# 4

# **Basil**

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**Abstract**: Basil (*Ocimum basilicum*) is an annual spicy herb indigenous to India which has been cultivated for several millennia for its aromatic and medical uses. This chapter gives a definition of basil before examining the chemical composition of the different varieties of the plant. Production of basil is discussed in relation to soil and climate requirements, propagation, planting and fertilizing; organic production is also covered. The chapter then looks at postharvest handling and storage of the leaves and essential oil and extraction of the oil. The culinary and medicinal uses of basil and its functional properties are described before the chapter finishes with a discussion of quality issues and toxicity.

**Key words**: basil, *Ocimum basilicum*, essential oil, functional properties, quality, toxicity.

# 4.1 Introduction: the origin of basil

Basil (Ocimum basilicum, Lamiaceae) (also known as sweet basil) is an annual spicy herb, indigenous to India. Several species of Ocimum are cultivated in India, where their medicinal and aromatic uses have been known for several millennia. The ancient Ayurvedic surgeon Sushruta classified Ocimum as a green leafy vegetable, while Bhavamisra, a famous Ayurvedic Acharya, referred to Ocimum basilicum as 'barbari' (Pushpangadan et al., 1993). It is also mentioned in classical Ayurvedic texts such as Sushruta Samhita, Charaka Samhita, Bhavaprakasham, and Ashtangahridayam, among others. Sweet basil is native to India and tropical Asia, and now grows wild in tropical and sub-tropical regions (Ayurnepal, 2012) including Central Africa and South East Asia (Simon, 1998). It is cultivated commercially in many warm and temperate countries worldwide, including France, Hungary, Greece and other southern European countries, Egypt, Morocco and Indonesia. It is also cultivated in several US states (Christman, 2010), including Arizona, New Mexico and North Carolina, as well as in California, where a superior quality of leaf is grown (Prakash, 1990).

### **Basil can be classified as follows** (Kartesz, 2009):

- kingdom: *Plantae* plants
- sub-kingdom: *Tracheobionta* vascular plants

superdivision: Spermatophyta – seed plants
 division: Magnoliophyta – flowering plants

• class: Magnoliopsida – dicotyledons

sub-class: Asteridaeorder: Lamiales

• family: Lamiaceae – mint family

• genus: *Ocimum* L. – basil

#### 4.1.1 Definition of basil

Ocimum basilicum is an erect, almost glabrous herb, which grows to between 30 and 90 cm high. The leaves are ovate, lanceolate, cucuminate, toothed or entire, glabrous on both surfaces and glandular. When mature, they reach approximately 5 cm in length, excluding the petiole, which is approximately 2 cm long. The upper surface is smooth and lustrous; on the lower surface along the midrib and on the petiole short, stiff hairs occur sparingly (Prakash, 1990).

The flowers are white or pale purple and are borne in long terminal racemose inflorescences, in simple or many branched racemes. The greenish corolla is small and inconspicuous. The calyx is partly grown together with the branches, and enlarges itself after flowering, remaining dry on the plant with the branches. The capitate hairs have commonly a two-celled head with a stalk so short as to appear sessile.

Polymorphism and cross-pollination under cultivation have given rise to a number of sub-species and varieties differing in height, habitat and growth, degree of hairiness and colour of stems and in their leaves and flowers.

# 4.2 Chemical composition of the basil plant

The flowers yield an average of 0.4 % oil while the whole plant contains 0.1–0.25 % oil (figures refer to Indian basil). By taking the initial three to four harvests of flowers (including main and sub inflorescences) and final harvest of the whole herb, approximately 3–4 t of flowers and 13 t of whole herb per hectare can be obtained, corresponding to about 13 kg of the flower oil and about 27 kg of whole herb oil, a total of 40 kg of oil per hectare. The oil of sweet basil produced both from the herb and flowers has commercial value: it has a clove-like scent with an aromatic and somewhat saline taste, and is used as a flavouring agent and as a perfume (Pruthi, 1976; Farrell, 1985).

Pushpangadan and Bradu (1995) reported that the essential oil composition of *O. basilicum* differed between varieties, finding different varieties of the herb to be rich in methyl chavicol, linalool, methyl cinnamate and geraniol. Based on thin-layer chromatography (TLC) and gas chromatographic studies they established that *O. basilicum* L. var. *minima* Benth. contained geraniol (45%) and eugenol (25%) as the major compounds; *O. basilicum* L. var. *glabratum* Benth., chemotype No. 1 contained methyl chavicol (38%) and linalool (35%); *O. basilicum* L. var. *glabratum* Benth., chemotype No. 2 contained linalool (47%) and eugenol (20%) as the major components; *O. basilicum* L. var. *glabratum* Benth., chemotype No. 3 contained

linalool (40%), eugenol (20%) and camphor (20%); *O. basilicum* L. var *purpurascence* Benth. contained methyl cinnamate (20%) and linalool (60%); *O. basilicum* L. var *tryrsiflora* Benth. contained methyl cinnamate (35%) and linalool (60%); *O. basilicum* L. var *crispum* Benth. contained methyl chavicol (50%) and linalool (28%); and *O. basilicum* L. var *darkapal* contained geraniol (35%), linalool (35%) and eugenol (25%).

Ji-Wen *et al.* (2009) studied the composition of essential oil obtained from the aerial parts of *O. basilicum* Linn. var. *pilosum* (*Willd.*) Benth., an endemic medicinal plant growing in China. They identified linalool (29.68 %), (*Z*)-cinnamic acid methyl ester (21.49 %), cyclohexene (4.41 %), α-cadinol (3.99 %), 2,4-diisopropenyl-1-methyl-1-vinylcyclohexane (2.27 %), 3,5-pyridine-dicarboxylic acid, 2,6-dimethyl-diethyl ester (2.01 %), β-cubebene (1.97 %), guaia-1(10),11-diene (1.58 %), cadinene (1.41 %), (*E*)-cinnamic acid methyl ester (1.36 %) and β-guaiene (1.30 %) all present in this oil.

Koba *et al.* (2009) described five chemotypes of *O. basilicum* (Lamiaceae) from Togo. They are the estragole type; the linalool/estragole type; the methyleugenol type; the methyleugenol/t-anethole type and the t-anethole type.

Thus the essential oil composition of *O. basilicum* varies according to the variety, geographic origin, harvesting season, etc. This considerably affects the aroma and flavour characteristics of the oil. A literature search conducted by Lee *et al.* (2005) revealed that the oil constituents belonged to different classes of compounds, including mono and sesquiterpene hydrocarbons, oxygenated mono and sesquiterpenes, aliphatic alcohols, aldehydes, esters, ketones, acids, aromatic compounds, and so on. A list of the compounds identified in basil oil is presented in Table 4.1.

## 4.3 Production of basil

#### 4.3.1 Soil and climate requirements

Ocimum species thrive well in a variety of soils and climatic conditions. Soils suitable for cultivation are rich loam to poor laterite, and saline and alkaline to moderately acidic. Well-drained soil helps to encourage improved vegetative growth. Basil flourishes well under fairly to high rainfall and humid conditions, and long days and high temperature have been found to be favourable for plant growth and higher oil production. O. sanctum can be grown in partially shaded conditions, but this leads to a low oil yield. The above factors make tropical and sub-tropical climates ideal for basil cultivation (Pushpangadan and Bradu, 1995).

#### 4.3.2 Seed and propagules

Since the *Ocimum* species are generally highly cross-pollinated, a certain amount of heterozygosity is essential for vigorous growth, high oil yield and high-quality oil. These characteristics are mostly controlled by polygenes whose effect is mostly additive. The seeds are therefore likely to deteriorate in future generations unless the selected lines from which the polycross seed is produced are maintained. It is also crucial that fresh seed is collected from the polycross lines each time. For fresh

 Table 4.1
 Different compounds identified in basil oil

Monoterpene hydrocarbons	$\alpha$ -Pinene, sabinene, myrcene, $p$ -cymene, limonene, $\alpha$ -terpinene, (Z)- $\beta$ -ocimene, (E)- $cis$ -ocimene, $\gamma$ -terpinene, terpinolene
Oxygenated monoterpenes	1,8-Cineole, linalool <i>cis</i> -furanoid, linalool oxide <i>cis</i> -furanoid, <i>trans</i> -sabinene hydrate, linalool <i>trans</i> -furanoid, linalool oxide <i>trans</i> -furanoid, ocimene oxide, camphor, 3,7-dimethyl-1,6-octadien-3-ol, linalool, linalyl acetate, <i>trans</i> - <i>p</i> -menth-2-en-1-ol, bornyl acetate, carvacryl methyl ether, exo-methylcamphenilol, 4-terpineol, <i>cis</i> -dihydrocarvone, hotrienol, terpinen-1-ol, l-menthol, <i>trans</i> -pinocarveol, d-terpineol, lavandulol, <i>trans</i> -verbenol, <i>p</i> -menth-1,8-dien-4-ol, terpinyl formate, α-terpineol, borneol, verbenone, exo-2-hydroxycineole acetate, dihydrocarveol, α-citral, exo-2-hydroxycineole, l-carvone, linalool oxide <i>cis</i> -pyranoid, <i>trans</i> -piperitol, linalool oxide <i>trans</i> -pyranoid, citronellol, yrtenol, nerol, <i>trans</i> -carveol, <i>p</i> -cymen-8-ol, geraniol, geranyl acetate, guaiacol, exo-2-hydroxycineole, piperitenone, l-perillyl alcohol, cuminyl alcohol, fenchone, estragole, <i>t</i> -anethole, carvacrol, thymol, bornyl acetate, methyleugenol, geranyl formate
Sesquiterpene	β-Cubebenec, δ-cadinene, valencene, α-amorphene, δ-selinene,
hydrocarbons	dehydroaromadendrene, β-elemene, α-copaene, β-caryophyllene, α-humulene, (–) calamenene (E)-α-bergamotene, α-caryophyllene, germacrene D, β-selinene, α-zingiberene, bicyclogermacrene, α-muurolene, germacrene A, germacrene D, γ-cadinene
Oxygenated sesquiterpenes	<ul> <li>Γ-Cadinol, spathulenol, caryophyllene oxide, α-humulene oxide, elemol, viridiflorol, spathulenol, α-cadinol, Γ-murolol, β-bisabolol, β-bisabolol isomer, α-eudesmol, isospathulenol, β-eudesmol, caryophylla-4(12),8(13)-dien-5β-ol, dihydroactinidiolide, caryophylla-3,8(13)-dien-5α (or β)-ol</li> </ul>
Aliphatic alcohols	1-Penten-3-ol, 3-methyl-3-buten-1-ol, (Z)-2-pentenol, 3-methyl-2-buten-1-ol, hexanol, (Z)-3-hexenol, 3-octanol, cyclohexanol, 1-octen-3-ol, octanol
Aliphatic aldehydes	Hexanal, (E)-2-hexenal, (E,Z)-2,4-heptadienal, (E,E)-2,4-heptadienal
Aliphatic esters Aliphatic ketones	Methyl 2-methylbutyrate, ( <i>Z</i> )-3-hexenyl acetate 3-Octanone, 3-hydroxy-2-butanone, 6-methyl-5-heptenone, 6-methyl-(E,E)-3,5-heptadien-2-one, β-ionone, <i>cis</i> -jasmone, <i>trans</i> -β-ionone-5,6-epoxide, methyl jasmine
Aliphatic acids Aromatic compounds	Butanoic acid, octanoic acid, decanoic acid Benzaldehyde, methyl benzoate, phenyl acetaldehyde, 1-methoxy- 4-(2-propenyl) benzene, methyl salicylate, p-methylacetophenone, cuminaldehyde, anethol, safrole, benzyl alcohol, phenethyl alcohol, methyl cinnamate, methyl eugenol, α,α-dimethylphenylethyl alcohol, anisaldehyde, trans- cinnamaldehyde, methyl cinnamate, p-cresol, ethyl cinnamate, eugenol, 2-isopropyl-5-methylphenol (thymol), 2-isopropyl-2- methylphenol (carvacrol), 5-isopropyl-3-methylphenol, 4-allylphenol, dillaiole, p-methoxycinnamaldehyde
Miscellaneous compounds	2,6-Dimethylpyrazine, <i>c</i> -butyrolactone, myristicin (Lee <i>et al.</i> , 2005)

plantings, the growers must take fresh seeds from the pedigree stock. Selected lines can be multiplied vegetatively by growing tender shoot tips. Good-quality planting seeds can be obtained from reputed seed companies.

### 4.3.3 Nursery practices

Plantations can be created either by raising the seedlings in the nursery and then planting them in the field or by direct sowing of seeds in the field.

## Direct sowing

Experiments have shown that direct sowing is more efficient, economical and profitable than raising seedlings in a separate nursery and then transplanting them to the field. The seeds (75–250 g/ha) are mixed with dry sand to ensure an even distribution. Before sowing, the field is ploughed into long narrow furrows at a spacing of 50–60 cm; the seed is then sown in rows by hand or drilled. After the seeds are sown, the field is worked to cover the seeds. The field must be flooded with water within 24 hours of sowing, if there is no rain.

The seeds germinate within 10–15 days. After 20–25 days, when the seedlings have grown to 15–20 cm high, the first weeding and thinning or gap-filling can be carried out, if required.

### Nursery sowing

An alternative method is the raising of seedlings in a nursery before transferring them to the field. The nursery seed beds should be prepared and treated with farmyard manure. For 1 ha of land, approximately 200–300 g seeds should be planted. Once the seeds are sown, more manure, mixed with soil, should be thinly spread over the seeds. The beds should then be irrigated with a sprinkler hose. After approximately 8–12 days, the seeds will germinate, and the seedlings will be ready to be transplanted to the field around 6 weeks after planting. The plants can be sprayed with a 2% urea solution 15–20 days prior to transplanting: this helps to ensure healthy plants for transplanting.

## **Transplanting**

Approximately 6–7 weeks after planting, the seedlings are ready to be transplanted. At this stage, the seedlings should have four to five leaves. Seedlings are usually transplanted in April; however, if the seedlings have been raised in hot beds, transplanting may be carried out in March.

Spacing of 40– $60 \, \mathrm{cm}^2$  has been found to be most suitable for *Ocimum* species. For *O. basilicum*, a spacing of  $60 \times 60 \, \mathrm{cm}$  was recommended by Singh *et al.* (1970) for Assam and by Gulati *et al.* (1977) for Haldwani in Uttar Pradesh, India.

When the second irrigation is performed, the seedlings are already well established. This is the stage at which gaps should be filled and any weak plants replaced, for the purposes of uniformity. During the summer, plants should be irrigated three times a month; outside of this period, irrigation should be carried out as necessary, except during the rainy season when no irrigation is required. Over the year, irrigation should be carried out approximately 12–15 times.

#### 4.3.4 The effect of manures and fertilizers

Between 20 and 25 kg of nitrogen and 10--15 kg  $P_2O_5$  per hectare ensures good vegetative growth, herb and oil yield. The application of up to 75 kg/ha of nitrogen has also been found to be beneficial for optimum herb and oil yield.

### 4.3.5 Organic basil

The use of inorganic fertilizers should be avoided as far as possible since this may reduce the therapeutic property of basil. Organic materials such as farmyard manure, biogas slurry, compost, neem cake and other oil seed cakes, biofertilizers, green manure and cover crops can be used as substitutes for inorganic fertilizers. The use of organic manure will increase the organic matter of the soil, reducing the bulk density and increasing the water-holding capacity. Both of these factors improve the fertility of the soil, especially when the soil is sandy in texture and low in organic matter. These should preferably be incorporated into the soil at the start of summer, as their decomposition rate increases with warm weather. Moreover, the use of organic manure is preferable as it conserves nutrients by preventing leaching losses, and releases them as a continual process. Organic manures also help in reducing soil erosion, while crop rotation and green manures involving legumes add to soil fertility. For the control of pests, bio-insecticides such as tobacco extract, turmeric extract, garlic extract, garlic—neem extract, neem seed oil emulsion and neem seed kernel extract can be used.

## 4.4 Post-harvest handling and production of basil

In countries with a Mediterranean climate, three to five cuttings per year may be taken of most *Ocimum* species, with the first 90–95 days after planting, and subsequent harvesting taking place at intervals of 60–75 days. In the more northern temperate zones, however, only one or two cuttings are usually possible in a year, one early in the summer with a low yield, and one normally just prior to flowering or in full bloom, depending on the intended use (Simon, 1995).

#### 4.4.1 Post-harvest handling and storage for fresh or dried leaves

If the basil is grown for its leaves, either fresh or dried, the main cutting is taken just prior to flowering, while the leaves are still young. The plant is cut at least 10–15 cm from the ground, above the bottom two to four sets of true leaves, in order to allow for re-growth. For large-scale and commercial purposes, harvesting may be carried out with a modified sickle bar or jerry mover with an adjustable cutting height. To ensure a continuous supply of fresh leaves, the field harvest and planting dates are normally staggered (Simon, 1995).

Once harvested, the leaves should first be washed and cleaned to remove weeds and extraneous materials. For the fresh market, only the highest quality plant material should be used, i.e. that with the best colour and aroma retention. The leaves can be preserved by hanging the foliage upside down in small bunches and air drying in a warm, dry, well-ventilated room. Foliage can also be dried by spreading flat on

a drying rack under the same conditions. Once the basil is thoroughly dried, the leaves are stripped from the stems and stored whole or chopped in an air-tight container away from heat sources and bright light. In terms of colour and aroma retention, the best results are obtained when the plant is dried below 30°C under vacuum in suitable containers. The leaves can also be preserved by storing them in small plastic bags in a freezer, or in jars of oil. If the leaves and flowers are to be processed, they should be dried at below 35°C before milling or grinding to ensure the best colour retention.

Basil cannot be stored for long periods after harvest, as this will result in a reduction in quality. However, storage at 10 °C in PVC packaging reduces mould and yeast colonies and can maintain the microbiological quality of basil for up to 9 days after harvesting (Franceli et al., 2005). At 5°C, the post-harvest shelf-life of basil is only 3-4 days; after this the visual quality deteriorates as the symptoms of chilling injury begin to appear. Lange and Cameron (1997) showed that an extended postharvest shelf-life of up to 7 days can be obtained by chill-hardening the plants before harvesting and packaging. Chill-hardening should be carried out at 10 °C for 2 hours at the end of the light period and 2 hours at the beginning of the dark period each day for 2 days. The same study further examined the effect of chill-hardening on 4–6 week-old plants. The plants were chill-hardened over the course of a week at different periods of the day: 4-5 week-old plants chill-hardened at the beginning of the day displayed an extended shelf-life (1-1.5 days longer), but other periods of pre-harvest chill-hardening either had no effect or actually decreased the shelf-life. Furthermore, packaged basil that was chill-hardened post-harvest for 1 day at 10°C in darkness before being transferred to 5 °C had a shelf-life 5 days longer than the non-chill-hardened product. Post-harvest chill-hardening is a promising, effective and convenient method of extending the post-harvest shelf-life of packaged sweet basil.

Amodio *et al.* (2005) investigated the regulatory effects of the post-harvest use of 2.5 kPa  $\rm CO_2$  and l-methylcyclopropene (l-MCP), which inhibits ethylene action, at 0.7 µmol on accelerated senescence in basil (Kenigsbuch *et al.*, 2009). They found that a reduction in the oxygen content of the storage atmosphere over a period of 20 days led to basil leaves of higher quality than those stored in air. No significant improvement was observed on the addition of 3 %  $\rm CO_2$ , although this concentration also did not cause  $\rm CO_2$  injury.

## 4.4.2 Post-harvest handling and storage for essential oil

When basil is grown for its essential oil, the plant is harvested during full bloom. The time of the harvest plays an important role in the quantity and quality of oil produced. The crop is usually harvested on bright sunny days in order to achieve a good oil yield and high-quality oil; harvesting should be avoided on rainy days or the day after rains. The crop should be cut at 15–20 cm above the ground level, and only the flowers and leaves used for processing, since the oil content in the stem is negligible. In a study by Jose *et al.* (2006), it was shown that a higher yield of essential oil can be obtained if the crop is harvested between 8:00 h and 12:00 h. The leaves should then be washed and any weeds and other material removed, just as for fresh cut basil. The length and manner of storage of the crop before processing also affects

the essential oil content. Basil is usually field-dried for 1–3 days before processing. However, the longer the basil shoots are stored, the greater the decrease in the chlorophyll and essential oil content of the plant, which is accompanied by an increase in eugenol and linalool content. Jose *et al.* (2006) found that when the biomass is dried at  $40\,^{\circ}\text{C}$  for a period of 5 days, the concentration of linalool is raised from  $45.18\,\%$  to  $86.80\,\%$ .

#### 4.4.3 Extraction of the essential oil

The extraction of essential oils from plants is discussed in detail in the work of Baby and George (2009), as follows:

Extraction of volatile oils from plants was known to almost all ancient civilizations. The extraction techniques have undergone constant refinements throughout the ages. The most common methods now employed are hydrodistillation, steam distillation, supercritical extraction and microwave extraction. The extraction technique is a careful choice based on the nature of the plant specimen and thermal lability of the target oil constituents.

#### Steam distillation

Steam distillation is carried out by passing dry steam through the plant material whereby the steam volatile compounds are volatilized, condensed and collected in receivers. Steam distillation has been in use for essential oil extraction for many years. Hydrosteam distillation is carried out when the perfumery plant material is susceptible to direct steam. In this technique the plant material is supported on a screen or a perforated grid placed at some distance above the bottom of the still. Distillation is carried out with low pressure steam which replaces the volatile compounds from the intact plant material.

## Hydrodistillation

Hydrodistillation is carried out by boiling the plant material with water and the volatile compounds in the plant material are carried away along with steam which is then condensed and collected in suitable receivers.

## Supercritical fluid extraction

Supercritical fluid extraction uses  $CO_2$  under high pressure to extract essential oils. This is an environmentally friendly process. The plant specimen is placed in a stainless steal tank and  $CO_2$  injected into this tank. Under high pressure  $CO_2$  liquefies and acts as a solvent to extract the essential oil from the plant specimen. When the pressure is decreased,  $CO_2$  returns to the gaseous state leaving the volatile oil. Supercritical fluid extraction is gentler on plant samples compared to steam distillation and results in fresher, cleaner and crisper oils which smell similar to the natural plant aroma.

#### Microwave extraction

Solvent free microwave extraction (SFME) is another recently developed green technology in which a combination of microwave heating and dry distillation at atmospheric pressure results in the extraction of essential oils from plant samples.

SFME results in substantial savings in time, energy, solvents and plant material. The oil obtained by microwave extraction can be used directly for GC and GC–MS studies without further purification. Studies have shown that the oil obtained by SFME is similar in yield and quality to that obtained by hydrodistillation for several hours.

## Hydrodistillation and steam distillation of basil oil

While all of the above methods may be used for the extraction of basil oil, hydrodistillation and steam distillation are most commonly used. The flowers or/and whole herbs are put into a distillation unit and hydrodistilled or steam distilled. It takes about 4 hours to complete one charge. The oil, being lighter than water, can easily be separated from the mixture. Once the oil has been removed, the distillate should be distilled a second time as a small quantity of oil may remain. Care should be taken to see that the distillation unit is clean and free from contamination with residual oils from other sources.

### Essential oil quality

As discussed above, the quality and quantity of the essential oil produced may be affected by a number of factors before processing, including time of harvest and storage method. Once the oil has been extracted, further measures may be taken to ensure a high-quality oil.

Basil oil contains a large number of terpenic hydrocarbons. If these hydrocarbons are removed, the oil is terpene-free and thus fetches a better price in the market. In order to prepare terpene-free oil, basil oil is subjected to fractional distillation under vacuum in fractionating columns. The fractionating columns are also used to isolate major components present in the oil.

Over time, essential oils may darken and deteriorate as a result of contact with light, heat and air. This deterioration can be caused by oxidation, resinification, polymerization, hydrolysis of esters and the interaction of functional groups. Basil oil, along with all other essential oils, must therefore be stored in a cool dark environment, in opaque air-tight containers. It is also crucial to ensure that the oil is free from moisture and any other impurities prior to storage.

## 4.5 Main uses of basil

## 4.5.1 Basil as a flavouring agent in foods

Basil leaves are widely used for flavouring purposes (Niir Board, 2005) in soups, meat pies, fish dishes, certain cheeses, tomato salads, cooked cucumber dishes, cooked peas, squash, and string beans as well as vinegars and oils. Chopped basil may be sprinkled over lamb chops before cooking. Basil is an important seasoning in tomato paste products in Italy, and is often used with or as a substitute for oregano in pizza toppings, spaghetti sauces, meat balls, or in macaroni and cheese bakes.

The essential oil of *O. basilicum* obtained by distillation is used in a number of food products as a flavouring agent and is also used in perfumery thanks to its aromatic characteristics. It contains cineol, pinene, methyl chavicol, d-camphor and ocimene (Eltohami, 1997). The major aroma constituents of basil are

3,7-dimethyl-1,6-octadien-3-ol (linalool; 3.94 mg/g), 1-allyl-4methoxy benzene (estragole; 2.03 mg/g), methyl cinnamate (1.28 mg/g), 4-allyl-2-methoxyphenol (eugenol; 0.896 mg/g), and 1,8-cineole (0.288 mg/g) (Lee *et al.*, 2005).

Examples of food products that may be flavoured with basil essential oil include confectionery, baked goods, condiments, spiced meats, ice creams, puddings, liquors and non-alcoholic beverages (Prakash, 1990). The oil may also be used as a flavouring for certain dental and oral hygiene products.

#### 4.5.2 Uses of basil in traditional medicine

The leaves and infusions of *O. basilicum* are widely used in traditional medicine. In some Mediterranean areas, such as Eastern Morocco, they are used to decrease plasma lipid content (Zhang *et al.*, 2009), while the Santhal tribe of India use sweet basil for headache, earache, cough, cold, inflammation, snake bite and rabies (Pushpangadan *et al.*, 1993). Other reported medicinal uses of basil leaves include the treatment of diarrhoea, dysentery, constipation, flatulence and worms (Simon *et al.*, 1999); as an analgesic and insect repellent (Pushpangadan *et al.*, 1993); to relieve the symptoms of bronchitis, flu, colds, coughs and sinusitis; and as a cure for rheumatism, muscle aches, gout and exhaustion. The leaves are also reported to be effective in the treatment of warts; an ointment made of basil leaves can be used as a treatment for insect bites and can be applied directly to the skin as a cure for acne (Waltz, 1999).

The juice expressed from the leaves also has a number of therapeutic uses: it relieves the symptoms of cold and cough, and those of croup, when mixed with honey. It is also used as a treatment for toothache, earache and headache and can be mixed with camphor to stop nasal haemorrhage. It is said to give lustre to the eyes, and forms an excellent nostrum for the cure of ring worms, scorpion sting and snake bite.

Basil seeds steeped in water and eaten are said to be cooling and very nourishing. The seeds are chewed as a treatment for snake bite (Kirtikar and Basu, 1935). The washed and pounded seeds are used in poultices for unhealthy sores and sinuses, and are also used in *sharbat* for the treatment of chronic constipation and in internal piles. A teaspoon full of seed infused in a tumbler of water with a little sugar, when taken daily, acts as a demulcent in genito-urinary disease; a cold infusion of seeds is said to relieve afterpains of childbirth; and an infusion of seed is also given in fever (Dastur, 1970). The aqueous extract of the seeds is used as a diuretic (Pushpangadan *et al.*, 1993). Finally, the roots of the plant are used for bowel complaints in children (Chopra *et al.*, 1956).

#### Use in Ayurvedic medicine

O. basilicum is referred to as 'barbari' by Bhavamisra. According to Ayurveda, the plant is used for diseases caused by aggravation of Kapha and Vata while the seeds are used for pacifying aggravation of Vata and Pitta. The medicinal properties of O. basilicum are described in a number of classical Ayurvedic texts such as the Sushruta Samhita, Charaka Samhita, Ashtangahridaya, Bhavaprakasham, Danwanthari Nighandu and Kaiyadeva Nighandu (Pushpangadan et al., 1993). In Ayurveda, the whole plant is used to treat cough, asthma, bronchitis, ophthalmia, giddiness, inter-

mittent and malarial fever, catarrh, otalgia, cephalalgia, dyspepsia and spasmodic affections (Udayan and Balachandran, 2009). The plant is considered stomachic, stimulant, carminative, diaphoretic, expectorant, diuretic and antipyretic (Kirtikar and Basu, 1935).

## 4.6 Functional properties of basil

#### 4.6.1 Basil as an antioxidant

Rosmarinic acid isolated from the leaves of O. basilicum has been found to be responsible for its antioxidant activity. The nature of the antioxidant activity of rosmarinic acid in the liposome system was examined by Jayasinghe  $et\ al.\ (2003)$ : the results showed that one rosmarinic acid can capture 1.52 radicals and that there is a synergistic effect between  $\alpha$ -tocopherol and rosmarinic acid. Durga  $et\ al.\ (2009)$  showed that various concentrations (50, 100, 250 and 500 µg/ml) of acetone and ethanol extracts of O. basilicum displayed antioxidant activities, which varied by concentration. The 500 µg/ml concentration of ethanol extract displayed 75.87% activity, which is very close to the level displayed by a 500 µg/ml concentration of  $\alpha$ -tocopherol (82.14%), the reference compound. The activity of the extract increased with the increase in polar solvent, suggesting that polyphenols, flavanone and flavonoids affect the activity level. The ethanolic extract of the leaves of O. basilicum showed significant antilipid peroxidation effects in vitro, besides exhibiting significant activity in superoxide radical and nitric oxide radical scavenging in goat liver (Meera  $et\ al.\ 2009$ ).

#### 4.6.2 Basil as an antimicrobial agent

Basil has shown strong inhibitory effect against multi-drug resistant clinical isolates from the genera Staphylococcus, Enterococcus, and Pseudomonas (Opalchenova and Obreshkova, 2003). In a study by Adiguzel et al. (2005) ethanol, methanol, and hexane extracts from O. basilicum were investigated for their in vitro antimicrobial properties. The result showed that none of the three extracts tested have antifungal activities, but they do have anticandidal and antibacterial effects. Both the hexane and methanol extracts, but not the ethanol extracts, inhibited three isolates from the 23 strains of Candida albicans studied. The hexane extract showed a stronger and broader spectrum of antibacterial activity, followed by the methanol and ethanol extracts, which inhibited 10,9 and 6% of the 146 bacterial strains tested, respectively (Adiguzel et al., 2005). Chiang et al. (2005) used extracts and purified components of O. basilicum to identify possible antiviral activities against DNA viruses (herpes viruses (HSV), adenoviruses (ADV) and hepatitis B virus and RNA viruses (coxsackievirus B1 (CVB1) and enterovirus 71 (EV71)). The results showed that crude aqueous and ethanolic extracts of O. basilicum and selected purified components, namely apigenin, linalool and ursolic acid, exhibit a broad spectrum of antiviral activity. Of these compounds, ursolic acid showed the strongest activity against HSV-1, ADV-8, CVB1 and EV71, whereas apigenin showed the highest activity against HSV-2, ADV-3, hepatitis B surface antigen and hepatitis B e-antigen and linalool showed the strongest activity against ADV-II. No activity was noted for carvone, cineole,  $\beta$ -caryophyllene, farnesol, fenchone, geraniol,  $\beta$ -myrcene and  $\alpha$ -thujone (Chiang *et al.*, 2005). The essential oil from *O. basilicum* and its purified compounds, especially linalool, have antigiardial activity. Linalool (300 µg/ml) was able to kill 100% parasites after one hour of incubation, which demonstrates its high antigiardial potential (de Almeida *et al.*, 2007).

Kaya et al.'s (2008) study used the disc diffusion method to test the antimicrobial activity of chloroform, acetone and two different concentrations of methanol extracts of O. basilicum L in vitro against 10 bacteria and four yeast strains. While no effect was observed with the chloroform and acetone extracts, the methanol extracts of O. basilicum proved effective against the bacteria and yeast strains tested. Inhibition zones against strains of Pseudomonas aeruginosa, Shigella sp., Listeria monocytogenes, Staphylococcus aureus and two different strains of Escherichia coli were observed. The volatile oils of O. basilicum inhibited the growth of Klebsiella pneumoniae at a concentration of 0.51% in the agar; Streptococcus viridians and S. albus at 1.10% and P. aeruginosa at 10.0%. Proteus vulgaris was inhibited at 0.67% by O. basilicum in dental isolates (Ahonkhai et al., 2009).

Hossain et al. (2010) examined the antibacterial activity of the essential oils (10 μL/disc of 1:5, v/v dilution with methanol) and methanol extracts (300 μg/disc) of O. basilicum. The results showed that both the essential oils and methanol extracts exerted antibacterial activity against Bacillius cereus, B. subtilis, B. megaterium, S. aureus, L. monocytogenes, E. coli, Shigella boydii, S. dysenteriae, Vibrio parahaemolyticus, V. mimicus and Salmonella typhi murium. The zones of inhibition were 11.2–21.1 mm and the MIC values were 62.5–500 μg/mL. Basil oil had the strongest antimicrobial activity against Salmonella enteritidis SE3. The composition of the oil, as revealed by GC-MS analysis, was found to be: linalool (64.35%), 1,8-cineole (12.28%), eugenol (3.21%), germacrene D (2.07%), α-terpineol (1.64%) and p-cymene (1.03%). Rattanachaikunsopon and Phumkhachorn (2010) experimentally inoculated nham, a fermented pork sausage, with S. enteritidis SE3, applied basil oil and stored the product at 4°C. It was found that the oil inhibited the S. enteritidis in a dose-dependent fashion, as follows: a concentration of 50 ppm reduced the number of bacteria from 5 to 2log cfu/g after 3 days of storage; a concentration of 100 ppm resulted in an unmeasurable level of bacteria after 2 days; and a 150 ppm concentration led to unmeasurable levels of bacteria after 3 days.

A further study of the antimicrobial properties of basil oil was carried out by Edris and Farrag (2003). Linalool and eugenol, two major constituents of the oil, were tested against a number of fungi that cause deterioration and severe decay in peaches during marketing, shipping and storage, namely *Sclerotinia sclerotiorum* (Lib.), *Rhizopus stolonifer* (Ehrenb. exFr.) Vuill and *Mucor* sp. (Fisher) in a closed system. Linalool alone showed a moderate antifungal activity, while no antifungal activity was observed when eugenol alone was used. When linalool and eugenol were mixed in a ratio similar to that found in basil oil, enhanced antifungal activity was observed, indicating a synergistic effect.

#### 4.6.3 Basil as a larvicidal agent

A study was carried out on the larvicidal effect of crude CCl<sub>4</sub>, methanol and petroleum ether leaf extracts of *O. basilicum* against *Anopheles stephensi* and *Culex*  *quinquefasciatus*. Petroleum ether extract was found to be most effective against the larvae of both mosquitoes, with LC<sub>50</sub> values of 8.29, 4.57; 87.68, 47.25 ppm and LC<sub>90</sub> values of 10.06, 6.06; 129.32, 65.58 against *A. stephensi* and *C. quinquefasciatus* being observed after 24 and 48 hours of treatment, respectively. These extracts are highly toxic against mosquito larvae from a range of species (Prejwltta *et al.*, 2009).

The larval toxicity and smoke repellent potential of O. basilicum Linn. at different concentrations (2, 4, 6, 8 and 10%) against the different instar (I, II, III and IV) larvae and pupae of Aedes aegypti were also evaluated. The LC<sub>50</sub> values of O. basilicum for I instar larvae were 3.734, II instar 4.154, III instar 4.664, IV instar 5.124 (Murugan et al., 2007). A laboratory investigation using plants such as Vetiveria zizanioides (Linn.) (Poaceae), O. basilicum (Linn.) (Lamiaceae) and the microbial pesticide spinosad against the malarial vector O. stephensi Liston showed 85% mortality. The observed mortality rate suggests that the above extract can be used as a biopesticide. The LC<sub>50</sub> of second, third and fourth instar larvae of O. stephensi were 0.276%, 0.285% and 0.305%, respectively (Aarthi and Murugan, 2010).

The direct toxicity of the essential oil *O. basilicum* L. to females of six species of predacious mites of the family phytoseiidae was tested. The phytoseiid mites tested were *Typhlodromus athiasae* Porath and Swirski, *Euseius yousefi* Zaher and El-Borolossy, *Amblyseius zaheri* Yousef and El-Borolossy, *A. deleoni* (Muma and Denmark), *A. swirskii* Athias-Henriot and *A. barkeri* (Hughes). Sweet basil oil was highly toxic to females *E. yousefi* and was relatively intoxic to females *A. swirskii*. The essential oil has a toxic effect on the predator species, *T. athiasae* and *A. barkeri*. With the exception of *A. zaheri*, females of all predacious mites tested suffered a depression in reproduction and food consumption when treated with sweet basil oil at a concentration of 2 % (Momen and Ame, 2003).

#### 4.6.4 Health-promoting properties

O. basilicum has a number of beneficial effects on the cardiovascular system: it may contain polar products with the ability to lower plasma lipid concentrations (Harnafia et al., 2009), which could prove effective in the treatment of hyperlipidaemia, artherosclerosis and related diseases, which are becoming an increasing health concern in developing countries. Amrani et al. (2006) showed that O. basilicum extract displayed significantly stronger hypolipidaemic activity compared to fenofibrate treatments. O. basilicum aqueous extract displayed a very high antioxidant power (Amrani et al., 2006), and was shown to produce a \(\beta\)-adrenergic effect in albino rats (Muralidharan and Dhananjayan, 2004).

In a study by Singh *et al.* (1999b), the fixed oil of *O. basilicum* was shown to display significant anti-inflammatory activity against paw edema in rats caused by carrageenan and other mediators, including arachidonic acid and leukotriene. A significant inhibitory effect was also observed in castor oil-induced diarrhoea in rats. The fixed oil of *O. basilicum* may therefore prove to be a useful anti-inflammatory agent which, thanks to its linolenic acid contents, is able to block both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (Singh, 1998, 1999b).

Singh (1999a) showed that the fixed oil of *O. basilicum* exerted a significant anti-ulcer effect against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress-induced ulceration in experimental animal models. Aspirin-induced gastric ulcers and secretion in pylorus ligated rats were also shown to be inhibited. The anti-ulcer activity of the oil is likely to be the combined result of its lipoxygenase inhibiting, histamine antagonistic and antisecretory effects.

In a preliminary experiment, the toxic and mutagenic potential of essential oil from basil and pure substances, linalool,  $\beta$ -myrcene and 1,8-cineole, were tested using *S. typhimurium* TA98, TA100 and TA102, with and without S9 mix (microsomal fraction of rat liver). No mutagenic effect of basil derivatives was detected in any tested strain.

The antimutagenic effects of essential oil from basil and its pure constituents were further evaluated in the Ames test using *S. typhimurium* TA100. UVC irradiation and three chemical mutagens, 4-nitroquinoline-N-oxide (4NQO), 2-nitropropane (2-NP), and benzo(a)pyrene (B(a)P) were used to induce mutagenesis. All tested basil derivatives significantly reduced UV-induced mutations. The maximum inhibition was in the range of 64–77 %. In the presence of S9, EO and 1,8-cineole showed moderate inhibition of 2-NP induced mutagenesis, while the remaining two substances had no effect. Linalool exhibited a high co-mutagenic effect with B(a)P, 1,8-cineole showed moderate inhibitory effect against B(a)P-induced mutations, while EO and  $\beta$ -myrcene were ineffective (Olivera *et al.*, 2007). In a sample of 17 Thai medicinal plants, sweet basil oil had the highest antiproliferative activity with an IC(50) value of 0.0362 mg/ml (12.7 times less potent than Fluorouracil (5-FU)) in the P388 cell line (Manosroi *et al.*, 2006).

# 4.7 Quality issues and toxicity

## 4.7.1 Toxicity studies

Estragole is a natural constituent of basil oil. Several studies with oral, intraperitoneal or subcutaneous administration to CD-1 and B6C3F1 mice have shown that estragole is carcinogenic. The 1-hydroxy metabolites are stronger hepatocarcinogens than the parent compound. Controversial results are reported for the mutagenicity of estragole. However, the formation of hepatic DNA adducts *in vivo* and *in vitro* by metabolites of estragole has been demonstrated (Vincenzi *et al.*, 2000).

Smith *et al.* (2005) developed a guide for evaluating the safety of essential oils that are used as flavourings in foods, based on the chemical composition of the oil and on the extent to which it varies in the product. The guide classifies the chemically-identified constituents of the oil, biosynthesized by common pathways, into congeneric groups. Each congeneric group is evaluated and its safety judged on the basis of data on absorption, metabolism and toxicology from the members of the group. The guide further evaluates the intake of the group of unidentified constituents assuming that the essential oil is to be consumed in food, and on the basis of the toxicity data on the oil or an oil of similar chemotaxonomy (Baby and George, 2009).

## 4.7.2 Quality specifications

The physical and chemical characteristics of the oil vary depending on the geographic origin, variety, time of harvest and the plant parts used for extraction. The oil obtained from the inflorescence is of higher quality than the oil obtained from the whole plant. The physical constants of the European oil are reported by various authors in Tables 4.2 and 4.3. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) of *O. basilicum* are generally recognized as safe (GRAS) for their intended use.

Table 4.2 The physical constants of European basil oil

Light yellow
0.895-0.930
1.477-1.495
$-22^\circ$ to $-86^\circ$
3–15
0–4
1–2 parts of 80 % alcohol

**Table 4.3** EOA standards for Oil of Basil (*Ocimum basilicum*)

Property	Specifications
Preparation	By steam distillation of the flowering tops or the whole plants
Physical and chemical constants	
Appearance and odour	Light yellow liquid with a spicy odour
Specific gravity at 25 °C	0.95-0.973
Optical rotation at 25 °C	$0^{\circ}$ to $+2^{\circ}$
Refractive index at 20 °C	1.5120 to 1.5190
Acid value	Not more than 1
Saponification value	4–10
Ester value after acetylation	25–45
Descriptive characteristics Solubility	
Benzyl benzoate	Soluble in all proportions
Fixed oils	Soluble in all proportions in most fixed oils
Mineral oil	Soluble with turbidity
Propylene glycol	Soluble up to 5 % with slight haziness
Glycerine	Insoluble
Stability	
Alkali	Unstable
Acids	Unstable in the presence of strong mineral acids
Containers	Glass or aluminium containers
Storage	Store in tight full containers in a cool place protected from light

### 4.8 References

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