8 Clove

N.K. Leela and V.P. Sapna

8.1. Introduction

Clove (*Syzygium aromaticum* (L.) Merril. & Perry, syn. *Eugenia aromaticum* or *E. caryophyllata*) is one of the most ancient and valuable spices of the Orient. It is a member of the family *Myrtaceae*. The clove of commerce is its dried unopened flower buds. The word 'clove' was derived either from the Latin word *clavus*, or the French form *clou*, meaning 'nail'. The buds resemble irregular nails.

Area and production of clove in India for the period 1995 to 2005 are indicated in Table 8.1. There was a marginal increase in the area under cultivation from 2270 to 2528 ha over these years, whereas production decreased from 2455 to 1815 t. Tanzania, Indonesia, Madagascar, Cameroon and Sri Lanka are the major clove-exporting countries. In recent years, world production of clove has averaged around 80,000 t a year. Indonesia is the world's largest producer at 50,000–60,000 t per annum. It is used mainly in the preparation of *kretek* cigarettes. Singapore is the entrepôt for the clove trade. Saudi Arabia, the USA, France and India are the major importing countries.

8.2. Botany and Uses

Clove is a medium-sized tree, which grows to a height of 10–20 m, that can live up to 100 years or more. The bark is grey, the leaves are elliptical in shape and fragrant with crimson flowers. The flowers are hermaphrodite with a fleshy hypanthium surrounded by sepals. The fruit is a purple drupe, about 2.5 cm long.

Cloves are best used whole. The flavour deteriorates quickly once it is powdered. Whole and ground cloves are used to enhance the flavour of meat and rice dishes. They are used widely in curry powders and masalas. In North Indian cuisine, cloves are used in almost every sauce or side dish made, mostly mixed with other spices. In South India, they find extensive use in 'biriyanis' to enhance the flavour of the rice. They are highly valued in medicine as a carminative and stimulant. Cloves are said to be a natural anthelmintic.

The spice is used throughout Europe and Asia and is smoked in a type of cigarette, known locally as *kretek*, in Indonesia and in occasional coffee bars in the West, mixed with marijuana to create marijuana spliffs. Clove cigarettes (Indonesian *kretek*) are cigarettes made with a complex blend of tobacco, cloves and a flavouring 'sauce'. Cloves are also an important incense material in Chinese and Japanese culture.

Oil of clove is used extensively for flavouring all kinds of food products, such as meats, sausages, baked goods, confectionery, candies, table sauces, pickles, etc. Clove oil

Table 8.1. Area and production of clove in India.

Year	Area (ha)	Production (t)
1994/95	2270	2455
1995/96	2300	2439
1996/97	2222	1836
1997/98	2273	1698
1998/99	2308	1056
1999/2000	2795	1633
2000/01	1881	979
2001/02	1891	1048
2002/03	2127	1374
2003/04	2431	1811
2004/05	2528	1815

Source: DASD (2007).

is used in aromatherapy and oil of cloves is used widely to treat toothache. It is used in medicine for its antibacterial, antiseptic and antibiotic properties. The oil has many industrial applications and is used extensively in perfumes, soaps and as a clearing agent in histological work. It is an ingredient in many toothpastes and mouthwashes. It is also used for flavouring oral preparations and chewing gums. The chief constituent of the oil, eugenol, is used in the preparation of synthetic vanillin and isoeugenol (Pruthi, 1976).

8.3. General Composition

The composition of the clove varies according to the agroclimatic conditions under which it is grown, processed and stored. The dried clove bud contains carbohydrates, fixed oil, steam-volatile oil, resins, tannins, proteins, cellulose, pentosans and mineral elements. Carbohydrates comprise about two-thirds of the weight of the spice (Purseglove *et al.*, 1981). The dried dark and flower buds also contain nutrients like proteins, minerals, vitamins, etc. Nutrient composition of 100 g of clove is indicated in Tables 8.2 and 8.3. It is evident from Table 8.2 that 61% of clove is carbohydrates, 20% is fat and the rest is contributed by secondary metabolites, vitamins and minerals. Cloves are an excellent source of manganese, a very good source of dietary fibre, vitamin C, vitamin K and ω-3 fatty acids and a good source of calcium and

Table 8.2. Nutrient composition of 100 g of clove.

Composition	USDA (ground)
Water (g)	5.40–6.86
Food energy (Kcal)	323
Protein (g)	5.98
Fat (g)	20.06
Carbohydrate (g)	61.22
Ash (g)	5.88
Ca (g)	0.646
P (mg)	105
Na (mg)	243
K (mg)	1102
Fe (mg)	8.68
Thiamin (mg)	0.115
Riboflavin (mg)	0.267
Niacin (mg)	1.458
Ascorbic acid (mg)	80.81
Vitamin A (RE)	53

Source: Tainter and Grenis (1993).

magnesium. Volatile oil can be extracted from the leaf, stem and buds of clove. Volatile oil is present in oval cavities, two or three rows below the epidermis. The major component of the volatile oil is a phenol, namely eugenol. Phenolic activity is greater at the outer glandular regions of the hypanthium than in the inner aerenchymatous spongy tissue.

8.4. Chemistry

Volatiles

Clove yields three types of volatile oil – oil extracted from the leaves, the stem and the buds. These oils differ considerably in yield and quality. The yield and composition of the oil obtained are influenced by its origin, season, variety and quality of raw material, maturity at harvest, pre- and post-distillation treatments and method of distillation. The chief component of the oil is eugenol.

Bud oil

Good-quality clove buds contain 15–20% essential oil (Gopalakrishnan *et al.*, 1988; Pino *et al.*, 2001; Raina *et al.*, 2001; Zachariah *et al.*, 2005). The oil is dominated by eugenol (70–85%), eugenyl acetate (15%) and

Table 8.3. Nutrient values and weights for edible portion of clove.

Nutrient	Units	Value per 100 g	Number of data points	Std. error	1.00 × 1 tsp / 2.1 g
Proximates:					
Water	g	6.86	343	0.241	0.14
Energy	kcal	323	0	0	7
Energy	kj	1350	0	0	28
Protein	g	5.98	73	0.106	0.13
Total lipid (fat)	g	20.07	299	0.273	0.42
Ash	g	5.88	384	0.049	0.12
Carbohydrate, by difference	g	61.21	0	0	1.29
Fibre, total dietary	g	34.2	0	0	0.7
Sugars, total	g	2.38	1	0	0.05
Sucrose	g	0.02	1	0	0.00
Glucose (dextrose)	g	1.14	1	0	0.02
Fructose	g	1.07	1	0	0.02
Maltose	g	0.00	1	0	0.00
Galactose	g	0.15	1	0	0.00
Minerals:					
Calcium	mg	646	6	64.353	14
Iron	mg	8.68	7	1.500	0.18
Magnesium	mg	264	5	4.867	6
Phosphorus	mg	105	4	2.887	2
Potassium	mg	1102	6	68.431	23
Sodium	mg	243	6	12.368	5
Zinc	mg	1.09	5	0.278	0.02
Copper	mg	0.347	0	0	0.007
Manganese	mg	30.033	0	0	0.631
Selenium	mcg	5.9	1	0	0.1
Vitamins:					
Vitamin C, total ascorbic acid	mg	80.8	1	0	1.7
Thiamin	mg	0.115	2	0	0.002
Riboflavin	mg	0.267	4	0.012	0.006
Niacin	mg	1.458	1	0	0.031
Vitamin B_6	mg	0.590	2	0	0.012
Folate, total	mcg	93	0	0	2
Folate, food	mcg	93	0	0	2
Folate, DFE	mcg_DFE	93	0	0	2
Vitamin B_{12}	mcg	0.00	0	0	0.00
Vitamin B_{12}, added	mcg	0.00	0	0	0.00
Vitamin A	IU	530	1	0	11
Vitamin A	mcg_RAE	27	0	0	1
Retinol	mcg	0	0	0	0
Vitamin E (α-tocopherol)	mg	8.52	1	0	0.18
Vitamin E, added	mg	0.00	0	0	0.00
Vitamin K (phylloquinone)	mcg	141.8	1	0	3.0
Lipids:					
Fatty acids, total saturated	g	5.438	0	0	0.114
14:0	g	0.022	0	0	0.000
16:0	g	3.967	0	0	0.083
18:0	g	0.847	0	0	0.018
Fatty acids, total monounsaturated	g	1.471	0	0	0.031
16:1 undifferentiated	g	0.089	0	0	0.002
18:1 undifferentiated	g	1.337	0	0	0.028

Continued

Table 8.3. *Continued*

Nutrient	Units	Value per 100 g	Number of data points	Std. error	1.00 × 1 tsp / 2.1 g
20:1	g	0.022	0	0	0.000
Fatty acids, total polyunsaturated	g	7.088	0	0	0.149
18:2 undifferentiated	g	2.586	0	0	0.054
18:3 undifferentiated	g	4.257	0	0	0.089
20:4 undifferentiated	g	0.045	0	0	0.001
22:5 n-3	g	0.022	0	0	0.000
Phytosterols	mg	256	0	0	5
Others:					
β-Carotene	mcg	84	0	0	2
α-Carotene	mcg	0	1	0	0
β-Cryptoxanthin	mcg	468	0	0	10
Lycopene	mcg	0	1	0	0
Lutein + zeaxanthin	mcg	0	1	0	0

Source: USDA (2005).

β-caryophyllene (5–12%), which together make up 99% of the oil. *β*-Caryophyllene, which was earlier thought to be an artefact of distillation, was first reported as a constituent of bud oil by Walter (1972). The constituents of the oil also include methylamylketone, methylsalicylate, *α*- and *β*-humulene, benzaldehyde, *β*-ylangene and chavicol. The minor constituents like methylamylketone, methylsalicylate, etc., are responsible for the characteristic pleasant odour of cloves. The physico-chemical properties of clove oils are shown in Table 8.4.

Gopalakrishnan *et al.* (1984) characterized six sesquiterpenes, namely: α-cubebene (1.3%), α-copaene (0.4%), *β*-humulene (9.1%), β-caryophyllene (64.5%), γ-cadinene (2.6%) and δ-cadinene (2.6%), in the hydrocarbon fraction of the freshly distilled Indian clove bud oil. The oil from the Malagasy Republic (Madagascar) was dominated by eugenol (72–73%), eugenyl acetate (6.3–7.8%) and caryophellene (15.7%) (Lawrence and Reynolds, 1989).

The clove bud and stem oils from Madagascar were also dominated by eugenol, eugenyl acetate and β-caryophyllene. The stem oil contained a higher level of eugenol, whereas the eugenyl acetate content was higher in the bud oil. The oil from clove bud contained 73.5–79.7% eugenol and 4.5–10.7% eugenyl acetate, while the stem oil contained 76.4–84.8% eugenol and 1.5–8.0%

Table 8.4. Physico-chemical properties of clove oil.

Characteristic	Bud oil	Stem oil	Leaf oil
Colour	Colourless to pale yellow	Yellow to dark brown	Straw coloured or very pale
Specific gravity (25°C)	1.051–1.054	1.050–1.055	1.040–1.054
Optical rotation	–1°35′ to –0°25′	–1°30′ to –0°32′	–1°40′ to –0°40′
Refractive index (20°C)	1.531–1.537	1.531–1.539	1.531–1.538
Solubility	Soluble in 1 vol. of 70% ethanol	Soluble in 1–2 vol. of 70% ethanol	Soluble in 1.0–1.5 vol. of 70% ethanol
Total phenols (%)	91–93	88–93	78–93

Source: Guenther (1950).

eugenyl acetate. Both contained 7.3–12.4% β-caryophyllene and 1.0–1.4% α-humulene (Gaydou and Randriamiharisoa, 1987).

The essential oils of clove buds of Indian and Madagascan origins were analysed by Srivastava *et al.* (2005). The oil from Madagascar was richer in eugenol (82.6%) and eugenyl acetate (6%) compared with that of India (70 and 2.1%, respectively), whereas the Indian oil contained a higher level of *β*-caryophyllene (19.5 against 7.2% in Madagascan oil) (Srivastava *et al.*, 2005).

The neutral fraction of the bud oil from Madagascar contained β-caryophyllene (75.64%), *α*-humulene (14.12%) and δ-cadinene (2.34%) as the major components (Muchalal and Crouzet, 1985).

Pino *et al.* (2001) identified 36 compounds of the volatile oil of clove buds. The major components of the bud oil were eugenol (69.8%), β-caryophyllene (13%) and eugenyl acetate (16.1%) (Pino *et al.*, 2001). The chief components of clove oil from various regions are listed in Table 8.5, which indicates quantitative variations of the individual components of the oil from different regions. Zachariah *et al.* (2005) reported that clove buds from India contained 12.9–18.5% oil, of which 44–55% was eugenol, whereas the pedicels contained 3.0–7.7% oil with 60.0–72.4% eugenol.

Wild uncultivated trees in Molucca yielded 3.0–7.7% bud oil. The oil contained no eugenol and was quite different from the bud oil from cultivated trees (Guenther, 1950).

Analysis of clove bud oil extracted with liquid and supercritical carbon dioxide showed significant qualitative and quantitative compositional differences compared to oil obtained by the conventional hydrodistillation process. The parameters such as pressure, temperature, contact time, etc., affect the extraction of the bud flavour from the spice (Gopalakrishnan *et al.*, 1990). Guan *et al.* (2007) compared the essential oil obtained by four different extraction techniques; namely, hydrodistillation, steam distillation, solvent extraction and supercritical carbon dioxide extraction (SFE). The study showed that temperature had the largest effect on the eugenol content of the extracts and particle size had the maximum effect on oil yield. Among these techniques, the oil obtained by SFE and steam distillation had a desirable, pale yellow colour. Hydrodistilled oil had the lowest content of eugenol and eugenyl acetate. Extraction yield of SFE was twice as high as that obtained by steam and hydrodistillation. The SFE method yielded the highest content of eugenol + eugenol acetate in the oil. Clove oil obtained by steam distillation yielded the highest eugenol content, followed by SFE. Hydrodistillation yielded oil with a high *β*-caryophyllene content, whereas the SFE-extracted oil had the lowest *β*-caryophyllene content. GC-MS analysis of the clove oils obtained by different methods showed that the composition of the clove oil was almost similar, but the relative concentration of the identified compounds was apparently different. Among the four methods evaluated, SFE was the optimum method for obtaining high quality oil (Guan *et al.*, 2007). The oil yield was influenced largely by particle size and the eugenol content by temperature.

Leaf oil

Clove leaves yield 3.0–4.8% essential oil (Raina *et al.*, 2001). In Zanzibar, oil is distilled from dried fallen leaf or fresh leaf

Table 8.5. Major constituents of clove oil from different locations.

Constituent	A	B	C	D
β-Caryophyllene	4.35	5.13	9.86	12.50
α-Humulene	0.54	0.60	1.10	1.36
Eugenol	88.95	92.35	86.89	84.77
Eugenol acetate	5.54	1.25	1.59	ng

Note: A: Madagascan clove bud oil; B: Zanzibar clove stem oil; C: Madagascan clove leaf oil; D: Indonesian clove leaf oil; ng = not given.

after trimming the upper part of the tree. Crude leaf oil is harsh and woody, with a phenolic, sweet aroma quite different from bud oil. Rectified oil is clear pale yellow in colour with a sweeter, less harsh, dry woody odour close to that of eugenol. The oil contained 94.4% eugenol followed by β-caryophyllene (2.9%), nerol (0.79%) and β-caryophyllene oxide (0.67%) (Raina *et al.*, 2001). The leaf oil from Cuba contained 31 volatile compounds. Eugenol (78.1%) and β-caryophyllene (20.5%) were the main constituents in the oil (Pino *et al.*, 2001). Cuban leaf oil contained a higher amount of β-caryophyllene compared with that from Little Andaman.

The leaf oil from Madagascar contained 22 constituents, the chief constituents being eugenol (82.0%) and β-caryophyllene (13.0%). It contained a higher level of β-caryophyllene compared with bud oil (7.2%) (Srivastava *et al.*, 2005; Table 8.7). A commercial sample of leaf oil obtained in Germany contained 76.8% eugenol and 17.4% β-caryophyllene as the chief components (Jirovetz *et al.*, 2006).The constituents of various clove oils are indicated in Tables 8.6 and 8.7.

The essential oil content during the different stages of leaf growth revealed that the eugenol content in the leaves increased from 38.3 to 95.2% with maturity, while the contents of eugenyl acetate (51.2 to 1.5%) and caryophyllene (6.3 to 0.2%) decreased (Gopalakrishnan and Narayanan, 1988). Clove bud and leaf oil contain various classes of compounds, e.g. monoterpenes, sesquiterpenes, aldehydes and ketones (Vernin *et al.*, 1994), which are indicated in Table 8.8.

Clove stem oil

Clove stem yields 6% volatile oil (Gopalakrishnan *et al.*, 1988). The oil is a pale to light yellow liquid containing 80.2% eugenol and 6.6% β-caryophyllene, besides several minor components. Stem oil is used mainly in flavouring and perfumery and also to adulterate bud oil. Stem oil from Madagascar contains 77.10% eugenol and 11.20% β-caryophyllene as the major compounds (Gaydou and Randriamiharisoa, 1987). The chief volatile compounds from clove are indicated in Fig.8.1.

Fruit oil

Ripe fruits yield 2% of oil, which is comprise of 50–55% eugenol.

Clove bud concrete

Clove bud concrete is another important value-added product from buds, extracted using petroleum ether and benzene. It is olive to pale brown, having a sweet, rich spicy aroma similar to that of dried buds.

Clove concrete, on treatment with benzene/petroleum ether, produces a viscous, olive-green semi-solid (at low temperature), namely bud *absolute*, soluble in alcohols of different proportions. *Absolute* lacks caryophyllene and contains the same constituents as those present in unprocessed bud. Clove oleoresin is an extremely concentrated product, containing all the flavouring ingredients soluble in the solvent used, and is much closer to the original clove odour and flavour. Menon and Narayanan (1992) studied the glycosidically bound volatiles in clove leaves and buds using hydrolysing enzymes. When β-glucosidase was used for hydrolysis, eugenol was the major compound liberated from both buds and leaves, with *cis*- and *trans*-isoeugenol, nerolidol and farnesol in minor amounts, whereas hydrolysis using α–amyloglucosidase yielded farnesol as the major compound (Table 8.9).

Non-volatiles

So far, a few non-volatiles have been isolated from clove, which include tannins, sterols, triterpenes and flavonoids. These are listed below. Wild uncultivated trees of the Moluccas contained the crystalline compounds eugenone, eugenine, eugenitin and isoeugenitol (Guenther, 1950).

Tannins

Cloves contain 10–13% tannin, which has the same chemical composition as

Table 8.6. Volatiles of clove oils.

Component	% Composition			
	Leaf oil[1]	Bud oil[2]	Stem oil[2]	Leaf oil[2]
α-Pinene	–	0.42	–	–
β-Pinene	–	0.44	0.16	0.09
2-Hexanone	–	0.48	0.13	0.09
2-Heptanone + 1,8-cineol	–	0.50	0.11	0.10
α-Terpinene + limonene	–	0.53	0.14	–
p-Cymene	–	0.56	0.09	0.07
2-Heptanol	–	0.60	–	–
2-Nonanol	–	0.64	–	0.02
Benzaldehyde	–	0.69	–	0.01
β-Terpineol (t)	–	0.81	0.01	0.02
α-Cubebene	–	0.90	0.66	0.70
α-Terpineol	–	0.92	0.93	0.96
β-Caryophyllene	2.91	1.00	7.22	7.59
Benzyl alcohol	–	1.10	0.59	0.57
δ-Cadinene	–	1.18	0.31	0.44
α-Caryophyllene	–	1.34	0.07	0.22
Isoeugenol	–	1.51	–	1.00
Eugenol acetate	–	1.54	24.59	16.71
Farnesol (c,t)	–	1.59	–	–
Farnesol (t,t)	–	1.66	–	0.93
Vanillin	–	1.82	0.89	1.15
Asarone (t)	–	2.06	1.17	1.47
(*E*)-β-Ocimene	0.03	–	–	–
Linalool	0.08	–	–	–
Terpinen-4-ol	0.03	0.87	–	0.01
Nerol	0.79	–	–	–
Eugenol	94.41	1.41	59.14	60.82
α-Copaene	0.04	–	–	–
α-Humulene	0.36	10.60	1.24	1.44
(*E*,*E*)-α-Farnesene	0.06	–	–	–
γ-Cadinene	0.18	1.14	0.45	0.45
(*E*)-Nerolidol	0.03	–	–	–
β-Caryophyllene oxide	0.67	–	–	–
Humulene oxide II	0.07	–	–	–
t-Cadinol	0.07	–	–	–
Cadalene	0.18	–	–	–
Hexadecyl acetate	0.09	–	–	–

Source: [1]Raina *et al.* (2001); [2]Gopalakrishnan *et al.* (1988).

gallotannic acid. Eugeniin and ellagitannin were isolated from cloves by Nonaka *et al.* (1980). Tanaka *et al.* (1993) isolated eugenol glucoside gallate, a chromone *C*-glycoside, galloyl and hexahydroxy diphenyl esters of 2,4,6-trihydroxy acetophenone-3-glucopyranoside from clove leaves. Further, two ellagitannins, namely, syzyginin A (1,2,3-tri-*O*-galloyl–4,6-(*S*)-tergalloyl-β-D-glucoside) and syzyginin B, were also isolated from the leaves by Tanaka *et al.* (1996).

Triterpenes

Cloves contain about 2% of the triterpene, oleanolic acid. Narayanan and Natu (1974) isolated maslinic acid from clove buds. From clove, 2α-hydroxyoleanolic acid was isolated by Brieskorn *et al.* (1975).

Table 8.7. Essential oil composition of clove bud and leaf from India and Madagascar.

Compound	Bud oil (India)	Bud oil (Madagascar)	Leaf (Madagascar)
n-Octane	–	0.1	–
α-Pinene	–	0.1	–
(*E*)-β-Ocimene	–	t	–
Methyl benzoate	–	t	–
Linalool	0.1	t	–
m-Methyl acetophenone	t	0.1	–
Methyl salicylate	0.3	0.1	0.1
Nerol	t	0.1	–
Carvone	0.1	0.2	0.1
Chavicol	t	0.1	0.1
Linalyl acetate	t	0.1	0.1
Anethole	t	–	–
Eugenol	70.0	82.6	82.0
n-Butyl benzoate	1.3	–	–
α-Cubebene	–	t	–
Methyl eugenol	–	t	–
α-Ylangene	–	t	–
iso-Eugenol-1	0.8	0.1	0.1
Vanillin	t	–	–
α-Copaene	0.1	0.1	–
β-Caryophyllene	19.5	7.2	13.0
(*E*)-α-Bergamotene	1.3	0.2	0.4
α-Humulene	1.9	0.8	1.5
allo-Aromadendrene	0.3	0.1	–
Germacrene D	0.1	–	–
Eugenyl acetate	2.1	6.0	0.4
α-Selinene	0.1	0.3	–
Calamenene	0.1	0.1	–
γ-Cadinene	0.8	0.2	0.3
δ-Cadinene	0.2	–	–
(*E*)-Nerolidol	0.1	0.4	0.2
Caryophyllene oxide	0.4	0.3	0.5
Humulene epoxide I	–	0.1	–
Humulene epoxide II	0.1	t	0.1
Cubenol	–	0.1	–
t-Cadinol	0.1	0.1	0.2
t-Muurolol	–	t	–
epi-α-Cadinol	–	0.1	0.1
α-Cadinol	0.1	0.1	0.1
n-Heptadecane	–	0.1	0.2
Benzyl *n*-octanate	–	–	0.1
Myristic acid	–	–	0.1
iso-Propyl myristate	–	–	0.1
Oleic acid	–	–	0.1

t = trace.
Source: Srivastava *et al.* (2005).

Sterols

Sterols isolated from clove include sitosterol, stigmasterol and campesterol (Brieskorn *et al.*, 1975).

Flavonoids

Achromone *C*-glucoside, isobiflorin (5,7-dihydroxy–2-methoxychromone-8-*C*-β-D-glucopyranoside) and biflorin were isolated from

Table 8.8. Classification of clove essential oil constituents.

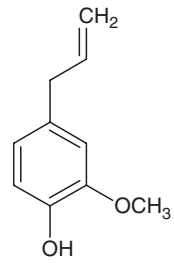
Structural type	Compounds
Monoterpenes	α-Thujene, α-pinene, myrcene, limonene, *p*-cymene
Sesquiterpenes and their derivatives	*t*-α-Bergamotene, α-cubebene, α-copaene, β-caryophyllene, germacrene D, α-amorphene, β-selinene, α-farnesene, viridiflorene; β-himalachene, valencene, γ-cadinene, zonarene, calamenene, calacorene, caryophyllene and humulene oxides, palustrol, α-cadinol, 4,4-dimethyl-8-bicyclo(6.2.3.0) tridecan-1-ol, 4(12),8(13)-caryophylladien-5β-ol,11,11-dimethyl-8-methylene bicyclo (1.2.0)-3-undecen-5β-ol
Aledehydes and ketones	Valeraldehyde, caproaldehyde, benzaldehyde 2 (or) 3-methoxybezaldehyde, coniferaldehyde, cuminaldehyde, geranial.
	2-Hexanone, 2-heptanone, 2-octanone, 2-nonanone, 6-methyl-5-hepten-2-one, fenchone, carvone, acetophenone, 2-hydroxy-4,6-dimethoxy-5-methylacetophenone
Esters	Ethyl caproate, methyl caprylate, ethyl caprylate, methyl palmitate, methyl stearate, methyl linoleate, benzyl tiglate.
Acetates	*Sec*-heptyl, *sec*-nonyl, α-terpenyl, benzyl, phenyl, β-phenethyl, eugenyl, sterallyl
Benzoates	Methyl, ethyl, *n*-propyl, *sec*-heptyl, benzyl
Cinnamate	Ethyl
Salicylates	Methyl, benzyl
Alcohols	Methanol, 2-heptanol, 2-nonalol, linalool, benzyl alcohol
Phenols and their derivatives	Methyl chavicol, *trans*-anethol, methyleugenol, eugenol, chavicol, vanillin
Heterocycles	Furfural, 5-methylfur fural, dimethyl furfural, furfuryl alcohol, 5-methylfurfuryl alcohol, γ-decalactone
Other compounds	Naphthalene

the ethanolic extract of cloves (Zhang and Chen, 1997). From the ethanol extract of the seeds, apigenin 6-*C*-[β-D-xylopyranosyl-(1→2″)-β-D-galactopyranoside]-7-*O*-β-D-glucopyranoside and apigenin-6-*C*-[β-D-xylopyranosyl- (1→2″)-β-D-galactopyranoside]-7-*O*-β-D-(6-*O*-*p*-coumarylglucopyranoside) were isolated (Nassar, 2006). The flavonoids, kaempferol and rhamnetin, isolated from clove are antioxidants. Chemical structures of a few non-volatile constituents are indicated in Fig.8.2.

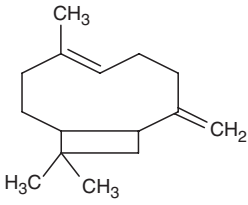
8.5. Medicinal and Pharmacological Uses

India's traditional Ayurveda healers have used cloves since ancient times to treat respiratory and digestive ailments. Like many culinary spices, cloves help relax the smooth muscle lining of the digestive tract and eating cloves is said to be aphrodisiac. Aqueous extract of clove flower bud inhibits its immediate hypersensitivity in rats by inhibition of histamine release from mast cells *in vivo* and *in vitro* (Kim *et al.*, 1998).

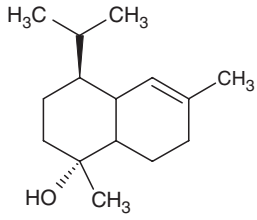
Cloves are more often used to assist the action of other herbal remedies rather than alone. When not available, allspice is substituted. It is spicy, warming, stimulant, anodyne, anaesthetic (topical), antiemetic, antigriping (added to other herbs), vermifuge, uterine stimulant, stomachic, aromatic, carminative, antiseptic, antiviral, antibacterial, antifungal, antispasmodic, expectorant, aphrodisiac and promotes salivation and digestive juices. The oil is expectorant, anaesthetic, emmenogogue; it affects the kidney, spleen and stomach and has preservative properties. Tea made from clove bud (other herbs/spices can be used or added to cloves, such as allspice, bay, cinnamon and marjoram) has been used to relieve bronchitis, asthma, coughs, a tendency to infection, tuberculosis, altitude



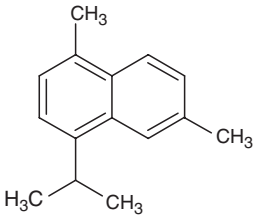
Eugenol



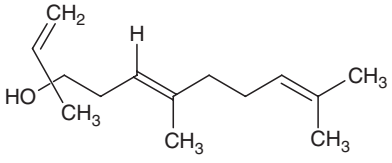
Caryophyllene



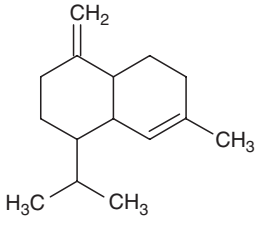
trans-cadinol



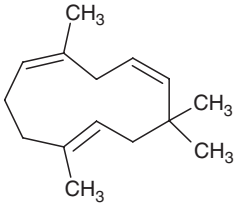
Cadalene



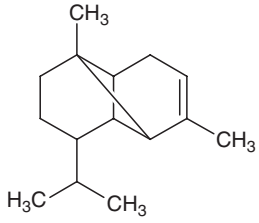
Nerolidol



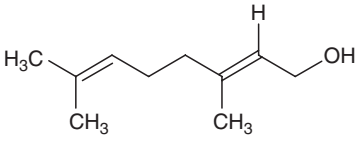
γ-Cadinene



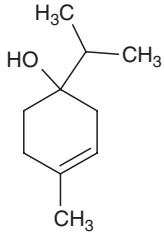
α-Humulene



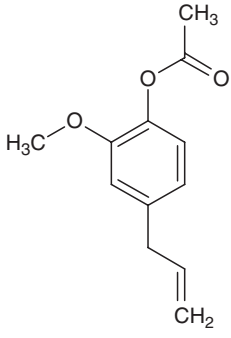
α-Copaene



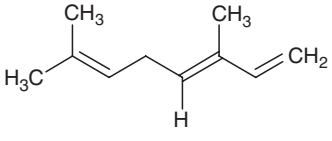
Nerol



4-terpineol



Eugenyl acetate



β-Ocimene

Fig. 8.1. Volatiles from clove.

Table 8.9. Aglycones in clove buds and leaves.

Component	Clove buds		Clove leaves	
	α-Amyloglucosidase	β-Glucosidase	α-Amyloglucosidase	β-Glucosidase
Pentan-3-ol	t	t	t	t
Hexen-3-ol	t	t	t	t
Heptan-3-ol	t	0.5	1.5	t
Octanol	t	t	t	t
Nonan-2-ol	t	t	t	t
Linalool oxide	t	0.5	t	0.4
Linalool	t	0.9	t	0.9
Benzyl alcohol	t	t	t	t
β-Phenylethyl alcohol	t	t	t	t
α-Terpineol	1.1	t	t	t
Nerol	0.5	t	t	0.5
Geraniol	0.3	2.6	t	0.8
Eugenol	3.4	62.6	12.0	76.1
cis-Isoeugenol	2.5	1.6	2.3	1.1
trans-Isoeugenol	2.0	3.7	2.6	3.6
cis-Nerolidol	4.1	1.8	0.8	0.3
Farnesol	54.3	1.9	59.8	2.0

t = trace.
Source: Menon and Narayanan (1992).

sickness, nervous stomach, nausea, diarrhoea, flatulence, indigestion, dyspepsia and gastroenteritis.

In Chinese medicine cloves are used as a kidney tonic (especially for impotence associated with deficient yang), to warm the body, increase circulation and as a digestive aid. They are also used for nausea, vomiting, flatulence, hiccups, stomach chills, fever, caries, toothache, cholera, colic, cracked nipples, diarrhoea, dyspepsia, halitosis (chewing on the whole clove), unusual uterine bleeding, nasal polyps and impotence. The root is used for a weaker effect. The oil is employed for diarrhoea, halitosis, hernia, nausea and toothache.

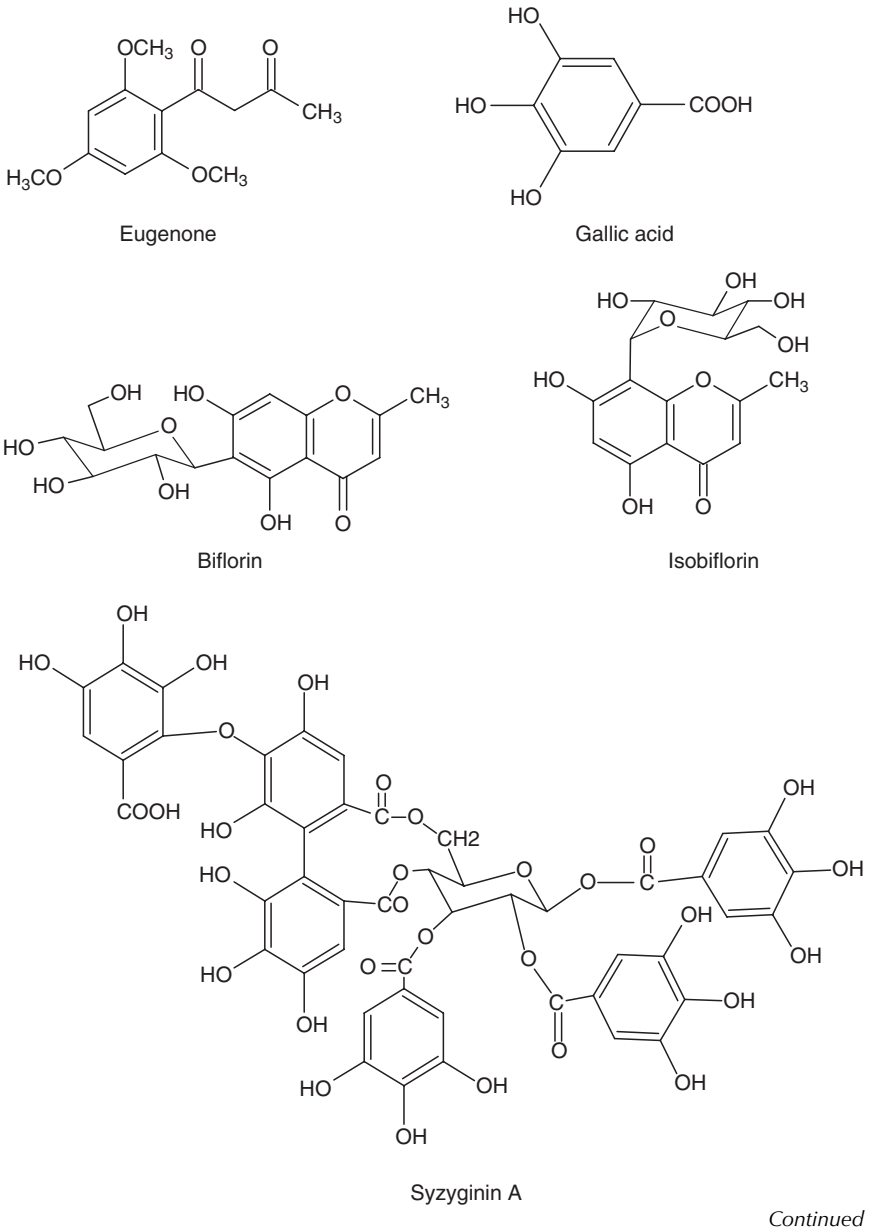
Ethanolic extract (50%) of clove produced a significant and sustained increase in the sexual activity of normal male rats, without any conspicuous gastric ulceration or adverse effects. Thus, the resultant aphrodisiac activity of the extract lends support to claims for its traditional usage in sexual disorders. In traditional Chinese medicine it is used to treat indigestion, diarrhoea, hernia, ringworm and other fungal infections. In Ayurveda, cloves are used to treat respiratory and digestive ailments, flatulence, nausea and vomiting. The medieval German herbalists used cloves as part of an antigout mixture. Clove is believed to have a cooling effect on the stomach. A paste of clove was applied to the forehead for relief from colds. It has powerful local antiseptic and mild anaesthetic actions.

Clove bud oil has various biological activities such as antibacterial, antifungal, antioxidant and insecticidal properties. The high level of eugenol present in the essential oil imparts strong biological and antimicrobial activity.

Clove oil is an active ingredient in several mouthwash products and a number of over-the-counter toothache pain-relief preparations. It is also used to disinfect root canals. For toothache, clove tea has been used in combination with chamomile or sage.

Eugenol is shown to alleviate neuropathic pain (Guénette *et al.*, 2007). Eugenol inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells (Raghavenra *et al.*, 2006).

Clove oil is used to prepare microscopic slides for viewing. It is used to treat



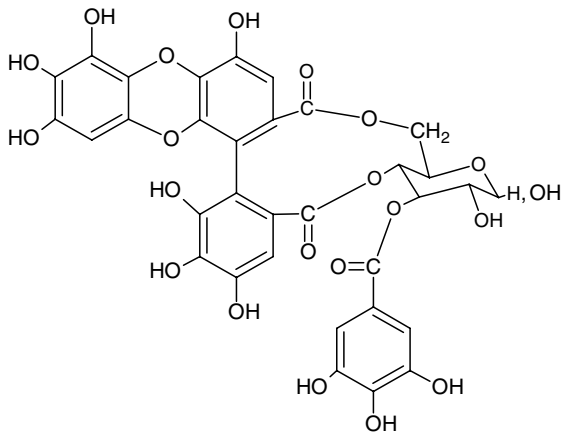
Continued

Fig. 8.2. Non-volatiles from clove.

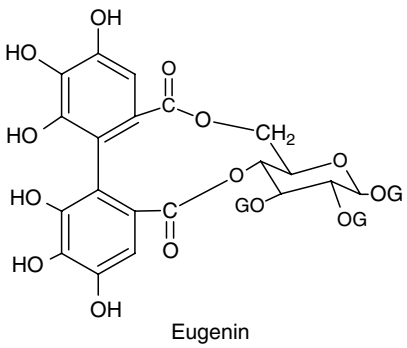
flatulence, colic, indigestion and nausea. Eugenol is used in germicides and perfumes, in the synthesis of vanillin and as a sweetener or intensifier. A recent review by Chaieb *et al*. (2007) lists the chemical composition and biological activity of clove essential oil.

Antimicrobial activity

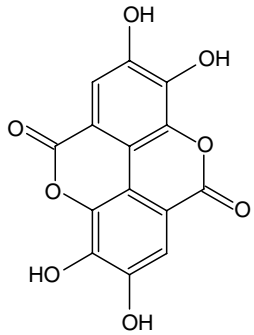
Clove exhibits potent antimicrobial activity against *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae* (De *et al*., 1999). Essential oils from clove and eugenol show various degrees of inhibition against



Syzyginin B



Eugenin
G = galloyl



Ellagic acid

Fig. 8.2. *Continued*

Aspergillus niger, S. cerevisiae, Mycoderma sp., *Lactobacillus acidophilus* and *B. cereus*, as estimated by the paper disc agar diffusion method (Meena and Sethi, 1994). The oil also inhibits the growth of *Fusarium verticilloides* (Veluti *et al.*, 2004). Clove oil (1% v/w) inhibits *Listeria monocytogenes* in chicken frankfurters (Mytle *et al.*, 2006). It has excellent antimicrobial properties and is used in food preservation (Smith Palmer *et al.*, 1998, 2001).

Clove extracts show high antifungal activity against *Rhizoctonia solani* (Lee Sang *et al.*, 2003). Clove oil and eugenol are reported to possess significant antifungal activity against rye bread spoilage fungi (Suhr and Nielsen, 2003). Clove oil shows antifungal activity against the fungi belonging to *Eurotium*, *Aspergillus* and *Penicillium* species, commonly causing deterioration of bakery products (Guynot *et al.*, 2003). Eugenol possesses antifungal activity against *Cladosporium herbarum*, *Penicillium glabrum*, *P. expansum* and *A. niger* (Martini *et al.*, 1996; Kong Qiu *et al.*, 2004).

Clove bud oil causes inhibition of both mycelial growth and aflatoxin production of *A. parasiticus* (Farag *et al.*, 1989; Gowda *et al.*, 2004). Clove oil, at concentrations > 100 µg/ml, results in reduction in the aflatoxin production in liquid cultures (Sinha *et al.*, 1993). Clove oil inhibits the growth and production of fumonisin B_1 by *F. proliferatum* (Veluti *et al.*, 2003).

Antibacterial activity

Cloves are one of Mother Nature's premium antiseptics. A few drops of the oil in water can stop vomiting and an infusion relieves

nausea. Essential oil of clove is effective against *Streptococci*, *Staphylococci* and *Pneumomocci* bacteria.The volatile oils of clove exhibited considerable inhibitory effects and antibacterial activity against several genera of bacteria, including animal and plant pathogens and food poisoning and spoilage bacteria (Deans and Ritchie, 1987; Dorman and Deans, 2000).

Clove kills intestinal parasites and exhibits broad antimicrobial properties, thus supporting its traditional use as a treatment for diarrhoea, intestinal worms and other digestive ailments. Clove essential oil is strongly antimicrobial, antiseptic, haemostatic and anti-inflammatory. Because of its strong antiparasitic action, clove is also included in Dr Huda Clark's protocol for elimination of parasites from the digestive system. It has also been found that a 0.05% solution of eugenol is sufficient to kill *B. tuberculosis*. Clove oil showed antimicrobial activity against some human pathogenic bacteria resistant to certain antibiotics (Arora *et al.*, 1999; Lopez *et al.*, 2005).

Antioxidant activity

Clove essential oil has the highest antioxidant capability of any essential oil, perhaps one of the highest known for a food or supplement. It has been included in some 'longevity' formulae for this reason. Clove and eugenol possess strong antioxidant activity, which is comparable to the activities of the synthetic antioxidants, BHA and pyrogallol (Dorman *et al.*, 2000). Essential oil from clove leaf possesses scavenging activity against the 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical at concentrations lower than the concentrations of eugenol, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). It also shows a significant inhibitory effect against hydroxyl radicals and acts as an iron chelator (Gulcin *et al.*, 2004; Jirovetz *et al.*, 2006). Clove oil is also commonly used for numbing tooth pain and the healing of mouth and gum sores. The oil can also be used to assist in the breaking of tobacco addiction (Ananda Aromatherapy). The antioxidant activity of clove bud extract and its major aroma components, eugenol and eugenyl acetate, are comparable to that of the natural antioxidant, α-tocopherol (Lee and Shibamoto, 2001). Eugenol inhibits 5-lipoxygenase activity and leukotriene-C4 in human PMNL cells (Raghavenra *et al.*, 2006).

Anti-inflammatory activity

Eugenol, the primary component of clove's volatile oils, functions as an anti-inflammatory substance. In animal studies, the addition of clove extract to diets already high in anti-inflammatory components (like cod liver oil, with its high ω-3 fatty acid content) brings a synergistic effect. In some studies, it further reduces inflammatory symptoms by another 15–30%. Clove also contains a variety of flavonoids, including kaempferol and rhamnetin, which also contribute to clove's anti-inflammatory and antioxidant properties. Another constituent of clove oil, β-caryophyllene, also contributes to the anti-inflammatory activity (Ghelardini *et al.*, 2001).

Anaesthetic effect

Clove oil is used as a safe anaesthetic for aquatic research. Tricaine or MS-222, the only anaesthetic registered in North America, is a very effective anaesthetic for several fish species but its application in the field is limited because the US Food and Drug Administration guidelines demand a 21-day withdrawal period after exposure to MS-222 before the fish enters the food chain. In this context, clove oil is found to be an alternative to MS-222 for use as a fish anaesthetic. Exposure of channel catfish (*Ictalurus punctatus*) to clove oil at a concentration of 100 mg/l induced anaesthesia within 1 min. The fish recovered from a 10 min period of anaesthesia within 4 min after removal from the anaesthetic solution.

Clove oil is therefore used as a safe anaesthetic for channel catfish (Waterstrat, 1999). The anaesthetic effect of clove oil and eugenol for use in aquaculture and aquatic

research was also reported by Soltani *et al.* (2001) and Jayathilake *et al.* (2003). Clove oil and eugenol were reported as an acceptable anaesthetic for rabbit fish (*Saiganus lineatus*), coral reef fish (*Pomacentrus amboinensis*) and rainbow trout (*Oncorhynchus mykiss*) for use in aquaculture and aquatic research (Soto and Burhanuddin, 1995; Munday and Wilson, 1997; Keene *et al.*, 1998). It was also found to be useful as a crab anaesthetic (Morgan *et al.*, 2001). *β*-Caryophellene is also reported to be an anaesthetic (Ghelardini *et al.*, 2001).

Mosquito-repellent activity

Clove oil exhibits repellent activity on *Anopheles albimanus*, *Aedes aegypti*, *A. dirus* and *Culex quinquefasciatus* (Barnard, 1999; Trongtokit *et al.*, 2005).

Insecticidal activity

Eugenol, isoeugenol and methyl eugenol cause contact toxicity to the storage pathogens, *Sitophilus zeamis* and *Tribolium costaneum*. These compounds have similar toxicity to *S. zeamis* at LD_{50} 30 µg/mg insect, while for *T. costaneum* the order of potency is isoeugenol > eugenol > methyl eugenol (Ho *et al.*, 1994; Huang *et al.*, 2002). The clove leaf and bud oils show potent insecticidal activity against the human headlouse (*Pediculus capitis*) (Yang *et al.*, 2003a,b).

Antithrombotic activity

Clove oil inhibits human platelet aggregation induced by arachidonic acid (AA), platelet-activating factor (PAF) or collagen. Clove oil is a more effective inhibitor for aggregation induced by AA and PAF (IC_{50}: 4 and 6 µM, respectively) than collagen (IC_{50}: 132 µM). It inhibits platelet aggregation and thromboxane synthesis and acts as an antithrombotic agent. Eugenol and acetyl eugenol are more potent than aspirin in inhibiting platelet aggregation induced by arachidonate, adrenaline and collagen. In arachidonate-induced aggregation eugenol is on par with indomethacin (Srivastava, 1990).

Anticancerous activity

Clove has strong anticancerous properties. The sesquiterpenes, *β*-cayophyllene, *β*-cayophyllene epoxide, *α*-humulene, *α*-humulene epoxide and eugenol present in clove oil showed potent anticarcinogenic activity by inducing the detoxifying enzyme, glutathione-*S*-transferase, in mouse liver and small intestine (Zheng *et al.*, 1992).

Antiviral activity

Clove is a potent antiviral agent and eugenin isolated from clove buds showed antiviral activity against *Herpes simplex* virus at a concentration of 10 µg/ml (Kim *et al.*, 2001; Chaieb *et al.*, 2007).

Antipyretic effect

Eugenol, the chief constituent of clove oil, has marked antipyretic activity when given intravenously, intragastrically and centrally to rabbits made febrile by interleukin-1. Eugenol was more effective in reducing fever than acetaminophen and it reduced fever primarily through a central action similar to that of common antipyretic drugs, such as acetaminophen (Feng and Lipton, 1987).

Toxicity studies

Cloves can cause local skin irritation, pulmonary oedema, mouth sensitivity and sudden lower airway closure. In addition, smoking clove cigarettes can damage soft tissues and injure the airway linings (Fetrow and Avila, 1999).

8.6. ISO Specifications

Quality requirements for various clove products is country specific. The American Spice Trade Association (ASTA) and Food and Drug Administration (FDA) recommendations for whole spice (clove) are illustrated in Table 8.10.

Table 8.10. ASTA cleanliness specifications.

Items	Suggested limit whole clove
Whole dead insect (mg/lb)	4.00
Mammalian excreta (mg/lb)	5.00
Other excreta (mg/lb)	8.00
Mould, % by weight	1.00
Insect-defiled/infested, % by weight	1.00
Extraneous, % by weight	1.00

The specific requirement for ground clove is for a minimum quercitannic content, maximum clove stem content and minimum 15% volatile ether extract. The ISO specifications of clove oil are illustrated in Table 8.11.

8.7. Conclusion

The spice clove and its value-added products are used extensively for flavouring food and confectionery. Clove oil has many industrial and pharmacological applications. Most of the studies conducted so far pertain to the clove volatiles and very little attention has been paid to the non-volatile constituents. Therefore, the phytochemical studies and biological activities of non-volatiles are worth examining. This may lead to identifying new properties and novel molecules.

Table 8.11. Specifications of clove products.

Oil of clove leaves	ISO 3141:1997	*Syzygium aromaticum*
Oil of clove buds	ISO 3142:1997	*S. aromaticum*
Oil of clove stems	ISO 3143:1997	*S. aromaticum*
Cloves, whole and ground (powdered)	ISO 2254:1980	*S. aromaticum*

Source: http://www.fao.org/inpho/content/documents/vlibrary/ad420e/AD420e37.htm.

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