CHAPTER II
HISTORICAL

2.1 *Artemisia vulgaris* L. var. *indica* Maxim.

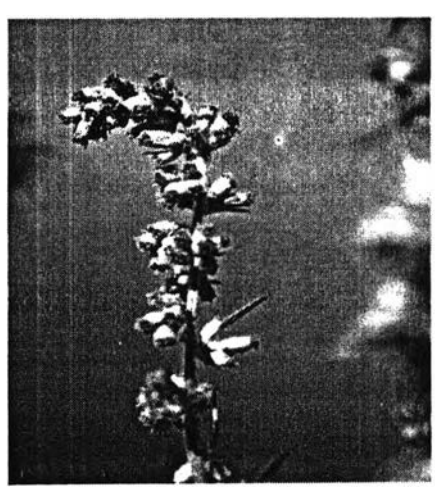
Artemisia vulgaris L. var. *indica* Maxim (syn. *Artemisia dubia* Wall. ex Bess.) (Fig. 7), belongs to the family Asteraceae. It is commonly known as Akajedaw, Fleabane, Indian wormwood, Mugwort, Mug-wort and Titepati. It has been called Kot chula lumpha, Phak hia, Ching hia, and Kot chula lumpha chin in Thai, and Ching-chu-chai in Chinese (Smitinand, 2001). It is native to Europe and continental Asia.

The description of this plant is; Perennial herb, Stem erect, ascending, branched, furrowed, and densely whitish hairy, Leaves alternate, short-stalked to sessile, pinnatipartile to bipinnate, densely white-lanate-arachnoid beneath, and thinly hairy above, Inflorescences terminal and axillaries panicles, heads sessile, corolla light green, Common weed in open localities, fallow fields, waste places, roadsides, rare in regularly cultivated fields, Propagated mainly by underground stolons, Blooming period: November-April (Harada *et al.*, 1987).

This plant has been used in various ways such as anthelmintic, asthma, scabies, skin rashes, headache, stomachache, homeostatic for nose bleeding and bleeding wound, antiseptic and antipyretic.

Aerial part

Leaves



Flowers

Figure 7 *Artemisia vulgaris* L. var. *indica* Maxim

(syn. *Artemisia dubia* Wall. ex Bess.)

The essential oil obtained from leaves hydrodistillation has been shown the characteristic sweet scent of (+)-davanone, which has been the major constituent.

(+)-Davanone ($C_{15}H_{24}O_2$) (Fig. 8), a sesquiterpene ketone, was first characterised by Sipma and van der Wal in 1968. It and related compounds have been synthesised by several groups and in recently, the total synthesis of (±)-davanone has been succeeded and described in 1999 by Molander and Hass (Molander and Hass, 1999). It showed a strong dose-dependent antispasmodic action (Perfumi *et al.*, 1995).

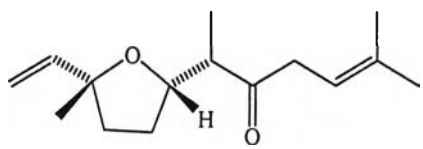


Figure 8 Chemical structure of (+)-davanone

Biosynthesis of davanone has been described in 1986 by Akhila *et al.* as shown in Fig. 9. Isopenthenylpyrrophosphate, IPP (I) condenses with dimethylallyl pyrrophosphate, DMAPP (II) to give geranylpyrrophosphate, GPP (III), which further condenses with another molecule of IPP to produce farnesylpyrrophosphate, FPP (IV), the traditional precursor of sesquiterpenes. It is well established now that FPP isomerises to nerolidylpyrrophosphate (V), and metabolises to many acyclic and cyclic sesquiterpenes. In this case, -OPP from C-5 of FPP would shift to C-3 to give nerolidylpyrrophosphate (analogy to geraniol-linalool interconversion). Nerolidyl-pyrrophosphate is expected to undergo cyclisation and oxidation at C-2 to metabolise davanone (VI) (Akhila *et al.*, 1986).

The essential oil production of *Artemisia* spp. cell and tissue cultures has been studied since 1990. However, there is no report about *Artemisia vulgaris* var. *indica* cell culture.

The selected examples of *Artemisia* spp. have been studied in essential oil production from cell and tissue culture are revealed in Table 12

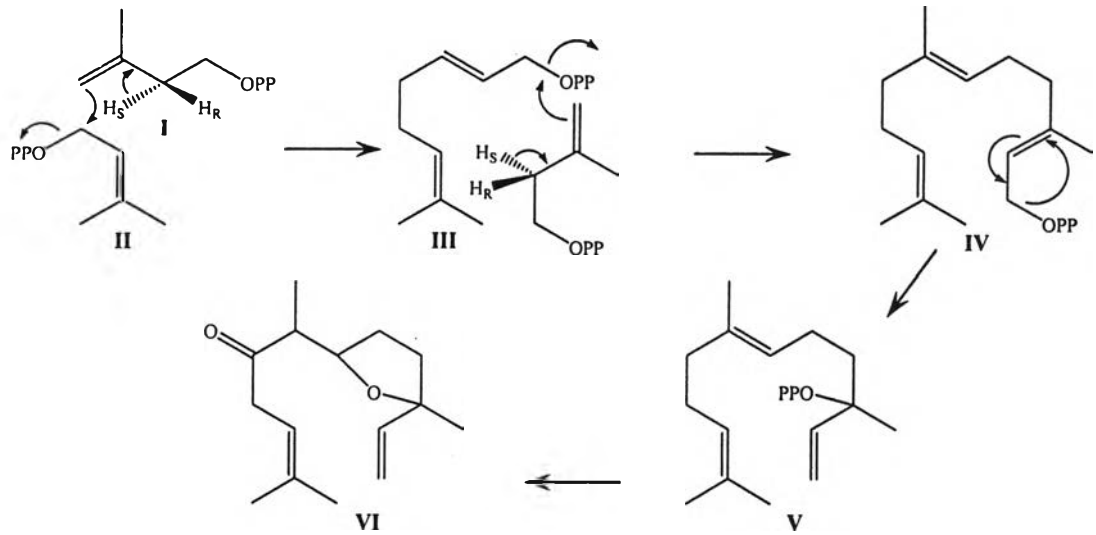


Figure 9 Possible biosynthesis pathway of davanone; I = Isopenthenylpyrrophosphate (IPP), II = Dimethylallylpyrrophosphate (DMAPP), III = Geranylpyrrophosphate (GPP), IV = Farnesylpyrrophosphate (FPP), V = Nerolidylpyrrophate, and VI = Davanone (Akhila *et al.*, 1986)

Table 12 Selected examples of *Artemisia* spp. had been studied in essential oil production from cell and tissue cultures

Plant species	**References**
Artemisia absinthium L.	Kennedy *et al.*, 1993, Nin *et al.*, 1996, and Nin *et al.*, 1997
Artemisia annua L.	Brown, 1994, and Fulzele *et al.*, 1995
Artemisia balchanorum L.	Bavrina *et al.*, 1994
Artemisia dracunculus L.	Cotton *et al.*, 1991
Artemisia pallen Wall.ex Bess.	Benjamin *et al.*, 1990

2.2 *Cuminum cyminum* L.

Cuminum cyminum L. (Fig. 10) belongs to the family Apiaceae. It is commonly known as Cumin. It is originally from the Mediterranean, and wildly distributed in North Africa, Iran, Turkey, Pakistan, Argentina, India, Indonesia, China and other countries in the warm region of the world (Tawfik and Noga, 2001, Tawfik and Noga, 2002, Ishikawa *et al.*, 2002, and De *et al*, 2003). In Thailand, it has been called Thian Khao or Yira (Smitinand, 2001).

The description of this plant is; an small annual herb about 50 cm (20 inches) high with deep green, narrow feather leaves and tiny white or pink flowers, followed by small oblong seeds.

Aerial part

Fruits

Figure 10 *Cuminum cyminum* L.

Known since Bible times, it is mainly used for its digestive properties. The Egyptians used it for headaches. It is a medicinal species and one of the oldest and economically most important spices (Jain *et al.*, 1992 and Lawrence, 1995), which has been used for a very long time in traditional medicine in the treatment of diarrhoea, dyspepsia and gastric disorder, and as an antiseptic agent (De *et al.*, 2003). The fruit has been used for medicinal purposed as a remedy for diarrhoea, flatulence, and indigestion (Norman, 1990). Its fruit has also been used as a popular aromatic herb and spices, such as a main constituent of curry powder, and traditional flavouring in a number of ethnic cuisines and food industries.

The essential oil obtained from fruits hydrodistillation has been called "cumin oil" which has been rich in cuminaldehyde (35-60%) and the other compounds such as α- and β-pinene, δ-3-carene, 1,8-cineole, α- and β-phellandrene, p-cymene, limonene, α- and γ-terpinene, α-terpineol, terpinene-4-ol, cuminyl alcohol, trans-dihydrocarvone (menthane type monoterpenoids), myrcene, linalool (acyclic monoterpenoids), β-caryophyllene, β-farnesene, β-elemene (sesquiterpenoids) (Baser *et al.*, 1992). Cumin oil shows a high antifungal activity against various pathogenic fungi (Afifi *et al.*, 1994 Hammad *et al.*, 1995, and Rahman *et al.*, 2000), and high antibacterial activity (Youssef and Hammad, 1995, Florist *et al.*, 1996, and De *et al.*, 2003). Therefore, it is also used as a fumigant or additive in the storage of foodstuffs. Cumin oil has an overpowering smell and should be used very sparingly yet it is considered non-toxic, non-irritant and non-sensitising but does have photo-toxic qualities, so should not be used when the skin will be exposed to sunlight. It should be also avoided during pregnancy and persons with a sensitive skin.

The biologically active compound of cumin oil has been characterised as cuminaldehyde; cuminal; p-isopropylbenzaldehyde; 4-isopropylbenzaldehyde ($C_{10}H_{12}O$) (Fig. 11).

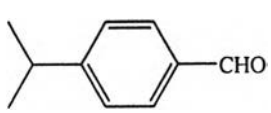


Figure 11 Chemical structure of cuminaldehyde

It has been identified as a potent mushroom tyrosinase inhibitor by inhibition of the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) by mushroom tyrosinase with an ID_{50} of 7.7 μg/ml (0.05 mM) (Kubo and Kinst-Hori, 1998). It has been also shown the aldose reductase inhibitor and α-glucosidase inhibitor effects with the IC_{50} of 0.00085 mg/ml and 0.5 mg/ml, respectively. Cuminaldehyde was about 1.8 and 1.6 times less in inhibitory activity than acarbose and quercitrin, respectively. Nonetheless, cuminaldehyde may be useful as a lead compound and a new agent for antidiabetic therapeutics (Lee, 2005).

While there has been an increasing demand on cumin in the recent years, its production is limited by several stress factors and has been decreased (Tawfik and Noga, 2001and Tawfik and Noga, 2002). The recent advances in cellular manipulations, DNA technology and genetic transformation could offer a potential approach for improvement of cumin resistance to these limiting stress factors. Therefore, interest in regeneration of cumin *in vitro* in presently is increasing (Dave *et al.*, 1996, Shukla *et al.*, 1997, Hussein *et al.*, 1998, Tawfik, 1998, Tawfik and Noga, 2001, and Tawfik and Noga, 2002). However, there had been any reported related with production of cumin oil, particularly in cuminaldehyde. In an attempt to produce this essential oil, the cell cultures of *Cuminum cyminum* have been established and many strategies have been applied to improve yield of cuminaldehyde.

2.3 *Fortunella japonica* (Thunb.) Swingle

Fortunella japonica (Thunb.) Swingle (Syn. *Citrus japonica* Thunb.) (Fig. 12) belongs to the family Rutaceae. It has many common names such as Kumquat, Round kumquat, or Marumi kumquat, and in China it has been called Kim kit. It is a novelty that is not cultivated extensively. It is native of China, cultivated throughout subtropical regions of the world. It is grown in India as ornamentals and for their fruit which are rich in pectin and excellent for marmalades and jellies (Talapatra *et al.*, 1974).

Figure 8 *Fortunella japonica* (Thunb.) Swingle (Syn. *Citrus japonica* Thunb.)

Fortunella japonica is an evergreen shrub or small trees, growing about 2-2.5 m in height. It has fine stems, few thorns, and dark green pointed leaves. The fruit varies in shape from round to slightly oval (around 2 cm diameter), averaging around 10-12 g. Its fruit is quite sour, but peel is edible, however, its size would make processing difficult (Umano, K., *et al.*, 1994).

The genus *Fortunella* is taxonomically close to the genus *Citrus*, however, flavonoid compositions of *Fortunella* spp. differ from those of *Citrus* spp. The principal flavonoids of the genus *Citrus* are flavone glycosides such as narirutin, naringin, hesperidin, and neohesperidin, and flavone aglycons such as nobiletin and tangeretin (Mizuno *et al.*, 1991, Kanes *et al.*, 1993, Tsuchida *et al.*, 1997, and Kawaii *et al.*, 1999), while a flavone glycoside, poncirin, has been reported, flavone glycosides are thought to be minor constituents in plants of the *Fortunella* genus. A number of C-glycosylated flavones have also been identified in *Fortunella japonica* and *Fortunella crassifolia* (Kumamoto *et al.*, 1985 and Tsuchida *et al.*, 1997), but quantities of flavonoids in *Fortunella* plants have been found to be rather low compared with plants of the *Citrus* genus (Kanes *et al.*, 1993 and Tsuchida *et al.*, 1997). Moreover, 3',5'-di-C-β-glucopyranosylphloretin, a dihydrochalcone derivative, has been found to be a flavonoid characteristic of the genus *Fortunella.* It has been accumulated in a large quantity in plants of the genus *Fortunella* but can not be found in any *Citrus* spp. except *Citrus madurensis* and *Citrus halimii* which have been thought to originate from natural hybrids between the genera *Citrus* and *Fortunella* (Ogawa *et al.*, 2001).

As well as being an ornamental plant, kumquat fruit is used for preserves, candy, syrup and fresh consumption, because of its special flavour and thick edible rind (Hodgson, 1967). The whole fruit including the peel can be eaten; the fruit is acid whilst the peel is sweet. Its fruit is usually cooked and used in jellies, preserved, or used as flavouring but it can also be eaten raw. Its fruit is also contained vitamin C up to 0.24 mg/ml (Chooper *et al.*, 1986). It has been used as medicinal plant in China; antiphologistic, antivinous, arminative, deodorant, and stimulant (Duke and Ayensu, 1985).

Its essential oil called "Kumquat oil" has a typical citrus oil character (Koyasako and Bernhard, 1983), but it gives a somewhat bitter aroma rather than the fruity aroma of orange or lemon (Shaw, 1979). The volatile constituents obtained from its peel by

steam distillation had been reported, and the most abundant compound was d-limonene (87-97%), and the other compounds are linalool, myrcene, geranyl acetate, α-pinene, and germacrene D.

d-Limonene or (+)-limonene ($C_{10}H_{16}$) (Fig. 13), a monocyclic monoterpenes, is the main constituent of orange and lemon peel oil (92-96%). It has been an attractive starting compound for industrially relevant fine chemicals and flavour compounds with identical carbon skeletons, such as carveol, carvone, and perillyl alcohol (Duetz *et al.*, 2001). It has been shown to be capable of preventing the development and causing the regression of chemically induced mammary carcinoma, and its dose for clinical activity is well tolerated in patient (Crowell *et al.*, 1994).

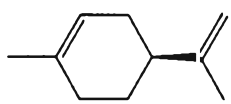


Figure 13 Chemical structure of d-limonene

The pathway of d-limonene biosynthesis has been shown in Fig. 14. Isopenthenylpyrophosphate, IPP (I) condenses with dimethylallylpyrophosphate, DMAPP (II) to give geranylpyrophosphate, GPP (III). GPP, the ubiquitous precursor of the monoterpenes, is cyclised by a monoterpene (d-limonene) synthase to d-limonene (IV) (Bouwmeester *et al.*, 1995).

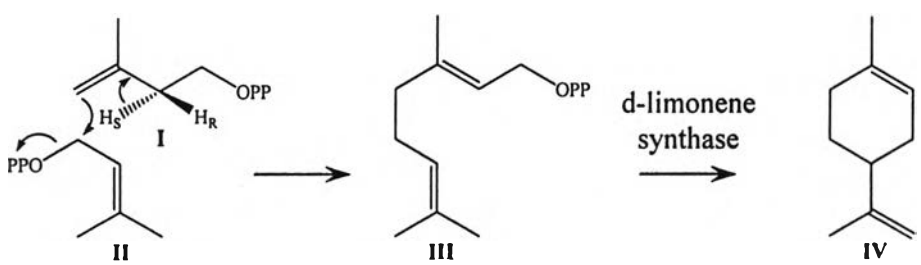


Figure 14 Biosynthesis pathway of d-limonene via geranylpyrophosphate formation (Bouwmeester *et al.*, 1995)

Peel and leaf oils of some *Citrus* had been analysed by gas-liquid chromatography at biweekly intervals from spring until harvest. The greatest changes were found in the peel oil where oxygenated constituents, particularly linalool, decreased markedly in relative concentration as the fruit matured, while d-limonene concentration exhibited a corresponding increase. The relationship between d-

limonene concentration and linalool concentration lead to suggest another possible biosynthesis pathway of d-limonene (Attaway *et al.*, 1967). Instead of alkylation and loss of pyrophosphate as shown in Fig. 14, assume that DMAPP adds across the double bond followed by concerted elimination of pyrophosphate from the isopentenyl group. This would give linalylpyrophosphate (LPP) as the product (Fig. 15), and then it further transformed to linalool and d-limonene.

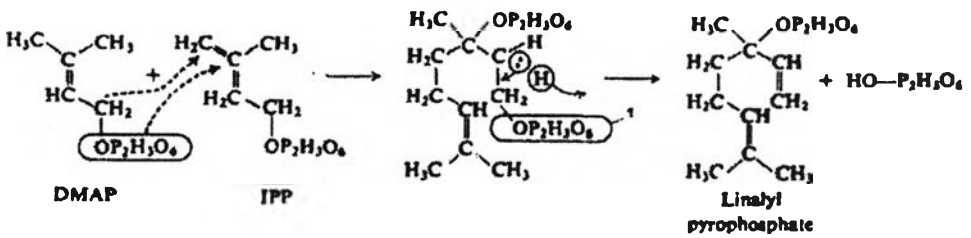


Figure 15 Biosynthesis pathway of linalylpyrophosphate (Attaway *et al.*, 1967)

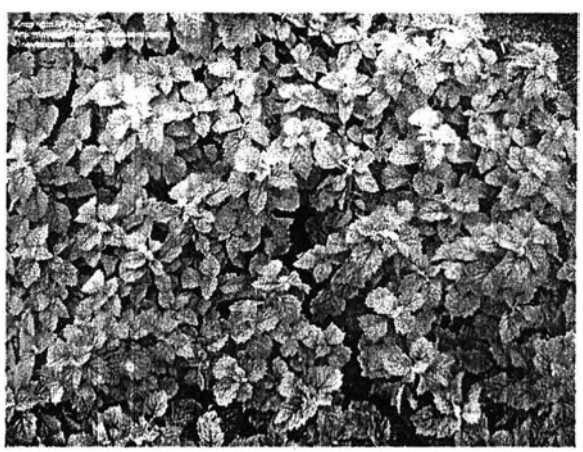
There have been some reports of cell cultures derived from other species of *Citrus* (Berger *et al.*, 1990, Niedz *et al.*, 1997, and Germana and Chiancone, 2003), however, to my knowledge, this is the first report on the cultivation of undifferentiated *Fortunella japonica*. Many strategies have been use in this experiment such as elicitation with chitosan and precursor feeding and biotransformation in attempt to improve d-limonene level, a biological active constituent in this essential oil.

2.4 *Pogostemon cablin* (Blanco) Benth.

Pogostemon cablin (Blanco) Benth. (Syn. *Pogostemon patchouli* Hook.) (Fig. 16) belongs to the family Lamiaceae. It has commonly known as Patchouli, Patchouly, Goung-Huo-Xiang, Kwang ho hsiang, and Kablin. It also has Ayurvedic names as Sanskrit-Patchpan, and Hindi-Patchouli. In Thai, it has been called Phim sen (Smitinand, 2001). "Patchouli" comes from the Hindustan word 'patch' meaning green and 'ilai' meaning leaf.

It is native to subtropical Himalayas, Southeast Asia and Far East and has been cultivated extensively in Indonesia, Malaysia, China, and Brazil.

Patchouli is a perennial bushy plant that grows up to 1 m (3 feet) with a sturdy, hairy stem and large, fragrant, furry leaves, about four inches long and five inches across. It has whitish flowers tinged with purple (Valkenburg and Bunyapraphatsara, 2001)



Aerial part

Leaves

Flowers

Figure 16 *Pogostemon cablin* (Blanco) Benth. (syn. *Pogostemon patchouli* Hook.)

Patchouli is an aromatic crop which yields an essential oil containing sesquiterpenes (Lawrence, 1981). It has been grown extensively for essential oil namely "patchouli oil" which has been used as fixative in perfumery raw material.

Patchouli oil is an important natural material in perfumery and food industry, and has been used in Asia historically to repel cloths moths and as a cold treatment (Akhila and Tewari, 1984). This oil also processes insecticidal activities, antifungal and bacteriostatic properties. The therapeutic properties of patchouli oil are antidepressant, anti-inflammatory, antiseptic, aphrodisiac, astringent, carminative, diuretic, febrifuge, fungicide, insecticide, sedative and tonic.

Patchouli oil is useful for fungal and bacterial infection; it helps for insect bites and could be used as an insect repellent. It has diuretic properties, therefore helpful for water retention, cellulite, and overweight. It has deodorizing action, and helps when feeling hot. It helps to cool down inflammation and assist with wound healing, scars and sores; it gives relief from acne, eczema and scalp disorders. It can be helpful with re-growth of skin cells and scar tissue. It can assist with stress related conditions and anxiety; and helpful in cases of substance.

In summary, patchouli oil has a beneficial effect on the skin, helps for infections and insect bites, water retention and can help with stress related problem and addictions.

Patchouli oil is non-toxic, non irritant and non-sensitizing but the smell of this oil may be a little persistent for some people and it may cause loss of appetite in some individuals.

Patchouli oil is extracted from the young leaves which are dried and fermented prior to stream distillation. Furthermore, patchouli oil has been also obtained from leaves hydrodistillation. Its yield varies from 1.8-3.0 % depending upon the quality and maturity of leaves. Patchouli oil improves with age and gives a fuller odor. The chemical constituents of patchouli oil are almost entirely composed of sesquiterpenes with patchouli alcohol being the most abundant, followed by α-patchoulene, β-patchoulene, γ-patchoulene, δ-patchoulene, pogostol, seychellence, α-bulnesene, β-bulnesene caryophyllene, α-guaiene, norpatchoulenol, and trace amounts of other epoxy and ketonic compounds (Henderson, W *et al.*, 1970 and Akhila and Tewari, 1984).

The biosynthesis pathway of some sesquiterpenes in patchouli oil has been has been shown in Fig. 17 (Akhila, 1988). Patchouli alcohol (1), seychellene (2), and cycloseychellene (3) are derived by cyclisation of cis-farnesyl pyrophosphate (4) via 4 intermediates. Furthermore, as shown in Fig.18, α-patchoulene (1), β-patchoulene (2), γ-patchoulene (3), and δ-patchoulene (4) are derived via cyclisation of cis-farnesyl pyrophosphate (5) via the other 2 intermediates (Akhila, 1987). In addition, total synthesis of (±)-patchouli alcohol and (±)-seychellene has been described in 1979 (Yamada *et al.*, 1979).

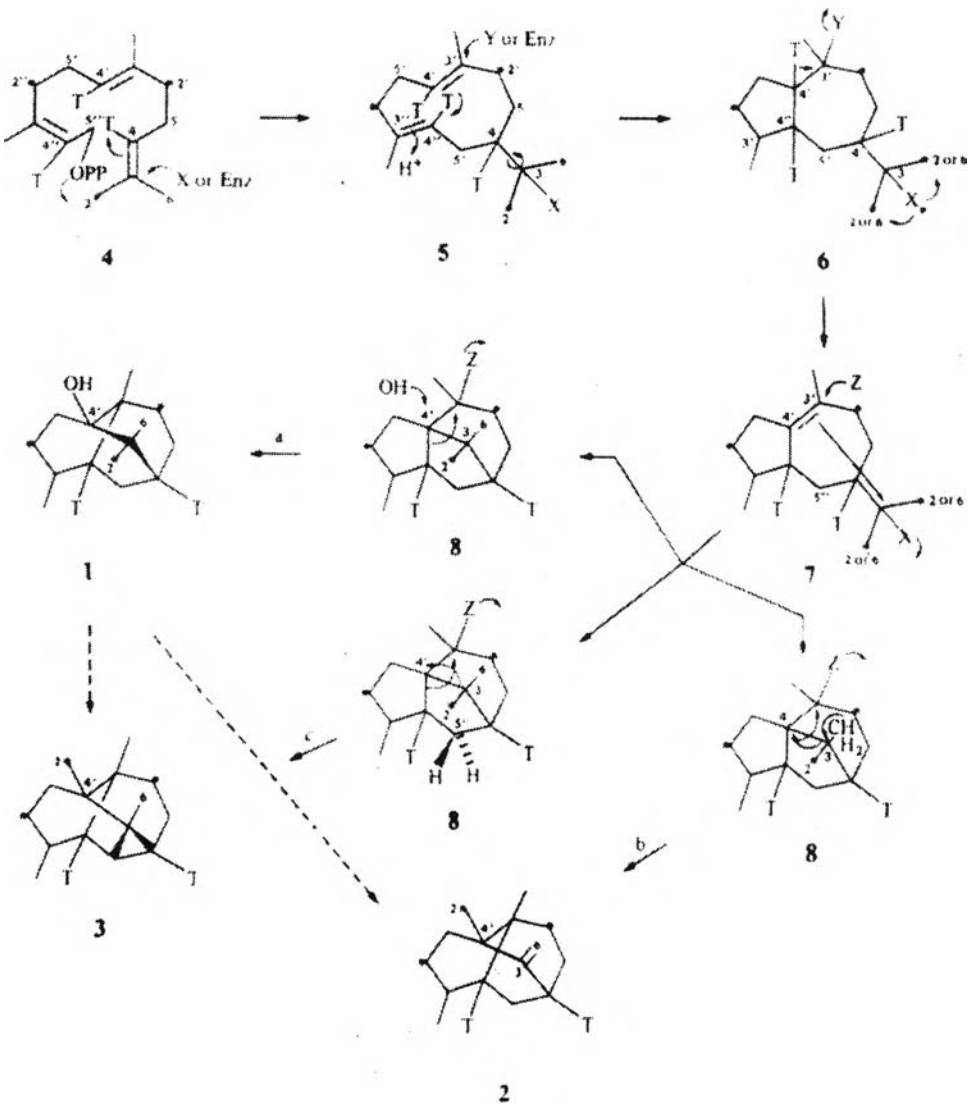


Figure 17 Biosynthesis pathway of patchouli alcohol (1), seychellene (2), and cycloseychellene (3) in *Pogostemon cablin* (Akhila, 1988)

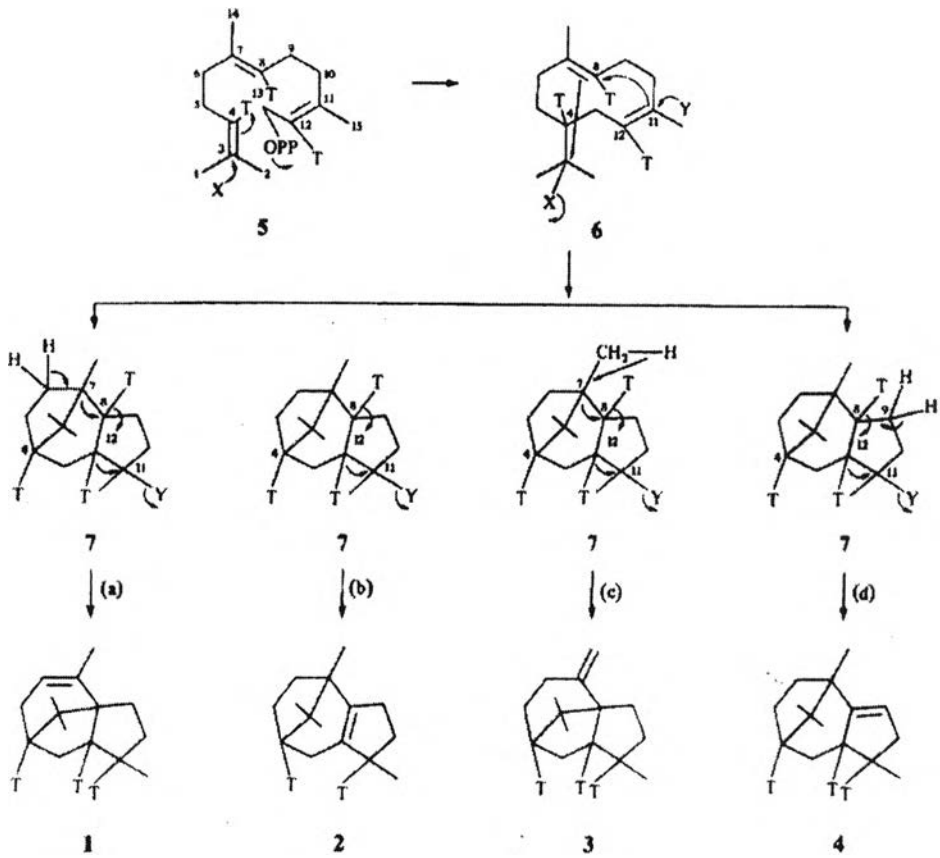


Figure 18 Biosynthesis pathway of α-patchoulene (1), β-patchoulene (2), γ-patchoulene (3), and δ-patchoulene (4) in *Pogostemon cablin* (Akhila, 1987)

The accumulation sites of sesquiterpenes in *Pogostemon cablin* had been studied under light and electron microscopic methods in 1970 (Henderson, 1970). This oil is secreted in a variety of specialised glandular cells, both on the leaf surface, and within the leaf. The accumulation sites of sesquiterpenes are not only in the external glandular trichomes, but also in specialized internal accumulatory cells, which appear to be a unique feature of the plant. In both cases the glandular cells are closely associated with the photosynthetic cells of the leaf, although some internal glands also occur in stem and even root tissue. In these cases glands are located in the phloem. It has been shown that, while the apical dome of the stem neither shows any specialise accumulatory structures nor contains any detectable sesquiterpenes, the second pair of primordial leaves has glandular trichomes and a sesquiterpene accumulation approximately twelve times higher than that in any other part of the plant.

Owning to the value of patchouli oil, particularly patchouli alcohol, many researchers have tried to produce it by biotechnological methods via *Pogostemon cablin* cell cultures (Jones and Krishnadethan, 1973, Kageyama *et al.*, 1995, and Misra, 1996). To my knowledge, there had been no research publication paper related to patchouli alcohol production. This plant cell cultures have been established in attempt to improved yield of patchouli oil, especially patchouli alcohol, and study biotransformation of terpenoids in this plant cell cultures.