, are separate from the leaf shoots. The spikes are about 16 cm tall, with the lowest green bracts, the middle bracts tipped with purple and the uppermost bracts entirely

Figure 1-2 *Curcuma zedoaria* (Berg.) Roscoe (Saralamp *et al.*, 2000; Basu, 1980).

1. Leafly shoot
2. Inflorescence
3. Rhizome

purple. The flowers, about five to each bract, are pale yellow in spikes. The flowering bracts, 3.8 cm long, are ovate, recurved, cymbiform, green tinged with red. The bracts of the coma are crimson or purple about 5 cm long. The calyx, about 8 mm long is obtuse, 3-toothed. The corolla is tube twice as long as the calyx, funnel-shaped. The lip is broad, suborbicular, deflexed, deep yellow. The capsule is ovoids, thin, smooth, bursting irregularely. Seeds are ellipsoid with a white lacerate aril (Kirtikar and Basu, 1980; Hooker, 1894).

2. Microscopy of *Curcuma longa* Linn. rhizome.

2.1 Intact rhizome.

Figure 1-3 (Ministry of Public Health, 1998; Asian countries, 1993) shows transverse section of the rhizome which depict the following characteristic features:

Epidermis consists of a layer of rectangular cells, covering trichomes, unicellular, up to 280 μm long. Hypodermis composes of 3-6 layers in the mature rhizome, but absent in the younger. Cork composes of 4-6 layers of rectangular cells. Cortex composed of thin-walled parenchymatous cells containing numerous starch grains, yellowish droplets and yellow coloring matter occasionally seen. Some of which contains oleoresin, starch grains, simple, flattened, rounded to oval or irregular in outline, 9-40 μm long and 7-20 μm wide, very faint transverse striations could be seen in some granules. Endodermis is a layer of thin-walled cells. Stele, thin-walled parenchymatous cells is similar to these found in cortex.

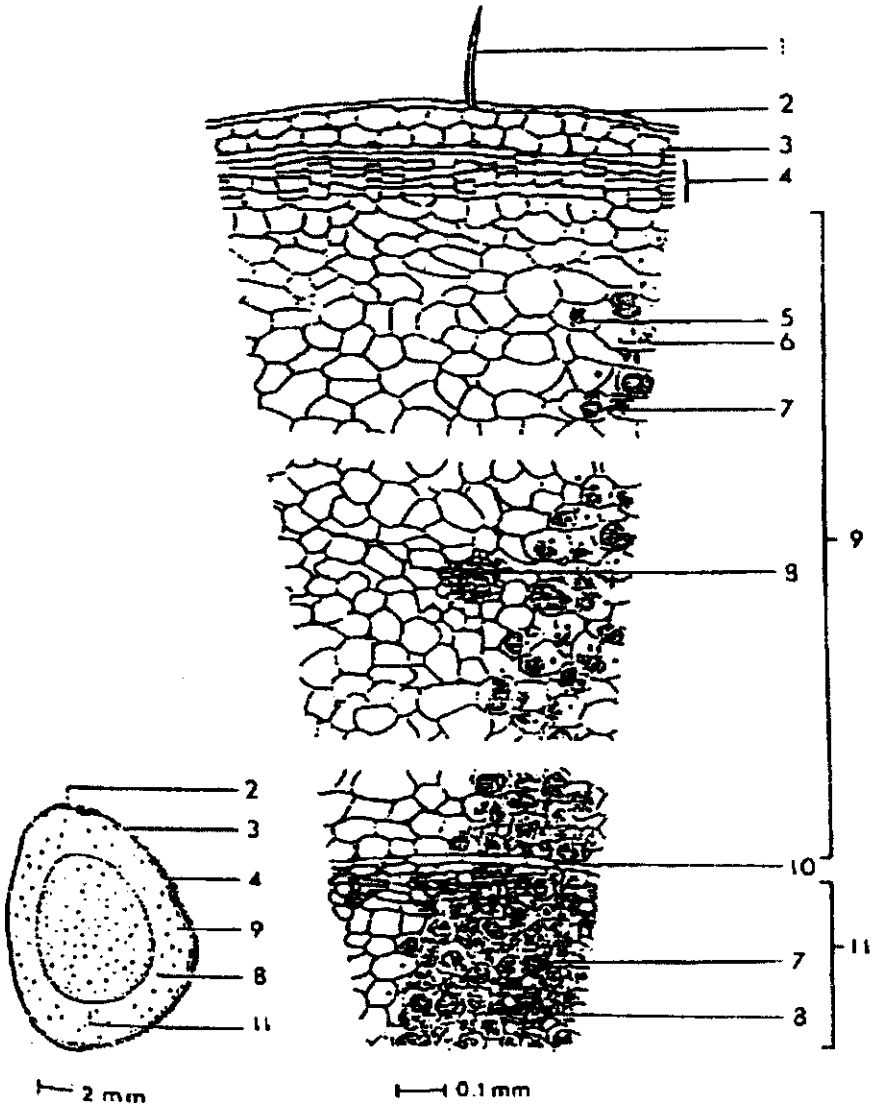


Figure 1-3 Transverse section of the rhizome of *Curcuma longa* Linn. (Ministry of Public Health, 1998; Asian countries, 1993).

1. Covering trichome
2. Epidermis
3. Hypodermis
4. Cork layers
5. Cortical parenchyma containing starch granules
6. Oil droplet
7. Oleoresin
8. Vascular bundle
9. Cortex
10. Endodermis
11. Stele

Fibrovascular bundles, non-lignified walled cells, scatter in cortex and stele vessels, spiral, sclariform and reticulate.

2.2 Powdered of turmeric rhizome.

Powdered turmeric is a bright golden-yellow with aromatic, pleasant odour and a pungent and aromatic taste. The diagnostic characters (Figure 1-4) (Ministry of Public Health, 1998; Betty and Derek, 1974) are:-

1. The abundant groups of parenchymatous cells, which are filled with gelatinized starch and permeated with a bright yellow colouring matter which is soluble in aqueous mounts are seen to be rounded to oval in outline with thin, slightly irregular walls.

2. The fairly abundant fragments of pale brown cork compose of thin-walled cells which in surface view appear large and polygonal. Fragments in sectional view show that the cork consists of 2-4 layers of cells and that it occurs inside the cortex. The epidermis and several layers of cortical cells are occasionally found associated with the cork.

3. The epidermis composes of a layer of straight-walled tabular cells, polygonal to elongated in surface view. The walls are sometimes slightly thickened and pitted; very occasional rounded stomata and cicatrices occur and covering trichomes may also be present. These fragments are rather indistinct and not easily detected.

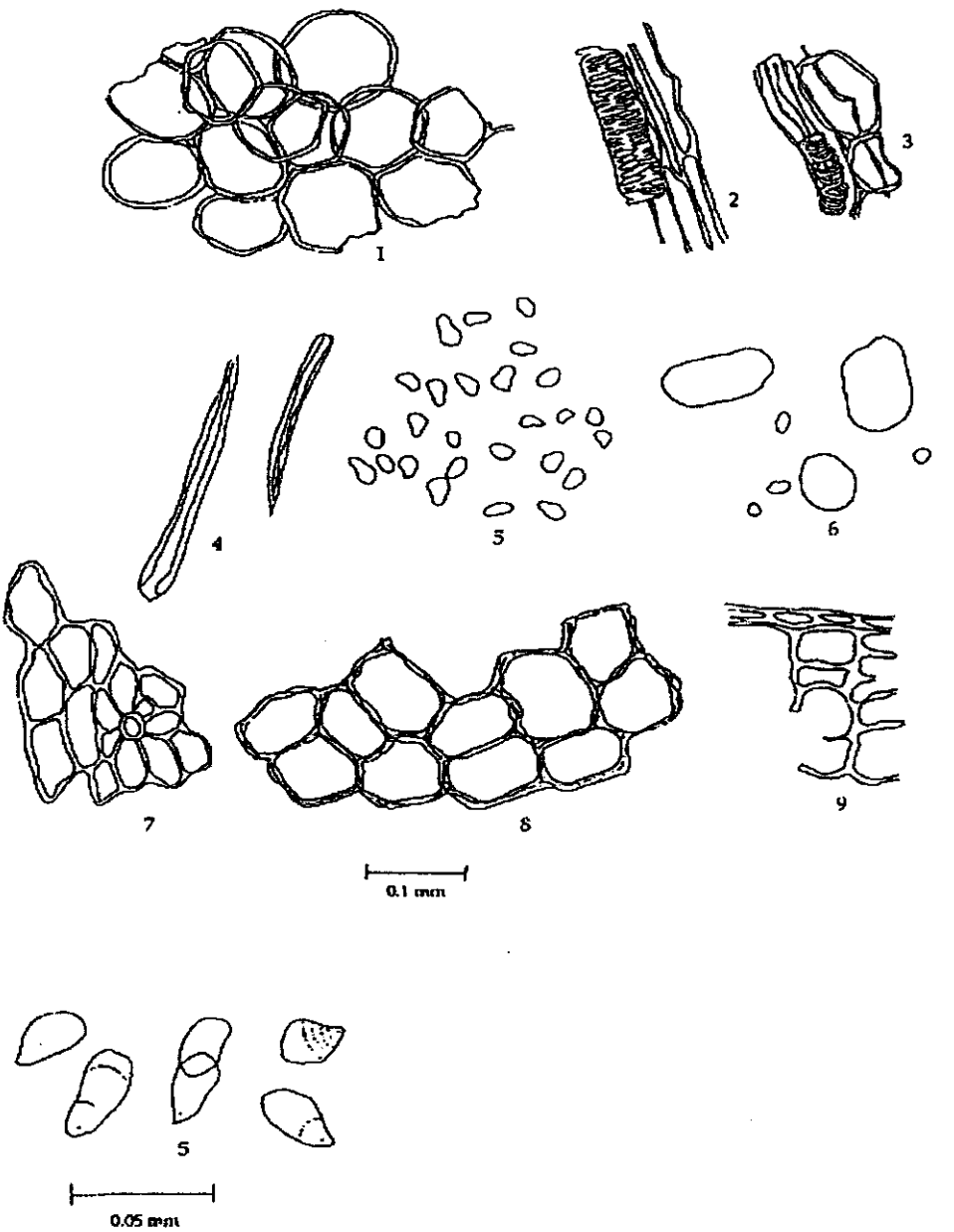


Figure 1-4 Powdered drug of the rhizome of *Curcuma longa* Linn. (Ministry of Public Health, 1998).

1. Parenchyma
2. Reticulate vessel
3. Spiral vessel
4. Unicellular trichomes
5. Starch granules
6. Altered starch grains
7. Epidermis in surface view
8. Cork in surface view
9. Epidermis and hypodermis in sectional view

4. The covering trichomes which, although not very numerous, are quite distinct; they are unicellular, elongated, conical and bluntly pointed with moderately thickened walls which may be faintly striated; the somewhat enlarged bases have pitted walls. The trichomes are found scattered and, occasionally, attached to fragments of the epidermis.

5. The vessels, which are fairly abundant are mostly large and reticulately thickened with regularly arranged rectangular pits. A few vessels with spiral or annular thickening also occur.

6. The very occasional starch granules (the majority of the starch is gelatininised) are mostly simple, flattened, oblong to oval or irregular in outline with a small point hilum situated at the narrow end; very faint transverse striations may be visible on a few of the granules.

3. Specification of high quality of powdered turmeric (Ministry of Public Health, 1998).

Commercially, powdered turmeric should consent with requirements given in Table 1-1.

Table 1-1 Specification of high quality of powdered turmeric (Ministry of Public Health, 1998).

Characteristic	Requirment	Method of test
Curcuminoids content	Not less than 5.0 % w/w	THP*
Volatile oil content	Not less than 6.0 % v/w	THP* appendix 7.3H
Moisture content	Not more than 10.0 % v/w	THP* appendix 4.12
Foreign matter	Not more than 2.0 % w/w	THP* appendix 7.2
Acid-insoluble ash	Not more than 1.0 % w/w	THP* appendix 7.6
Total ash	Not more than 8.0 % w/w	THP* appendix 7.7
Ethanol-soluble extractive	Not less than 10.0 % w/w	THP* appendix 7.12
Water-soluble extractive	Not less than 9.0 % w/w	THP* appendix 7.12

* = Thai Herbal Pharmacopoeia.

4. Chemical constituents and structures of compounds of turmeric and zedoary rhizomes.

There are a large number of publications in the literature reporting the chemical constituents in turmeric and zedoary rhizomes. The group of compounds commonly found in turmeric and zedoary are curcuminoids, volatile oil, carbohydrates, proteins, resin and mineral elements. Lists of the chemical constituents found in turmeric and zedoary are given in Tables 1-2 and 1-3, respectively. Structures of compounds of turmeric and zedoary are shown in Figures 1-5 and 1-6, respectively.

Table 1-2 Chemical constituents of *Curcuma longa* Linn.

Chemical groups	Chemical Constituents	References
Curcuminoids	Bisdemethoxycurcumin **(1)**	Purseglove *et al.*, 1981
	Curcumin **(2)**	Purseglove *et al.*, 1981
	Cyclocurcumin **(3)**	Kiuchi *et al.*, 1993
	Demethoxycurcumin **(4)**	Purseglove *et al.*, 1981
Volatile oil	Borneol **(5)**	Su *et al.*, 1982
- Monoterpenes	Camphene **(6)**	Farnsworth and Bunapraphatsara, 1992
	Camphor **(7)**	Farnsworth and Bunapraphatsara, 1992
	Car-3-ene **(8)**	Carron *et al.*, 1995
	1,8 –Cineole **(9)**	Carron *et al.*, 1995
	p-Cymene **(10)**	Su *et al.*, 1982
	p-Cymenene **(11)**	Su *et al.*, 1982
	Isoborneol **(12)**	Farnsworth and Bunapraphatsara, 1992
	Limonene **(13)**	Carron *et al.*, 1995
	Linalool **(14)**	Farnsworth and Bunapraphatsara, 1992
	α-Pinene **(15)**	Carron *et al.*, 1995
	β-Pinene **(16)**	Carron *et al.*, 1995
	α-Phyllandrene **(17)**	Carron *et al.*, 1995
	β- Phyllandrene **(18)**	Carron *et al.*, 1995
	Sabinene **(19)**	Su *et al.*, 1982
	α-Terpinene **(20)**	Carron *et al.*, 1995
	Terpinolene **(21)**	Carron *et al.*, 1995
	p-Tolylmethylcarbinol **(22)**	Farnsworth and Bunapraphatsara, 1992

Table 1-2 Chemical constituents of *Curcuma longa* Linn. (continued).

Chemical groups	Chemical Constituents	References
Volatile oil	α-Atlantone **(23)**	Su *et al.*, 1982
- Sesquiterpenes	γ-Atlantone **(24)**	Su *et al.*, 1982
- Bisaborane – type	Bisabola-3,10-diene-2-one **(25)**	Oshiro *et al.*, 1990
	2,5-Dihydroxy-bisabola-3,10-Diene **(26)**	Oshiro *et al.*, 1990
	4-Hydroxybisabola-2,10-diene-9-one **(27)**	Oshiro *et al.*, 1990
	4-Methoxy-5-hyhroxybisa-bola-2,10-diene-9-one **(28)**	Oshiro *et al.*, 1990
	Bisabolene **(29)**	Su *et al.*, 1982
	β-Bisabolene **(30)**	Richmond and Pombo, 1997
	Bisacumol **(31)**	Oshiro *et al.*, 1990
	Bisacurone **(32)**	Oshiro *et al.*, 1990
	ar-Curcumene **(33)**	Tang and Eisenbrand, 1992
	α-Curcumene **(34)**	Richmond and Pombo, 1997
	β- Curcumene **(35)**	Tang and Eisenbrand, 1992
	γ-Curcumene **(36)**	Tang and Eisenbrand, 1992
	Curlone **(37)**	Hiserodt *et al.*, 1996
	Dehydrocurcumene **(38)**	Kiso *et al.*, 1983
	β-Sesquiphyllandrene **(39)**	Richmond and Pombo, 1997

Table 1-2 Chemical constituents of *Curcuma longa* Linn. (continued).

Chemical groups	Chemical Constituents	References
- Sesquiterpenes	Turmerone **(40)**	Su *et al.*, 1982
-Bisaborane – type	ar-Turmerone **(41)**	Golding and Pombo, 1992
	α-Turmerone **(42)**	Golding and Pombo, 1992
	β- Turmerone **(43)**	Golding and Pombo, 1992
	ar-Turmerol **(44)**	Hiserodt *et al.*, 1996
	Turmeronol A **(45)**	Imai *et al.*, 1990
	Turmeronol B **(46)**	Imai *et al.*, 1990
	Zingiberene **(47)**	Farnsworth and Bunapraphatsara, 1992
	α- Zingiberene **(48)**	Richmond and Pombo, 1997
- Sesquiterpenes	α-Caryophyllene **(49)**	Su *et al.*, 1982
- Caryophyllane - type	β- Caryophyllene **(50)**	Su *et al.*, 1982
- Sesquiterpenes	Curcumenone **(51)**	He *et al.*, 1998
- Carabrane – type		
- Sesquiterpenes	Curzerenone **(52)**	Oshiro *et al.*, 1990
- Elemane – type		
- Sesquiterpenes	Curdione **(53)**	Su *et al.*, 1982
- Germacrane – type	Dehydrocurdione **(54)**	Oshiro *et al.*, 1990
	Germacrene **(55)**	Gopalan *et al.*, 2000
	Germacrene D **(56)**	Gopalan *et al.*, 2000
	Germacrone-13-al **(57)**	Oshiro *et al.*, 1990
	(4*S*,5*S*)-Germacrone-4,5-epoxide **(58)**	Oshiro *et al.*, 1990

Table 1-2 Chemical constituents of *Curcuma longa* Linn. (continued).

Chemical groups	Chemical Constituents	References
- Sesquiterpenes	Curcumenol **(59)**	He *et al.*, 1998
- Guaiane – type	Epiprocurcumenol **(60)**	Oshiro *et al.*, 1990
	Isoprocurcumenol **(61)**	Oshiro *et al.*, 1990
	Procurcumadiol **(62)**	Oshiro *et al.*, 1990
	Procurcumenol **(63)**	Oshiro *et al.*, 1990
	Zedoarondiol **(64)**	Oshiro *et al.*, 1990
Benzenoids	2-Hydroxy-5-methyl acetophenone **(65)**	Hiserodt *et al.*, 1996
	Dehydrozingerone **(66)**	Hiserodt *et al.*, 1996
	Vanillin **(67)**	Hiserodt *et al.*, 1996
Carbohydrates	Cellulose	Purseglove *et al.*, 1981
	Fructose	Farnsworth and Bunapraphatsara, 1992
	Glucose	Farnsworth and Bunapraphatsara, 1992
	Lectin	Farnsworth and Bunapraphatsara, 1992
	Pentosan	Purseglove *et al.*, 1981
	Starch	Purseglove *et al.*, 1981
	Ukonan A	Gonda *et al.*, 1990
	Ukonan B	Gonda *et al.*, 1990
	Ukonan C	Gonda *et al.*, 1990
	Ukonan D	Gonda *et al.*, 1992
Lipid	Fatty acid	Farnsworth and Bunapraphatsara, 1992
	Tetradecanoic acid	Richmond and Pombo, 1997

Table 1-2 Chemical constituents of *Curcuma longa* Linn. (continued).

Chemical groups	Chemical Constituents	References
Lipid (continued)	Stearic acid	Richmond and Pombo, 1997
Phenylpropanoid	Eugenol	Farnsworth and Bunapraphatsara, 1992
Proteins	Turmerin	Srinivas *et al.*, 1992
Steroids	Campesterol	Farnsworth and Bunapraphatsara, 1992
	Cholesterol	Farnsworth and Bunapraphatsara, 1992
Other compounds	Bitter principles	Purseglove *et al.*, 1981
	Glutamic acid	Farnsworth and Bunapraphatsara, 1992
	Mineral elements	Purseglove *et al.*, 1981
	Resin	Purseglove *et al.*, 1981
	Tocopherol	Farnsworth and Bunapraphatsara, 1992

Table 1-3 Chemical constituents of *Curcuma zedoaria* (Berg.) Roscoe.

Chemical groups	Chemical Constituents	References
Curcuminoids	Bisdemethoxycurcumin **(1)**	Syu *et al.*, 1998
	Curcumin **(2)**	Syu *et al.*, 1998
	Demethoxycurcumin **(4)**	Syu *et al.*, 1998
Volatile oil	Camphene **(6)**	Mau *et al.*, 2003
- Monoterpene	Camphor **(7)**	Mau *et al.*, 2003
	1,8-Cineole **(9)**	Mau *et al.*, 2003
	α-Pinene **(15)**	Mau *et al.*, 2003
	β-Pinene **(16)**	Mau *et al.*, 2003
	α-Terpineol **(68)**	Mau *et al.*, 2003
Volatile oil	β-Bisabolene **(30)**	Mau *et al.*, 2003
- Sesquiterpenes	Bisacumol **(31)**	Matsuda *et al.*, 2001a
- Bisaborane – type	Bisacurone **(32)**	Matsuda *et al.*, 2001a
	ar-Curcumene **(33)**	Tang and Eisenbrand, 1992
- Sesquiterpenes	α-Curcumene **(34)**	Mau *et al.*, 2003
- Bisaborane – type	β-Curcumene **(35)**	Tang and Eisenbrand, 1992
	ar-Turmerone **(41)**	Matsuda *et al.*, 2001b
	β-Turmerone**(43)**	Mau *et al.*, 2003
	Zingiberene **(47)**	Mau *et al.*, 2003
- Sesquiterpenes	α-Cadinol **(69)**	Mau *et al.*, 2003
- Cadinane – type	Curzeone **(70)**	Shiobara *et al.*, 1985
	Pyrocurzerenone **(71)**	Hikino *et al.*, 1975
- Sesquiterpenes	Curcarabranol A **(72)**	Yoshikawa *et al.*, 1998
- Carabrane – type	Curcarabranol B **(73)**	Yoshikawa *et al.*, 1998

Table 1-3 Chemical constituents of *Curcuma zedoaria* (Berg.) Roscoe (continued).

Chemical groups	Chemical Constituents	References
- Sesquiterpenes	Curcumenolactone A **(74)**	Matsuda *et al.*, 2001a
- Carabrane – type	Curcumenolactone B **(75)**	Matsuda *et al.*, 2001a
	Curcumenolactone C **(76)**	Matsuda *et al.*, 2001a
	Curcumenone **(51)**	Shiobara *et al.*, 1985
	4*S*-Dihydrocurcumenone **(77)**	Yoshikawa *et al.*, 1998
- Sesquiterpenes	Curzerene (Isofuranogerma-	Hikino *et al.*, 1968
- Elemane – type	Crene) **(78)**	
	Curzerenone **(52)**	Hikino *et al.*, 1968
	β-Elemene **(79)**	Mau *et al.*, 2003
	γ-Elemene **(80)**	Mau *et al.*, 2003
	β-Elemenone **(81)**	Mau *et al.*, 2003
	Elemol **(82)**	Mau *et al.*, 2003
	Epicurzerenone **(83)**	Hikino *et al.*, 1968
- Sesquiterpenes	Calarene **(84)**	Mau *et al.*, 2003
- Eudesmane – type	α-Calacorene **(85)**	Mau *et al.*, 2003
	Curcolonol **(86)**	Syu *et al.*, 1998
	β-Dictyopterol **(87)**	Matsuda *et al.*, 2001b
	β-Eudesmol **(88)**	Matsuda *et al.*, 2001a
	α-Selinene **(89)**	Mau *et al.*, 2003
	β-Selinene **(90)**	Mau *et al.*, 2003
	Zedoarofuran **(91)**	Matsuda *et al.*, 2001
- Sesquiterpenes	Curdione **(53)**	Matsuda *et al.*, 1998
- Germacrane – type	Dehydrocurdione **(54)**	Matsuda *et al.*, 1998
	Furanodiene **(92)**	Matsuda *et al.*, 1998
	Furanodienone **(93)**	Hikino *et al.*, 1975
	Furanogermenone **(94)**	Shibuya *et al.*, 1987

Table 1-3 Chemical constituents of *Curcuma zedoaria* (Berg.) Roscoe (continued).

Chemical groups	Chemical Constituents	References
- Sesquiterpenes	Germacrene B **(95)**	Mau *et al.*, 2003
- Germacrane – type	Germacrone **(96)**	Sakui *et al.*, 1992
	(+)-Germacrone-4,5-epoxide **(97)**	Matsuda *et al.*, 2001
	(4*S*,5*S*)-Germacrone 4,5-Epoxide **(58)**	Yoshihara *et al.*, 1984
	Glechomanolide **(98)**	Matsuda *et al.*, 2001
	13-Hydroxygermacrone **(99)**	Matsuda *et al.*, 1998; Shiobara *et al.*, 1986
	Isofuranodienone **(100)**	Matsuda *et al.*, 2001a
	Neocurdione **(101)**	Matsuda *et al.*, 1998
	Zederone **(102)**	Kouno and Kawano, 1985
- Sesquiterpenes	Aerugidiol **(103)**	Matsuda *et al.*, 1998
- Guaiane - type	Alismoxide **(104)**	Matsuda *et al.*, 2001a
	Curcumadiol **(105)**	Kouno and Kawano, 1985
	Curcumenol **(59)**	Hikino *et al.*, 1968
	Curcumol **(106)**	Kouno and Kawano, 1985
	3,7-Dimethylindan-5-carboxylic acid **(107)**	Syu *et al.*, 1998
	4-Epicurcumenol **(108)**	Matsuda *et al.*, 2001b
	7α, 11α-Epoxy-5β-hydroxy-9-guaiaen-8-one **(109)**	Matsuda *et al.*, 2001a
	Gajutsulactone A **(110)**	Matsuda *et al.*, 2001

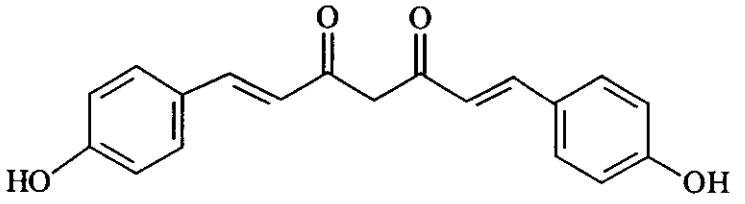
Table 1-3 Chemical constituents of *Curcuma zedoaria* (Berg.) Roscoe (continued).

Chemical groups	Chemical Constituents	References
- Sesquiterpenes	Gajutsulactone B **(111)**	Matsuda *et al.*, 2001
- Guaiane - type	Guaidiol **(112)**	Syu *et al.*, 1998
	Isocurcumenol **(113)**	Hikino *et al.*, 1968
	Isoprocumenol **(114)**	Matsuda *et al.*, 1998
	5-Isopropyllidene-3,8-Dimethyl-1(5H)-azulenone **(115)**	Mau *et al.*, 2003
	Isospathulenol **(116)**	Mau *et al.*, 2003
	Isozedoarondiol **(117)**	Matsuda *et al.*, 2001a
	Neocurcumenol **(118)**	Matsuda *et al.*, 2001b
	Procurcumenol **(63)**	Hikino *et al.*, 1968
	Spathulenol **(119)**	Mau *et al.*, 2003
	Zedoalactone B **(120)**	Matsuda *et al.*, 2001a
	Zedoarol **(121)**	Shiobara *et al.*, 1986
- Sesquiterpenes	Zedoarolide A **(122)**	Matsuda *et al.*, 2001b
- Guaiane - type	Zedoarolide B **(123)**	Matsuda *et al.*, 2001b
	Zedoarondiol **(64)**	Matsuda *et al.*, 1998; Kouno and Kawano, 1985
- Sesquiterpenes	Curcumanolide A **(124)**	Shiobara *et al.*, 1985
- Spirolactone - type	Curcumanolide B **(125)**	Shiobara *et al.*, 1985
- Sesquiterpenes	Curcumadione **(126)**	Matsuda *et al.*, 2001a
- Xanthane - type		
- Other- type	β-Farnesene **(127)**	Mau *et al.*, 2003
Sesquiterpenes	α-Humulene **(128)**	Mau *et al.*, 2003
Carbohydrate	Polysaccharide	Evan, 2002
Other compounds	2-Decanone**(129)**	Mau *et al.*, 2003

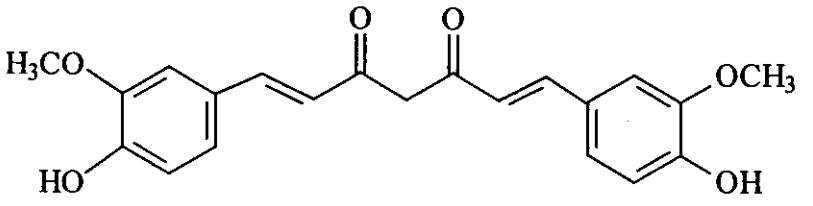
Table 1-3 Chemical constituents of *Curcuma zedoaria* (Berg.) Roscoe (continued).

Chemical groups	Chemical Constituents	References
Other compounds (continued)	Farnesol **(130)**	Mau *et al.*, 2003
	2-Nonanone **(131)**	Mau *et al.*, 2003
	2-Undecanone **(132)**	Mau *et al.*, 2003

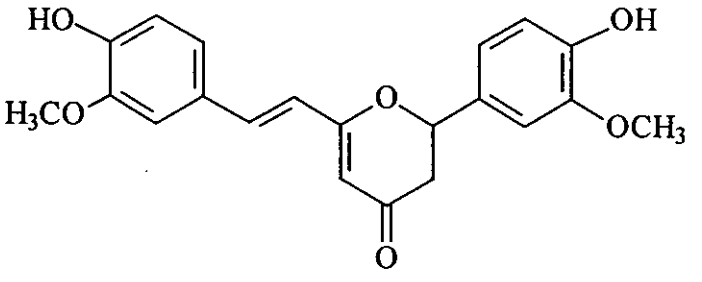
Curcuminoids



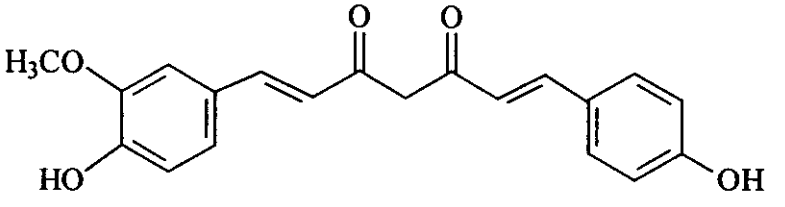
Bisdemethoxycurcumin **(1)**



Curcumin **(2)**



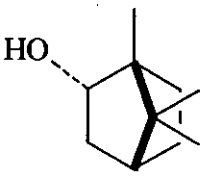
Cyclocurcumin **(3)**



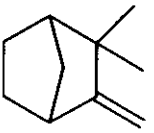
Demethoxycurcumin **(4)**

Figure 1-5 Structures of compounds of turmeric.

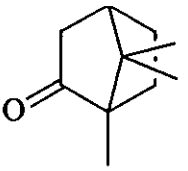
Monoterpenes



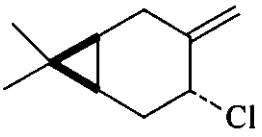
Borneol **(5)**



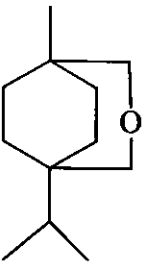
Camphene **(6)**



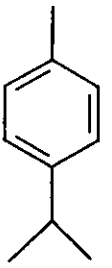
Camphor **(7)**



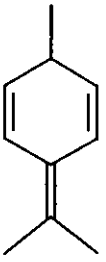
Car-3-ene **(8)**



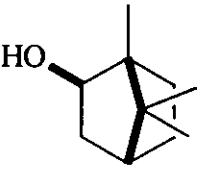
1,8-Cineole **(9)**



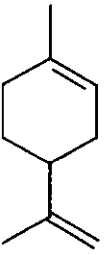
p-Cymene **(10)**



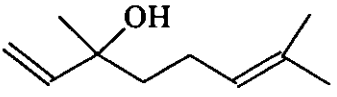
p-Cymenene **(11)**



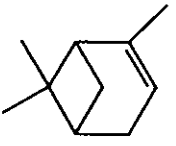
Isoborneol **(12)**



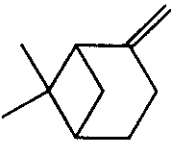
Limonene **(13)**



Linalool **(14)**



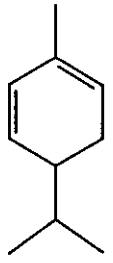
α-Pinene **(15)**



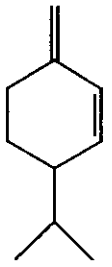
β-Pinene **(16)**

Figure 1-5 Structures of compounds of turmeric (continued).

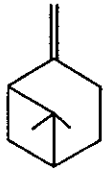
Monoterpenes



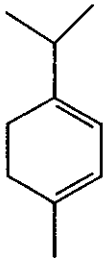
α-Phyllandrene **(17)**



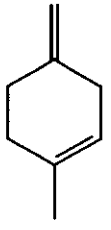
β-Phyllandrene **(18)**



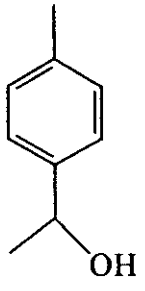
Sabinene **(19)**



α-Terpinene **(20)**



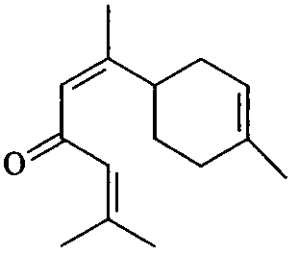
Terpinolene **(21)**



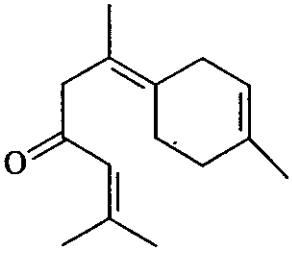
p-Tolylmethylcarbinol **(22)**

Sesquiterpenes

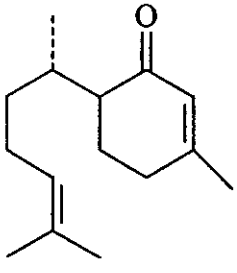
Bisaborane - type



α-Atlatone **(23)**



γ-Atlantone **(24)**

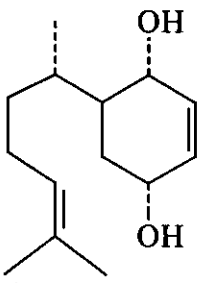


Bisabola-3, 10-diene-2-one **(25)**

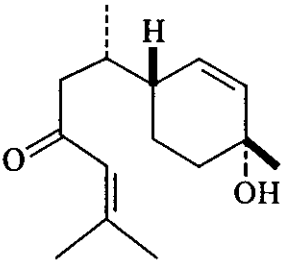
Figure 1-5 Structures of compounds of turmeric (continued).

Sesquiterpenes

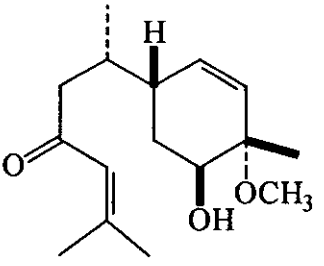
Bisaborane - type



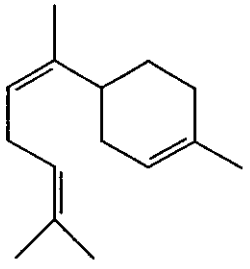
2,5-Dihydroxy-bisabola-3,10-diene **(26)**



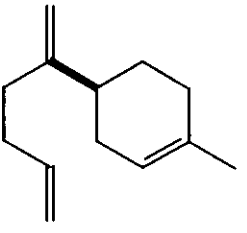
4-Hydroxybisabola-2,10-diene-9-one **(27)**



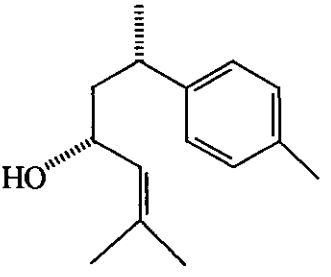
4-Methoxy-5-hydroxy-bisabola-2,10-diene-9-one **(28)**



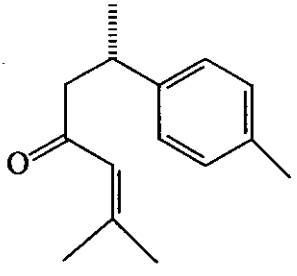
Bisabolene **(29)**



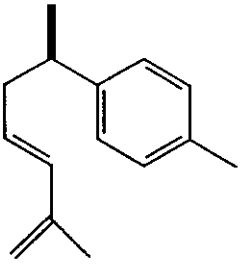
β-Bisabolene **(30)**



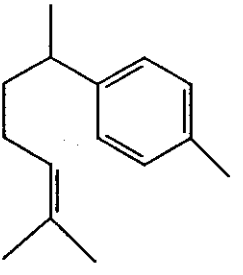
Bisacumol **(31)**



Bisacurone **(32)**



ar-Curcumene **(33)**

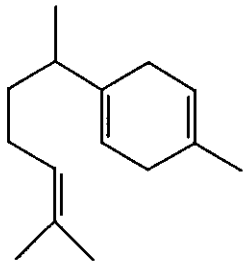


α-Curcumene **(34)**

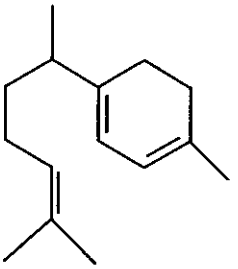
Figure 1-5 Structures of compounds of turmeric (continued).

Sesquiterpenes

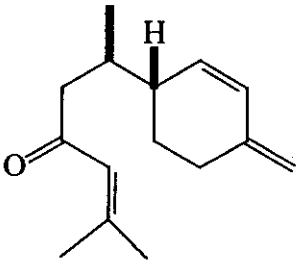
Bisaborane - type



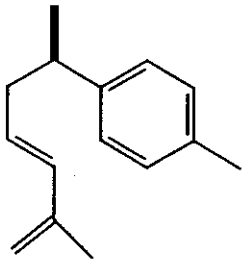
β-Curcumene **(35)**



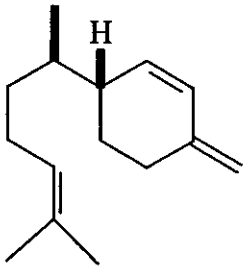
γ-Curcumene **(36)**



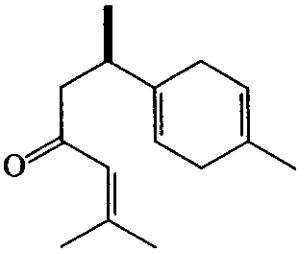
Curlone **(37)**



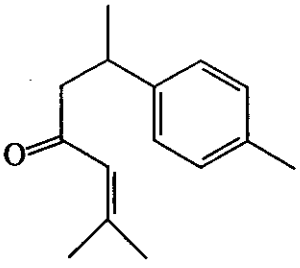
Dehydrocurcumene **(38)**



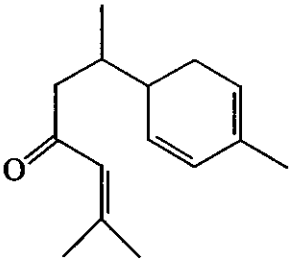
β-Sesquiphyllandrene **(39)**



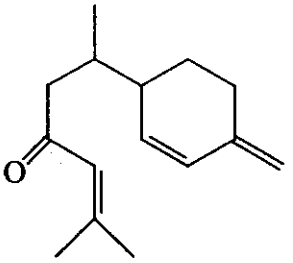
Turmerone **(40)**



ar-Turmerone **(41)**



α-Turmerone **(42)**

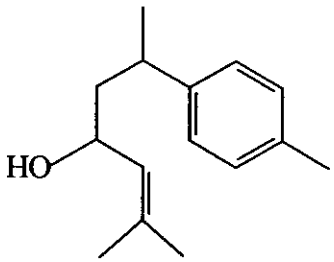


β-Turmerone **(43)**

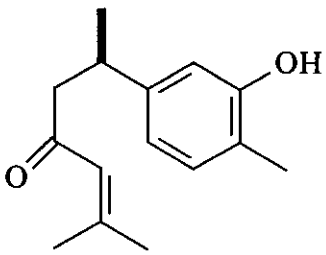
Figure 1-5 Structures of compounds of turmeric (continued).

Sesquiterpenes

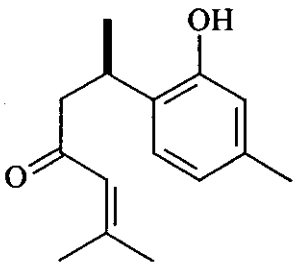
Bisaborane - type



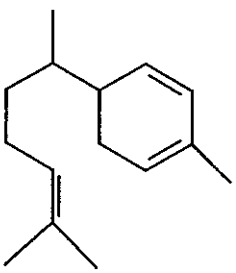
ar-Turmerol **(44)**



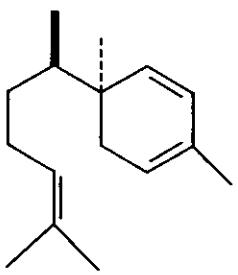
Turmeronol A **(45)**



Turmeronol B **(46)**

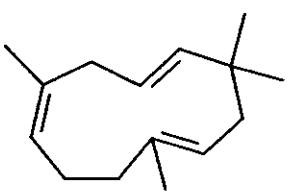


Zingiberene **(47)**

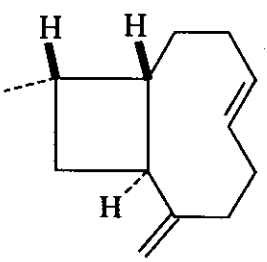


α-Zingiberene **(48)**

Caryophyllane - type



α-Caryophyllene **(49)**

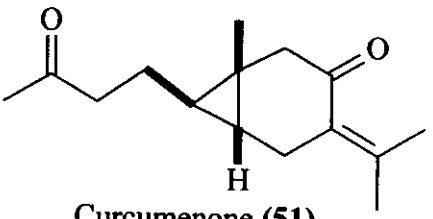


β-Caryophyllene **(50)**

Figure 1-5 Structures of compounds of turmeric (continued).

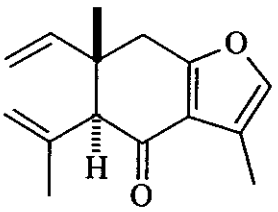
Sesquiterpenes

Carabrane - type



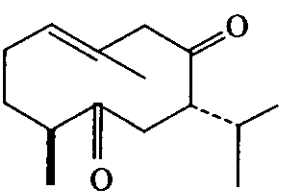
Curcumenone **(51)**

Elemane - type

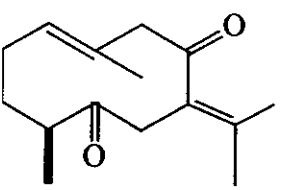


Curzerenone **(52)**

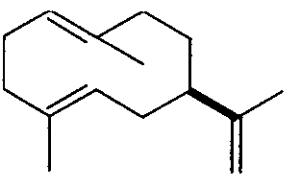
Germacrane - type



Curdione **(53)**



Dehydrocurdione **(54)**

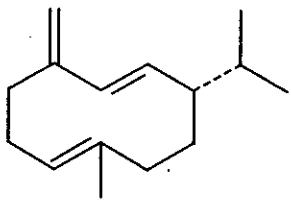


Germacrene **(55)**

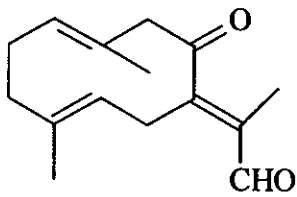
Figure 1-5 Structures of compounds of turmeric (continued).

Sesquiterpenes

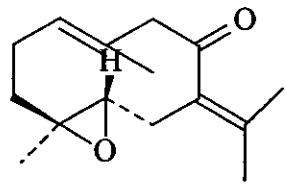
Germacrane - type



Germacrene D **(56)**

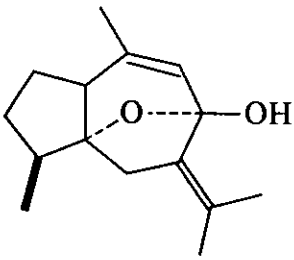


Germacrone-13-al **(57)**

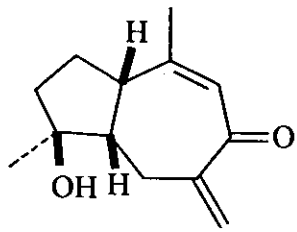


(4*S*,5*S*)-Germacrone-4,5-epoxide **(58)**

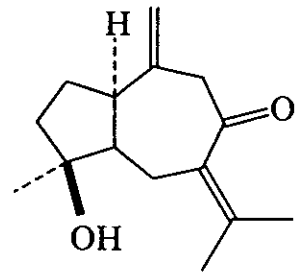
Guaiane - type



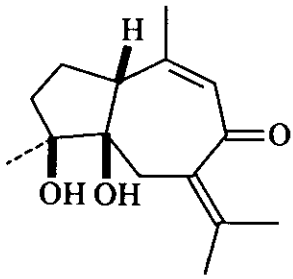
Curcumenol **(59)**



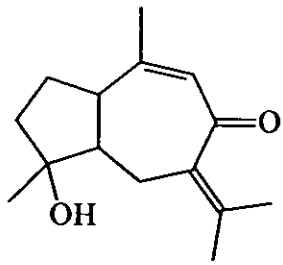
Epiprocurcumenol **(60)**



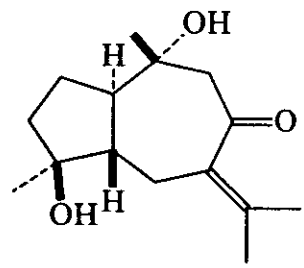
Isoprocurcumenol **(61)**



Procurcumadiol **(62)**



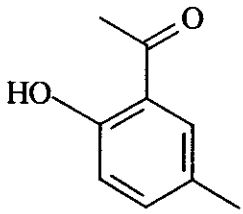
Procurcumenol **(63)**



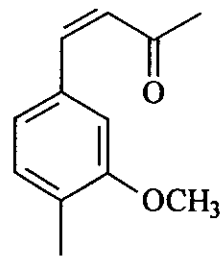
Zedoarondiol **(64)**

Figure 1-5 Structures of compounds of turmeric (continued).

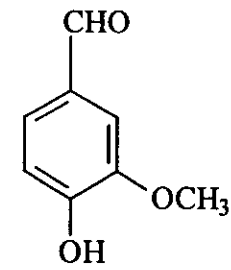
Benzenoids



2-Hydroxy-5-methyl acetophenone **(65)**



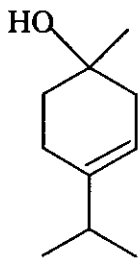
Dehydrozingerone **(66)**



Vanillin **(67)**

Figure 1-5 Structures of compounds of turmeric (continued).

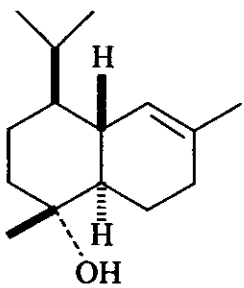
Monoterpenes



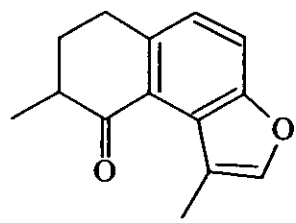
α-Terpineol **(68)**

Sesquiterpenes

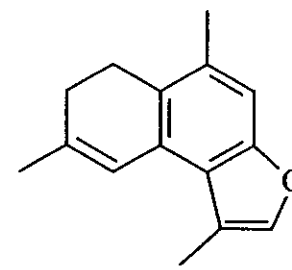
Cadinane - type



α-Cadinol **(69)**



Curzeone **(70)**

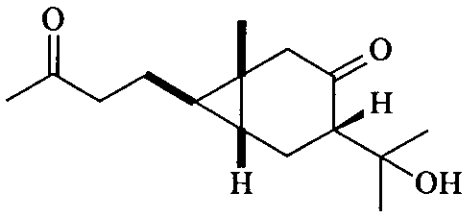


Pyrocurzerenone **(71)**

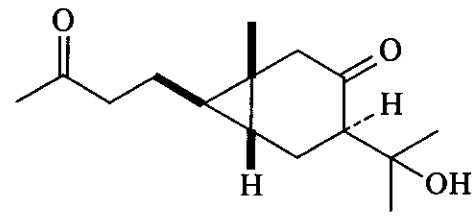
Figure 1-6 Structures of compounds of zedoary.

Sesquiterpenes

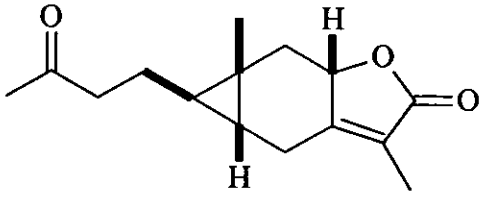
Carabrane - type



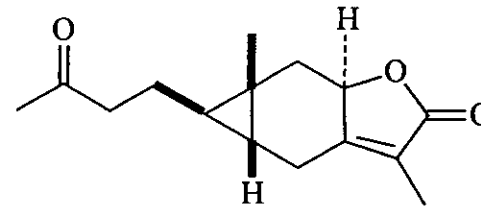
Curcurabranol A **(72)**



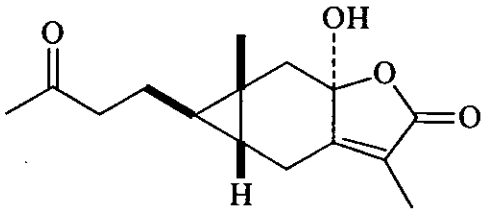
Curcurabranol B **(73)**



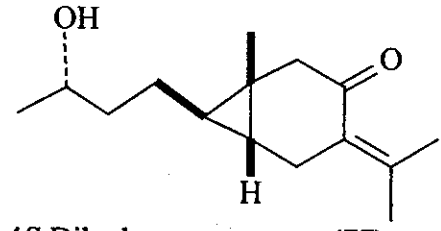
Curcumenolactone A **(74)**



Curcumenolactone B **(75)**



Curcumenolactone **(76)**

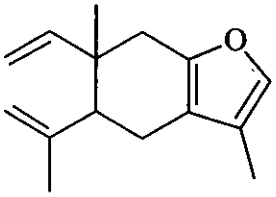


4*S*-Dihydrocurcumenone**(77)**

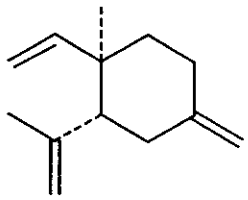
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

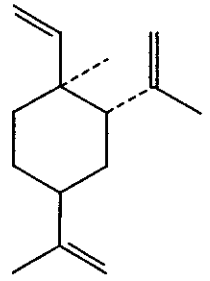
Elemane - type



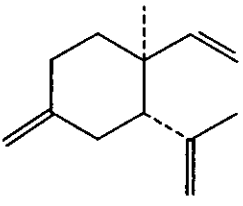
Curzerene (Isofuranogermacrene) **(78)**



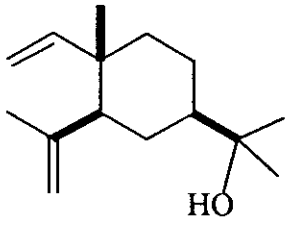
β-Elemene **(79)**



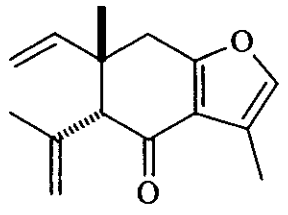
γ-Elemene **(80)**



β-Elemenene **(81)**

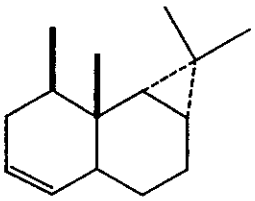


Elemol **(82)**

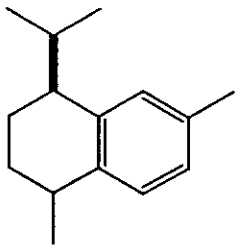


Epicurzerenone **(83)**

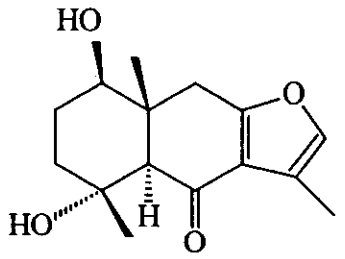
Eudesmane-type



Calarene **(84)**



α-Calacorene **(85)**

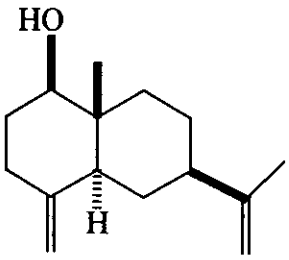


Curcolonol **(86)**

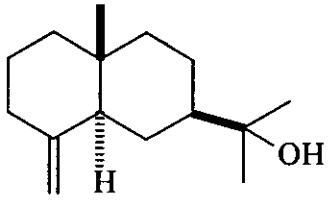
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

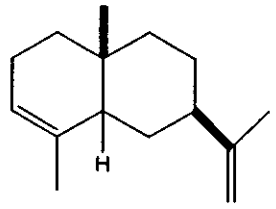
Eudesmane - type



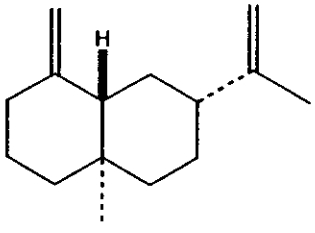
β-Dictyopterol **(87)**



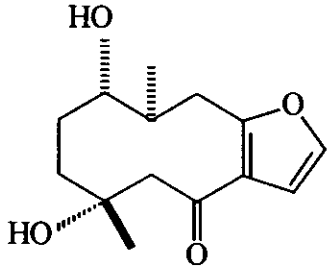
β-Eudesmol **(88)**



α-Selinene **(89)**

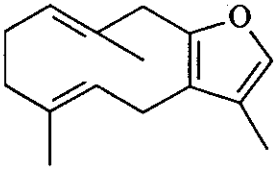


β-Selinene **(90)**

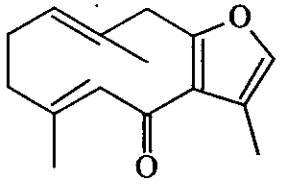


Zedoarofuran **(91)**

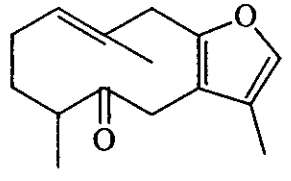
Germacrane - type



Furanodiene **(92)**



Furanodienone **(93)**

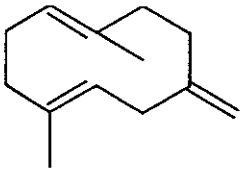


Furanogermenone **(94)**

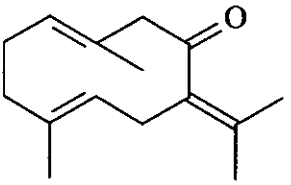
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

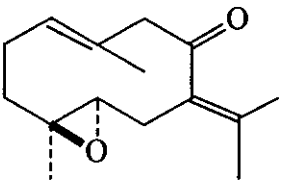
Germacrane - type



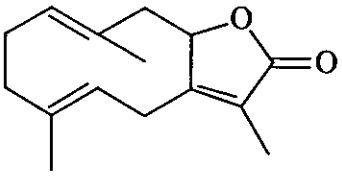
Germacrene B **(95)**



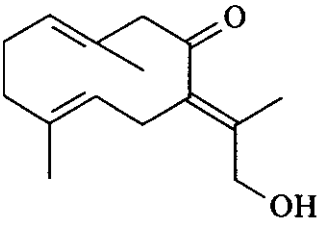
Germacrone **(96)**



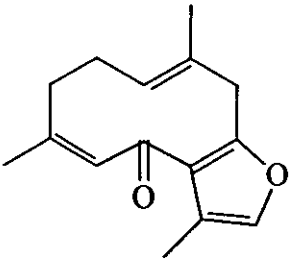
(+)-Germacrone-4,5-epoxide **(97)**



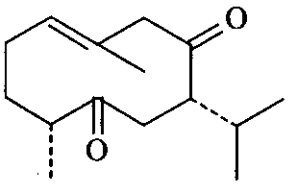
Glechomanolide **(98)**



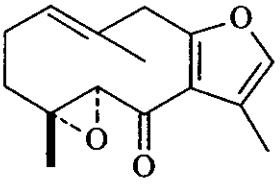
13-Hydroxy germacrone **(99)**



Isofuranodienone **(100)**



Neocurdione **(101)**

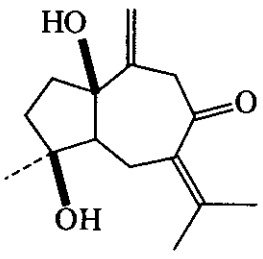


Zederone **(102)**

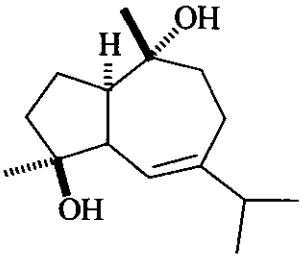
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

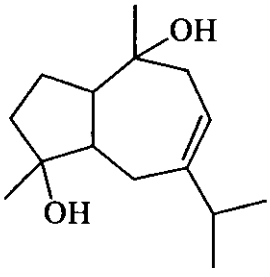
Guaiane - type



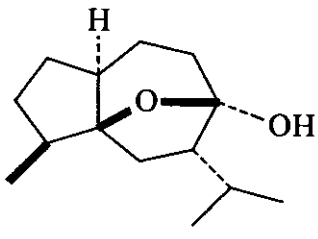
Aerugidiol **(103)**



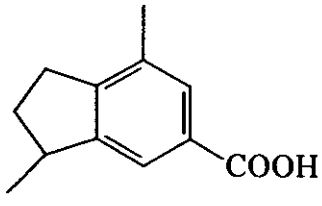
Alismoxide **(104)**



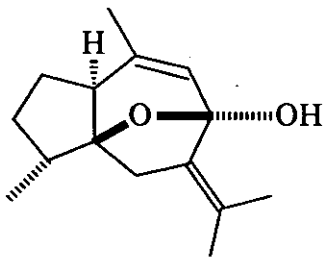
Curcumadiol **(105)**



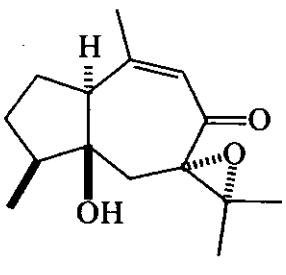
Curcumol **(106)**



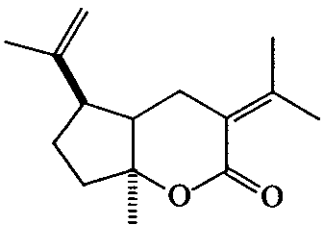
3,7-Dimethylindan-5-carboxylic acid **(107)**



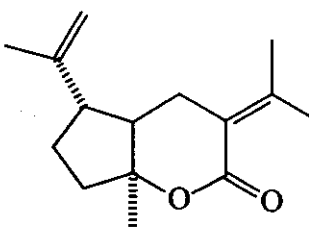
4-Epicurcumenol **(108)**



7α, 11α-Epoxy-5β-hydroxy- 9-guaiaen-8-one **(109)**



Gajutsulactone A **(110)**

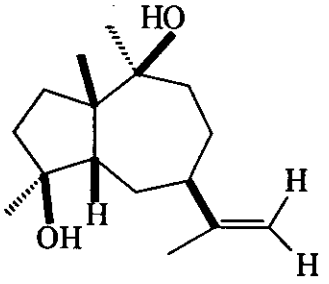


Gajutsulactone B **(111)**

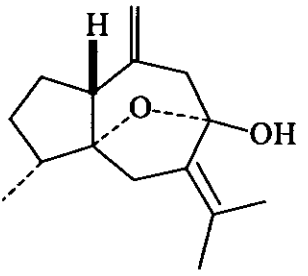
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

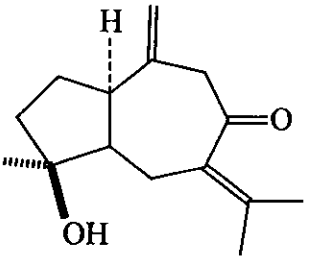
Guaiane - type



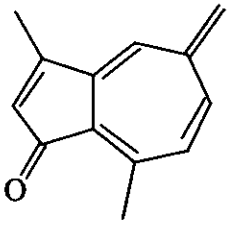
Guaidiol **(112)**



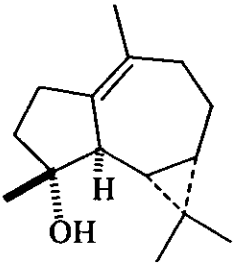
Isocurcumenol **(113)**



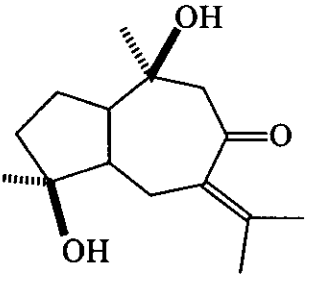
Isoprocumenol **(114)**



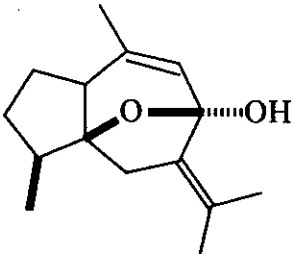
5-Isopropylidene-3,8-dimethyl-1 (5H)-azulenone **(115)**



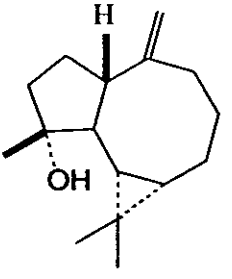
Isospathulenol **(116)**



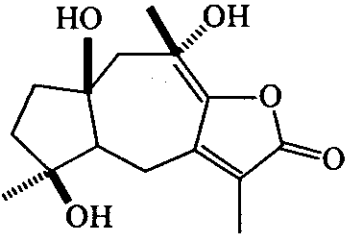
Isozedoarondiol **(117)**



Neocurcumenol **(118)**



Spathulenol **(119)**

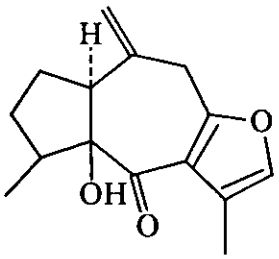


Zedoalactone B **(120)**

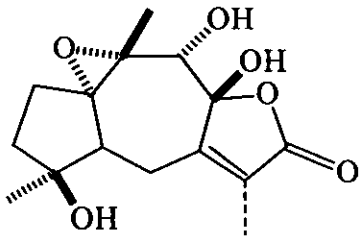
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

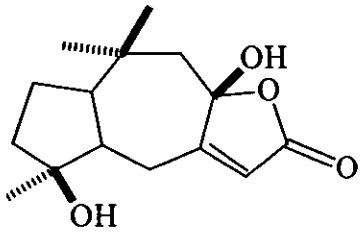
Guaiane - type



Zedoarol **(121)**

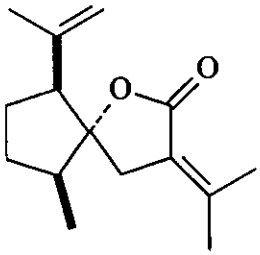


Zedoarolide A **(122)**

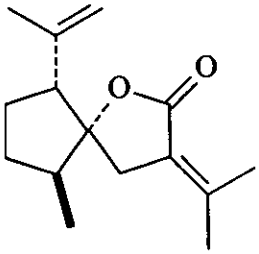


Zedoarolide B **(123)**

Spirolactone - type

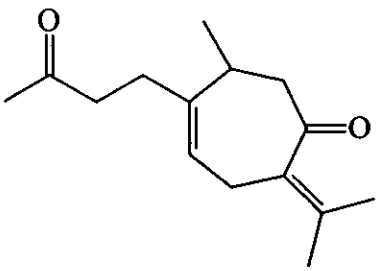


Curcumanolide A **(124)**



Curcumanolide B **(125)**

Xanthane - type

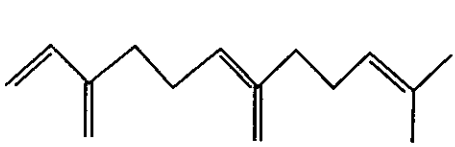


Curcumadione **(126)**

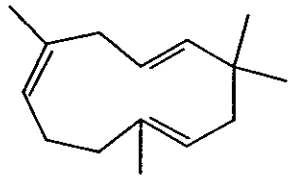
Figure 1-6 Structures of compounds of zedoary (continued).

Sesquiterpenes

Other - type sesquiterpenes

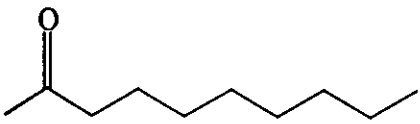


β-Farnesene **(127)**

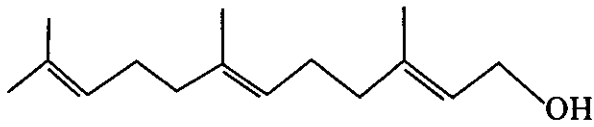


α-Humulene **(128)**

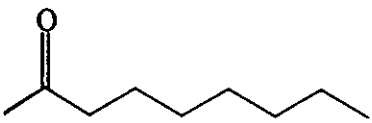
Other - type compounds



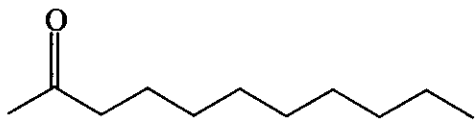
2-Decanone **(129)**



Farnesol **(130)**



2-Nonanone **(131)**



2-Undecanone **(132)**

Figure 1-6 Structures of compounds of zedoary (continued).

4.1 Chemical properties of curcuminoids.

Turmeric and zedoary rhizomes have three major components of curcuminoids which are responsible for theirs bright yellow color. They are curcumin {1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione}, demethoxycurcumin {1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione} and bisdemethoxycurcumin {1,7-bis(4-hydroxyphenyl)-1, 6-heptadiene-3,5-dione} (Khurana and Ho, 1988; Purseglove *et al.*, 1981). Curcuminoids are practically insoluble in water, while soluble in ethanol, ketone, acetic acid and chloroform. They are also more soluble in alkaline solvents and in extreamly acidic solvents, presumably due to the ionization of the phenolic or enolic groups and/or due to their degradation or change in their dissociation forms (Tønnessen *et el.*, 2002; Araujo and Leon, 2001; Bong, 2000).

4.2 Chemical properties of volatile oil.

Volatile oils obtained from turmeric and zedoary rhizomes are a yellow to orange-yellow in color. These oils are derived by steam-distillation and solvent-extraction methods (Mau *et al.*, 2003; Purseglove *et al.*, 1981). They contain many types of sesquiterpenes (e.g. zingiberene), and monoterpenes (e.g. camphor, 1,8-cineol) (Evan, 2002; Matsuda *et al.*, 2001a).

5. The uses of turmeric and zedoary rhizomes.

5.1 The uses of turmeric.

Curcuma longa Linn., known as turmeric, is an important spice among the peoples of India, East and South East Asia. It has been extensively used over centuries, both in food as a flavour, and as a common house hold medicine (Purseglove *et al.*, 1981; Perry, 1980).

In China and Japan, turmeric is used as stomachic, stimulant, carminative, hematic in all kinds of haemorrhages and a remedy for certain types of jaundice and other liver diseases. Externally it is applied to minor wounds and certain skin eruptions, and a decoction affords relief for burning sensation in eye diseases. It is considered to be very good for irregular menstruation, it promotes circulation, dissolves blood clots and is prescribed as a remedy for abdominal, chest and back pains (Perry, 1980; Loewengfeid and Bach, 1974).

In India, turmeric is boiled with milk and sugar for relief of a cold and is also used as a remedy for flatulence and liver complaints. It is an antacid and it acts as carminative, stomachic, appetizer and tonic. The juice of fresh rhizome has been used as an antiparasitic for many skin diseases (Loewengfeid and Bach, 1974).

In Indonesia, turmeric is a part of numerous native compound medicines. It is gargled as a mouthwash for inflamed gums and used for the treatment of itching.

A decoction is used as an antidysentery and anticholeretic medicine. It is applied topically for relief pain of insect bites (Perry, 1980).

In Philipines, the tincture or the juice may be given to treat bronchial catarrh and is used as an antiseptic by applying crushed rhizome to the affected area (Perry, 1980).

In Thailand, it is used as carminative, for treatment of peptic ulcer, dyspepsia, indigestion, flatulence, diarrhea, dysentery and also externally for itching and infected wounds by rubbing on the affected area. It is used as a mixture with a few drops of lime juice and alum or potassium nitrate for treatment of inflammation (Saralamp, 2000; Farnsworth and Bunapraphatsara, 1992).

5.2 The uses of zedoary.

Curcuma zedoaria (Berg.) Roscoe, known as zedoary has been widely cultivated as a vegetable or spice in East, South and Southest Asian countries including China, Vietnam, India, Japan and Thailand (Hong *et al.*, 2002; Saralamp *et al.*, 2000). In Chinese traditional medicine, zedoary has been prescribed as a stomachic, emmenagogue, or for the treatment of "Oketsu" syndrome, which is presumed to be caused by blood stagnation and for promoting menstruation in various preparations (Matsuda *et al.*, 1998; Yoshikawa *et al.*, 1998). In Japan, zedoary is listed in the Japanese Pharmacopoeia XIII as an aromatic stomachic, emmenagogus, or the treatment of "Oketsu" (Matsuda *et al.*, 2001a; Matsuda *et al.*,

1998; Shiobara *et al.*, 1985). Furthermore, zedoary also has been used as an important fragrance and spice (Matsuda *et al.*, 2001a).

In Thailand, zedoary has long been used in many preparations to relieve stomach discomfort, antidiarrheal, antiemetic and antipyretic. It is also used externally as an astringent for wounds (Saralamp *et al.*, 2000).

6. Biological activities of turmeric and zedoary rhizomes.

6.1 Biological activities of turmeric rhizome.

The active constituents of turmeric are curcuminoids, analogues of diarylheptanoids, and volatile oil, containing sesquiterpenes and monoterpenes. Curcuminoids consist of three constituents, the major constituent of which is curcumin, together with small quantities of demethoxycurcumin and bisdemethoxycurcumin.

There are a large number of publications in the literatures describing the biological activities of compounds extracted from turmeric. The biological activities of turmeric are as follows:

Antibacterial activities.

Turmeric oil was tested against cultures of *Staphylococcus albus*, *Staphylococcus aureus* and *Bacillus typhosus* (Araujo and Leon, 2001). It has been reported that alcoholic extracts inhibited growth of most organisms occuring in cholecytis including *Sarcinia*, *Lactobacillus*, *Corynebacterium*, *Escherichia*,

Streptococcus and *Bacillus* strains (Tang and Eisenbrand, 1992; Lutomski *et al.*, 1974). Curcumin (2.5-50 mg/ml) inhibited *Staphylococcus aureus* (Ammon and Wahl, 1991).

Anticancer activities.

Numerous studies have demonstrated the remarkable cancer preventive properties of curcumin. It is effective in inhibiting cancerous growths in various cancer inhibitor models (Jovanovic *et al.*, 2001). It has been found that curcumin has an efficacy in reducing chemically-induced tumors in mice (Soudamini and Kuttan, 1989). Huang *et al.* (1997) demonstrated that curcumin was a potent inhibitor on 12-*O*-tetradecanoyl-13 acetate (TPA)-induced oxidation of DNA bases in the epidermis, and on tumor promotion in mouse skin. Furthermore, it has been shown to have an action on rabbit osteoclast apoptosis; the results indicated that it drastically inhibited bone resorption in parallel with its stimulation of apoptosis in the cells (Araujo and Leon, 2001). Curcuminoids have been shown to be potent inhibitors of the proliferation of MCF-7 human breast and AK-5 tumor cells (Khar *et al.*, 1999; Simon *et al.*, 1998). An ethanolic extract of *C. longa* has been reported to inhibit the cell growth of chinese hamsters ovary cell (Kuttan *et al.*, 1985).

Antifungal and antiyeast activities.

The volatile oil apparently inhibits dermatophytes and pathogenic molds *in vitro* and markedly decreases erythema and scale induced by *Trichophyton*

rubum in guinea pigs, while pure curcumin has no antifungal and antiyeast activities (Apisariyakul *et al.*, 1995).

Antihepatotoxic activities.

Turmeric possesses antihepatotoxic activity which was later found to be due to curcuminoids. It has been reported that curcumin diminished carbon tetrachloride (CCl_4)-induced glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) (Kiso *et al.*, 1983).

Anti-HIV activities.

Mazumder *et al.* (1995) demonstrated that curcumin has an antiviral activity, being a HIV-1 integrase inhibitor and suggested that its analogs may be developed as anti-AIDs drugs. It has also been claimed that curcumin have anti HIV-1 and HIV-2 activities (Eigner and Scholz, 1999; Barthelemy *et al.*, 1998).

Antiinflammatory activities.

Ammon *et al.* (1992) demonstrated the antiinflammatory activity of curcumin as an inhibitor of leukotriene formation in rat peritoneal polymorphonuclear neutrophils, whereas, hydrocortisone did not show any effect. Curcumin was found to inhibit the 5-lipoxygenase activity in rat peritoneal neutrophils as well as the 12-lipoxygenase and the cyclooxygenase activities in human platelets (Ammon *et al.*, 1993). Curcumin has been reported to inhibit lipopolysaccharide-induced production of tumor necrosis factor-α and interleukin-

1β (IL-1) which play significant roles in many acute and chronic inflammatory diseases (Chan, 1995). Curcumin has been shown to have antiinflammatory activity in acute and chronic inflammation models, the potency of which is approximately equal to phenylbutazone in the carrageenin-induced edema test (Srimal and Dhawan, 1973). Curcumin was a more potent inflammation inhibitor than ibuprofen in comparative studies on inflammation-induced changes in rats. *In vitro*, it was found to be more potent than ibuprofen as a stabilizer of lysosomal membranes (Srivastava and Srimal, 1985). Volatile oil has been shown to have antiinflammatory activity against Freund's adjuvant-induced arthritis (Chandra and Gupta, 1972).

Antioxidant activities.

Free radicals have been shown to exert deleterious effects on skin and are thought to be involved in aging (Bonte *et al.*, 1997). There is a large volume of data that suggests that curcumin and other antioxidant compounds from the rhizome of turmeric may be useful for the prevention and/or treatment of some age-related degenerative process (Miquel *et al.*, 2002). Curcumin is a good antioxidant and lipid peroxidation inhibitor. It does this apparently by maintaining the activities of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Reddy and Lokesh, 1994). Curcuminoids have been reported to protect normal human keratinocytes by synergistically decreased in superoxide radical formation leading to diminish levels of cytotoxic hydrogen peroxide (Bonte *et al.*, 1997).

Antipeptic ulcer activities.

An ethanolic extract of turmeric was studied in rats for its ability to inhibit gastric secretion and to protect gastroduodenal mucosa against injuries. It was found that an oral dose of 500 mg/kg of the extract produced significant antiulcerogenic activity (Rafatullah *et al.*, 1999). Turmeric powder has been reported to protect the gastric mucosa against irritants (Scartezzini and Speroni, 2000).

Antiprotozoal activities.

Rasmussen *et al.* (2000) reported the efficacy of an ethanolic extract from turmeric against *Plasmodium falciparum* and *Leishmania major*. Curcumin was tested *in vivo* in mice and showed good activity in inhibiting the lesion size of the footpad of the infected animals, when compared with the same group without treatment (Araujo and Leon, 2001). The ethanolic extract of the turmeric has been reported having activity against *Entamoeba histolytica* (Ammon and Wahl, 1991).

Hypocholesterolemic activities.

Rao *et al.* (1970) demonstrated that curcumin has an hypocholesterolemic effect; it increased fecal excretion of cholesterol and bile acids in normal and in hypercholesterolemic rats. Turmeric extract has also been studied for hypocholesterolemic effect. The data showed that when a 5 or 10% of extract is fed to mice treated with a hypercholesterolemic diet, there was a significant decreased serum and liver value of cholesterol, triglycerides and total lipids

(Godkar *et al.*, 1996). Ramirez-Bosca *et al.* (2000) reported that daily oral administration of the turmeric extract significantly decreased the low density lipoprotein (LDL) and increased the high density lipoprotein (HDL) of healthy subjects.

Other activities.

Turmeric extract is also reported to be anticoagulant, antispasmodic, antivenum, cholagogue, immunological, nematocidal and wound healing activities. (Araujo and Leon, 2001; Bunapraphatsara, 1992; Farnsworth and Tang Eisenbrand, 1992; Ammon and Wahl, 1991)

6.2 Biological activities of zedoary.

Zedoary contains curcuminoids, including curcumin, demethoxycurcumin and bisdemethoxycurcumin and volatile oil including sesquiterpenes, and monoterpenes. The major sesquiterpene compounds, including dehydrocurdione, furanodiene, germacrone, curdione, curcumenol, neocurdione, curcumenol, isocurcumenol, aerugidiol, zedoarondiol and curcumenone were found to show biological activities (Mau *et al.*, 2003; Syu *et al.*, 1998; Yoshioka *et al.*, 1998). The biological activities of zedoary are as follows:

Analgesic activities.

The hydroalcoholic extract and curcumenol were evaluated in several pain models in mice, including formalin and capsaicin induced writhing. The results

showed that they exhibited potent and dose-related analgesic activity (Navarro *et al.*, 2002).

Anticancer activities.

Curcuminoids isolated from zedoary were demonstrated to be cytotoxic against human ovarian cancer OVCAR-3 cells. It has been found that polysaccharides and the protein-bound polysaccharides of this plant showed inhibition of sarcoma-180 and Echrlich ascites tumor in mice (Syu *et al.*, 1998).

Antihepatotoxic activities.

Sesquiterpenoids, such as furanodiene, curcumenone, dehydrocurdione, and curcumin were found to show a protective effect against D-galactosamine (D-GalN)/lipopolysaccharide-induced acute liver injury in mice and also inhibited the rising of serum aspartate aminotransaferase and alanine aminotransaminase (Morikawa *et al.*, 2002; Matsuda *et al.*, 2001a; Matsuda *et al.*, 1998).

Antiinflammatory activities.

Dehydrocurdione was tested for *in vivo* and *in vitro* antiinflammatory actions. The data was shown that oral administration of dehydrocurdione significantly reduced chronic adjuvant arthritis and inhibited the carrageenan-induced paw edema (Yoshioka *et al.*, 1998). Sesquiterpenes and curcuminoids were found to inhibit nitric oxide (NO) production from lipopolysaccharide (LPS)-activated macrophages. The inhibitory activity of these compounds against NO

production may be important evidence for the treatment of inflammation. Hong *et al.* (2002) and Lee *et al.* (2002) reported that sesquiterpenoids β-turmerone and ar-turmerone showed a potent inhibitory activity of cyclooxygenase-2 (COX-2) and nitric oxide synthase, both important mediators in the process of inflammation. They also inhibited lipopolysaccharide (LPS)-induced prostaglandin E_2 production in cultured mouse macrophage cell RAW 264.7 (Hong *et al.*, 2002).

Antioxidant activities.

Mau *et al.* (2003) reported that the volatile oil was moderate to good in antioxidant activity tested by different methods, good in reducing power and excellent in scavenging effect on 1,1-diphenyl-2-picrylhadrazyl (DPPH) radical but low in chelating effect on ferrous ion.

Other activities.

Zedoary extract has also been reported to have antimicrobial activities against *Staphylococcus aureus*, *Vibrio comma* and *Escherichia coli* (Syu *et al.*, 1998), antifungal (Gupta *et al.*, 1976), antimutagenic (Lee and lin, 1988), as well as vasorelaxant activities (Matsuda *et al.*, 2001b).

7. Pharmacokinetics of curcuminoids of turmeric and zedoary.

Oral administration of curcumin at a dose of 1 g/kg, resulted in 79% curcumin being excreted in the feces, while negligible amounts of curcumin appeared in the urine. Measurements of blood plasma levels and biliary excretions

showed that curcumin was poorly absorbed from the gut (Wahlstrom and Blennow, 1978). Curcumin injected intraperitoneally was excreted in the bile with glucuronides of tetrahydrocurcumin and hexahydrocurcumin as major metabolites, while, a minor biliary metabolite was dehydroferulic acid together with traces amount of ferulic acid (Holder *et al.*, 1978).

8. Toxicity of turmeric and zedoary.

8.1 Toxicity of turmeric.

Toxicological studies showed that whole turmeric or curcumin fed to rat at doses up to 125-fold those corresponding to normal human intake caused no adverse effects on growth, blood counts, or clinical blood chemistry such as serum aminotransferase or alkaline phosphatase. At the highest level tried (10% curcumin), the feeding efficiency was much lower than normal because of low diet intake possibly due to unpalatability (Sambaiah *et al.*, 1982). Acute toxicity studies on the alcoholic extract have also been conducted in different species of animals, including non-rodents. Neither the rhizome nor its alcoholic extract was toxic even at doses of 2.5 g/kg and 300 mg/kg, respectively (Shankar *et al.*, 1980). Curcumin was fed for 102-109 days to pigs at a daily dose of 60, 296 and 1551 mg/kg. The highest dose group showed a reduction in weight gain and in feed efficiency. Furthermore, statistically significant dose-related increases in weight of the liver and thyroid were recorded at all dose levels. Pericholangitis, hyperplasia of the thyroid and epithelial changes in the kidney and urinary bladder were

observed in the two higher dose groups (Bille *et al.*, 1985). Curcuminoids are cytotoxic inhibiting mitosis and leading to chromosome changes. In mice, chronic administration leads to significant changes in heart and lung weights and reduction in the red and white blood corpuscle counts (Bisset, 1994). Addition of either turmeric (0.5%) or curcumin (0.015%) into diets of mice showed no significant effects on the incidence of micronucleated polychromatic erythrocytes, structural and numerical aberrations in bone marrow chromosomes, pregnancy rate, number of live and dead embryos, total implants and mutagenic index. Rat fed cooked diets containing 0.5% or 0.05% turmeric showed no significant differences in the incidence of chromosomal aberrations in bone marrow (Vijayalaxmi, 1980).

8.2 Toxicity of zedoary.

Toxicological studies showed that curcumenol, sesquiterpene in volatile oil injected intravenously or directly into the cancer tissue produced no damage to the kidney or liver. Curcumenol was taken orally, occasional dizziness, nausea and vomitting, palpitation and fatigue may be reported (in patient) (Huang, 1993).

9. Factors affecting the curcuminoids and volatile oil contents in turmeric and zedoary rhizomes.

Since the quality of turmeric and zedoary are based directly on the curcuminoids and volatile oil content, some factors affecting the active constituents have been reported. The fresh rhizome of turmeric, which were dried in the laboratory and well preserved, contained higher curcuminoids and volatile oil

contents than the dried rhizome from old-styled drugstores (Chavalittumrong and Dechatiwongse, 1988). Khurana and Ho (1988) reported that curcuminoids were more stable to photooxidation as a dried powder than as an alcoholic extract. The quantities of the curcuminoids and volatile oil of the turmeric rhizome from Nakhon Pathom and Prachuap Khirikhan, Thailand were evaluated. It was found that turmeric of 5-month-old plant yielded the highest content of the active constituents (Chavalittumrong and Jirawattanapong, 1992). Tewtrakul (1993), concluded that the climatic and geographical conditions have an influence on the content and composition of curcuminoids and volatile oil in the turmeric rhizome. The effect of gamma irradiation on curcumin and volatile oil of turmeric was investigated. The result showed that irradiation treatment and storage time did not result in a significant change on curcumin content, water activity, pH and moisture content of investigated turmeric (Chosdu *et al.*, 1995; Chatterjee *et al.*, 1998). The gamma irradiation at 10 kGy, the dose recommended for microbial decontamination of spices, had no effect on the composition of the volatile oil as compared to the equivalent non-irradiated ones (Chatterjee *et al.*, 2000). The organoleptic properties of turmeric can differ somewhat according to the origin of plant and the age of the samples. The yield of oil and its physicochemical properties can also vary between individual samples (Purseglove *et al.*, 1981). Maga and Kim (1990) observed that water soluble turmeric color was stable during extrusion cooking at 125° and 155 °C. In contrast, the oil soluble compounds degraded during extrusion by the same procedure. Srinivasan *et al.* (1992) observed a loss of over 85% of curcuminoids during domestic cooking (boiling)

for 15 min either in presence or absence of a souring agent. Price and Buescher (1996) determined the decomposition of turmeric curcuminoids as affected by light, solvent and oxygen, the result shown that photodecomposition of purified curcumin, demethoxycurcumin and bisdemethoxycurcumin followed first-order kinetics. Rates of decomposition of the pigments were considerably lower in methanol than in acid brine. Curcuminoids were observed to be sensitive to light, however the combined effects of air and light were most deleterious and there was no influence of water activity on the stability of curcuminoids in curcumin- and turmeric oleoresin-microcrystalline cellulose model systems (Souza *et al.*, 1997). Alkaline degradation of the pigments curcumin, demethoxycurcumin and bisdemethoxycurcumin in aqueous solution were investigated. Alkaline degradation of the compounds corresponded to pseudo-first-order kinetics and degradation rate constants rapidly increased from pH 7.45 to 10.2 (Price and Buescher, 1997). The stability of curcumin under alkaline conditions was dramatically improved by complex formation between curcumin and cyclodextrins (Tønnessen *et al.*, 2002). Wang *et al.* (1997) investigated the kinetics of degradation of curcumin under various pH conditions, the result shown that the kinetic of curcumin degraded at various pH values followed a first order reaction and it decomposed rapidly in buffer systems at neutral-basic pH conditions, whereas, optimum stability of curcumin was obtained in pH lower than 7.0. *Trans*-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexanal, vanillin, ferulic acid and feruloyl methane were identified as its degradation products. The stability of curcuminoids in physiological media has also been reported, the data has shown

that the curcuminoids decomposed very rapidly (more than 90% within 12 hrs) when serum was omitted, but were more stable in the presence of serum (Pfeiffer *et al.*, 2003). Moreover, atmospheric oxygen can change the chemical composition of volatile oil by combining with some of their components. This process is called oxidation. It is speeded up by both heat and light (Tisserand and Balaces, 1995).

Objectives

The objectives of this study were as follows :

1. To determine the effect of growth stages on the contents of curcuminoids and volatile oil in turmeric and zedoary rhizomes.
2. To determine the effect of storage conditions on the contents of curcuminoids and volatile oil in turmeric and zedoary rhizomes.
3. To determine the kinetics of decomposition of curcuminoids content in turmeric and zedoary rhizomes.
4. To determine the antibacterial and antioxidant activities of turmeric and zedoary rhizomes over storage periods.