24 Bay Leaf

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24.1. Introduction

Bay (*Laurus nobilis* L.) leaf, or Turkish laurel, is an industrial plant used in foods, drugs and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fish (Kilic *et al.*, 2004). Bay leaves are also known as laurel leaves and are native to the Mediterranean countries. They are large, light to olive green elliptical leaves about 8 cm long and 3–4 cm wide. In 2000, Turkey exported 3600 t of *L. nobilis* leaves worth US$7.5 million (Tainter and Grenis, 1993; Kilic *et al.*, 2004). The plant is widely cultivated in Europe, America and in the Arabian countries, from Libya to Morocco (Kumar *et al.*, 2001). Extracts from these plants have great potential in protecting stored spices from *Aspergillus flavus* (Geeta and Reddy, 1990).

24.2. Botany

Laurus nobilis is an evergreen shrub or, more rarely, a tree attaining a height of 15–20 m. The smooth bark may be olive green or reddish-blue. The luxurious, evergreen leaves are alternate with short stalks, lanceolate or lanceolate oblong, acuminate, 5–8 cm or longer and 3–4 cm wide, coriaceous pellucid-punctate and with revolute, entire wavy margins (Kumar *et al.*, 2001). The leaves of *L. nobilis* are plucked and dried under shade for use as a flavouring material in a variety of culinary preparations, especially in French cuisine. The plants are important for aromatic oils and spices, edible fruits and timber (e.g. from species of the largest genus, *Ocotea*). The true laurel – that of history and classical literature – is *L. nobilis,* called also bay and sweet bay. It is native to the Mediterranean, where to the ancients it symbolized victory and merit and was sacred to Apollo. The fragrant leaves are sold commercially as bay leaf, a seasoning. American laurel is the evergreen California laurel (*Umbellularia californica*), also called pepperwood, bay tree and Oregon myrtle (Anon., 2005). Its aromatic bark is used occasionally for medicinal tea and its pulverized leaves for soup and condiments. Many of the evergreen laurels are grown as hedges and, because of their handsome foliage, are used by florists. Table 24.1 gives the nutritional composition of bay leaves per 100 g.

24.3. Chemistry

Extraction of volatiles

There are various methods of extraction of essential oils, such as steam distillation,

Table 24.1. Nutritional composition of bay leaves per 100 g.

Composition	USDA Handbook (crumbled)	ASTA
Water (g)	5.44	4.50
Food energy (Kcal)	313	410
Protein (g)	7.61	7.50
Fat (g)	8.36	8.80
Carbohydrates (g)	74.96	75.40
Ash (g)	3.62	3.70
Calcium (g)	0.83	1.00
Phosphorus (mg)	113	110
Sodium (mg)	23	20
Potassium (mg)	529	600
Iron (mg)	43.0	53.3
Thiamine (mg)	0.009	0.100
Riboflavin (mg)	0.421	0.420
Niacin (mg)	2.005	2.000
Ascorbic acid (mg)	46.53	47.00
Vitamin A activity (RE)	618	618

Source: Tainter and Grenis (1993).

supercritical extraction and microwave hydrodistillation; 83–96% recovery can be obtained by steam distillation (Borges *et al.*, 2003). A microwave-assisted hydrodistillation protocol was modified to extract essential oils from laurel leaves. The essential oils of this plant generally are obtained by hydrodistillation or steam distillation. The volatile compounds obtained by microwave-assisted hydrodistillation and hydrodistillation methods were analysed by GC and GC-MS. Both distillation methods and analytical results were compared. 1,8-Cineole (46.8–54.2%) was the main component in the leaf oil. Although the distillation was accomplished in a shorter time, the oil yields and 1,8-cineole contents were slightly higher in the microwave-assisted hydrodistillation compared with the usual hydrodistillation. Thus, microwave-assisted hydrodistillation appears to be an effective method for the production of essential oils (Kosar *et al.*, 2005).

Caredda *et al.* (2002) described the extraction conditions for leaf oil by supercritical carbon dioxide extraction as follows: pressure, 90 bar; temperature, 50°C; and carbon dioxide flow, Phi = 1.0 kg/h. Waxes were entrapped in the first separator set at 90 bar and 10°C. The oil was recovered in the second separator working at 15 bar and 10°C. The main components were 1,8-cineole (22.8%), linalool (12.5%), α-terpinyl acetate (11.4%) and methyl eugenol (8.1%). Comparison with the hydrodistilled oil did not reveal any significant difference. Collection of samples at different extraction times during supercritical extraction allowed the change of the oil composition to be monitored. Lighter compounds, such as hydrocarbon and oxygenated monoterpenes, were extracted in shorter times than the heavier hydrocarbon and oxygenated sesquiterpenes. Beis and Dunford (2006) described supercritical fluid extraction of seed oil. The oil yield of ground seeds varied from 14 to 28%, depending on the method and particle size used for oil recovery. Yields were similar for both petroleum ether and SC-CO_2 extraction. The extraction yield decreased significantly with increasing particle size. The amount of extract collected increased exponentially with increasing SC-CO_2 pressure. The highest extraction yield was obtained at the highest temperature studied, 75°C. More than 45% of the oil was lauric acid. SC-CO_2 is a viable technique to obtain high-purity *L. nobilis* L. seed oil, which is a potential ingredient for the cosmetic industry (Vasudevan *et al.*, 1997).

Constituents of essential oil

The volatiles of fresh leaves, buds, flowers and fruits were isolated by solvent extraction and analysed by capillary gas chromatography-mass spectrometry. Their odour quality was characterized by gas chromatography-olfactometry–mass spectrometry (HRGC-O-MS) and aroma extract dilution analysis (AEDA). In fresh bay leaves, 1,8-cineole was the major component, together with α-terpinyl acetate, sabinene, α-pinene, β-pinene, β-elemene, α-terpineol, linalool and eugenol. Besides 1,8-cineole and the pinenes, the main components in the flowers were α-eudesmol, β-elemene and β-caryophyllene, in the fruits (*E*)-β-ocimene and biclyclogermacrene, and

in the buds (*E*)-β-ocimene and germacrene D. The aliphatic ocimenes and farnesenes were absent in leaves. By using HRGC-O-MS, 21 odour compounds were identified in fresh leaves. Application of AEDA revealed (*Z*)-3-hexenal (fresh green), 1,8-cineole (eucalyptus), linalool (flowery), eugenol (clove), (*E*)-isoeugenol (flowery) and an unidentified compound (black pepper) with the highest flavour dilution factors (Kilic *et al.*, 2004).

Analysis of plant material from France indicated that the composition of the essential oil from the flowers showed differences compared with the essential oil from the leaves, having a high content of β-caryophyllene (10%), viridiflorene (12.2%), germacradienol (10.1%), β-elemene (9.7%) and (*E*)-ocimene (8%) (Fiorini *et al.*, 1997). Nhat *et al.* (1999) found a total of 49 compounds in the essential oil, the main constituents being 1,8-cineole [eucalyptol] (45.7%), α-terpinyl acetate (14.8%), sabinene (12.7%), α-pinene (4.8%), terpinene-4-ol (3.7%), α-terpineol (2.8%) and caryophyllene oxide (1.3%). The concentration of eugenol was lower than previously reported in the literature, which can affect the antioxidative and antimicrobial effect of the essential oil. Table 24.2 describes the percentage composition of the volatiles of the buds, flowers and fruits of *L. nobilis*. Fig. 24.1 illustrates the structures of the major volatiles of the bay leaf.

Table 24.2. Volatile compounds of buds, flowers and fruits of *L. nobilis* (%).

Compound	Bud	Flower	Fruit
Tricyclene	0.1	t	t
Thujone	0.1	0.2	0.1
α-Pinene	7.0	5.1	3.3
Camphene	3.4	2.4	1.7
Sabinene	2.4	1.7	1.7
β-Pinene	4.6	3.7	2.1
Myrcene	0.7	0.6	0.5
α-Phellandrene	t	0.1	t
Δ^3-Carene	–	0.4	–
ρ-Cymene	–	–	0.1
Limonene	t	t	t
1,8-Cineole	16.8	8.8	9.5
(*Z*)-β-Ocimene	0.1	0.3	–
Phenylacetaldehyde	0.1	–	–
(*E*)-β-Ocimene	8.1	2.7	22.1
(*E*)-Sabinene hydrate	0.1	t	t
Linalool	0.8	–	–
Pinocarvone	–	–	0.1
Borneol	0.7	0.4	0.3
α-Terpineol	–	t	0.4
Linalyl acetate	0.7	–	0.2
Bornyl acetate	2.0	2.1	1.1
2-Undecanone	–	–	0.1
δ-Terpinyl acetate	0.2	0.3	0.1
α-Terpinyl acetate	1.6	1.8	1.2
Eugenol	0.3	–	t
α-Ylangene	0.5	0.9	0.2
α-Copaene	0.2	0.3	0.1
iso-β-Elemene	0.1	0.4	0.1
β-Cubebene	–	–	t
β-Elemene	2.6	5.4	2.0
Eugenol methyl ether	0.3	–	0.1
(*E*)-β-Caryophyllene	0.9	5.1	0.3
(*E*)-Isoeugenol	0.3	0.5	0.2
α-Humulene	0.2	0.5	0.1
Alloaromadendrene	0.1	–	0.1
(*E*)-β-Farnesene	0.2	0.1	0.1
γ-Muurolene	–	–	t
GermacreneD	6.6	2.4	1.5
β-Selinene	0.1	0.3	0.1
Bicyclogermacrene	1.2	2.2	4.5
α-Farnesene	0.8	1.3	0.3
Germacrene A	0.8	1.1	0.6
γ-Cadinene	–	–	0.3
δ-Cadinene	t		0.1
Ni(sesquiterpene)	5.5	3.4	0.9
Elemol	0.4	–	–
Germacrene D-4-ol	0.7	–	–
α-Eudesmol	2.7	11.8	–
Costunolide	–	–	2.9

t = trace.
Source: Kilic *et al.* (2004).

Seasonal variation

Seasonal variations have also been observed in chemical composition. Anac (1986) studied the variation between fresh (March and June harvests) and dried (June harvest) leaves collected from mature trees in Istanbul. The respective essential oil yields were 0.86, 0.99 and 1.29%. Drying and harvest date also affected the qualitative composition; the content of the main component, 1,8-cineole, was 28.08, 40.62 and 42.70%, respectively, and the contents of the highly volatile components were somewhat higher in June than in March. A comparison with reported analyses indicated no major differ-

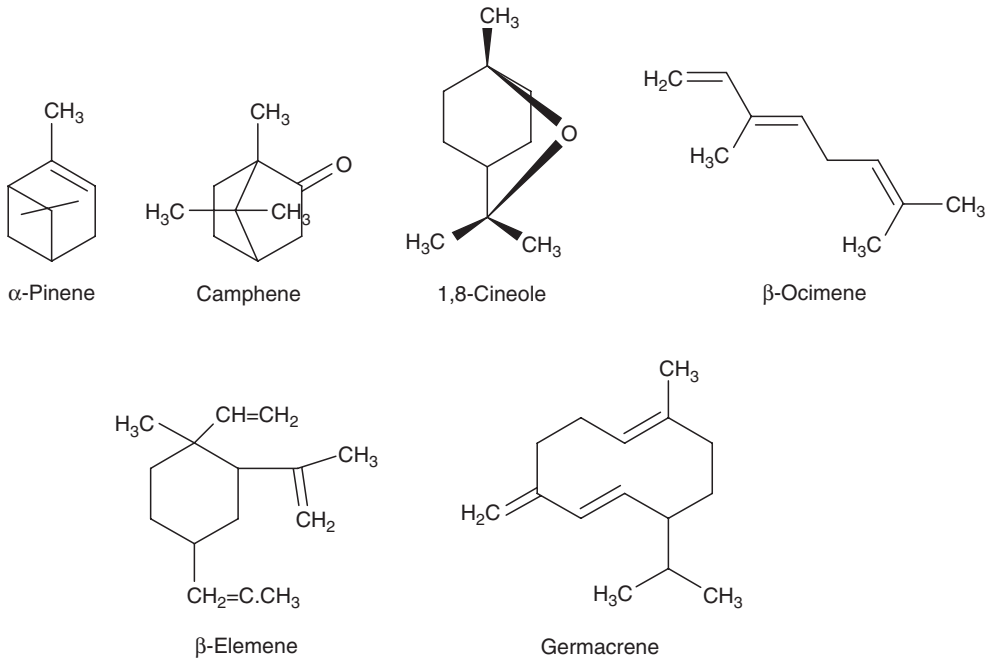

Fig. 24.1. Major volatiles in bay leaf.

ences between the Turkish oils and those normally found in commerce. Riaz *et al.* (1989) investigated the physical properties and chemical composition of essential oil extracted from *L. nobilis* leaves collected in March, July, September and November. Oil yield was lowest (0.13%) in March and highest in September (0.36%). GLC analysis showed 70 peaks corresponding to different terpenes, but only 27 of these were identified; data are reported for March, July and September samples. Cineole and eugenol were the major components in all three samples and there was very little difference in the amounts of all the components between the samples, except that linalyl acetate (a very minor component) was absent in the March samples. Kevseroglu *et al.* (2003) studied the ontogenetic and diurnal variability of laurel leaves. Leaves were collected from trees growing in Samsun (Turkey) every month from May to October, during the first 3 days of the month, in the morning (08.00 h), at noon (12.00 h) and in the evening (18.00 h). The leaves were dried in the shade for 3–4 weeks and then ground prior to essential oil analysis. Essential oil content in the leaves did not vary significantly during the day, but seasonal variation was significant. The highest essential oil percentage was found for leaves harvested in August (1.46%) and July (1.33%) and it was lowest for leaves collected during May and September (0.59 and 0.74%, respectively). There was a positive correlation between essential oil percentages in the leaves and atmospheric temperature.

The oil obtained from the dried leaves of *L. nobilis* (origin Tunisia; oil yield 1.5%) was analysed by capillary GC and GC-MS. The main components of the 24 constituents identified were 1,8-cineole [eucalyptol] (42.3%) and α-terpinyl acetate (11.2%) (Bouzouita *et al.*, 2001). Braun *et al.* (2001) identified 155 constituents: 76 monoterpenes, 46 sequiterpenoids, 10 phenylpropanoids and 23 others. δ-Terpinyl acetate was reported for the first time and was characterized using ^{1}H-, ^{13}C-NMR, GC-FTIR and GC-MS analysis.

24.4. Medicinal and Pharmacological Uses

Antimicrobial activity

Escherichia coli and many pathogens were inhibited by the essential oil of laurel (Baratta *et al.*, 1998; Friedman *et al.*, 2002; Bouzouita *et al.*, 2003; Mandeel *et al.*, 2003; Dadalioglu and Evrendilek, 2004). Trypanocidal constituents of the methanol extract of the dried leaves of *L. nobilis* L. resulted in the isolation of two guaianolides, dehydrocostus lactone (1) and zaluzanin D (2), and a new *p*-menthane hydroperoxide, (1*R*,4*S*)-1-hydroperoxy-*p*-menth-2-en-8-ol acetate (3). The minimum lethal concentrations of these compounds against epimastigotes of *Trypanosoma cruzi* were 6.3, 2.5 and 1.4 μM, respectively (Uchiyama *et al.*, 2002; Nakatani, 2003).

Inhibitors of nitric oxide production

Marino *et al.* (2005) isolated two new metabolites, 5α*H*,7α*H*-eudesman-4α, 6α,11, 12-tetraol (1) and 1*β*,15-dihydroxy-5α*H*, 7α*H*-eudesma-3,11(13)-dien-12,6 α-olide (2), from the methanolic extract of *L. nobilis* leaves (collected from spontaneous plants grown in Avellino, Campania, Italy). Their structures were determined through analysis of their one- and two-dimensional NMR spectral data (^{1}H- and ^{13}C-NMR, DEPT, COSY, HMQC, HMBC and ROESY). The relative stereochemistry was proposed based on the combined J-based configuration analysis and ROESY data. In addition, three known sesquiterpene lactones, santamarine (3), reynosin (4) and costunolide (5), along with blumenol C (6), were isolated and identified by spectral means. The isolated compounds 1–6 inhibited nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophages. The most active, compound 2, potently inhibited NO_2 release with an IC50 value of 0.8 μM. The methanolic extract of leaves of *L. nobilis* (bay leaf) inhibited nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages (Matsuda *et al.*, 2000). Through bioassay-guided separation, 14 known sesquiterpenes were isolated from the active fraction and were examined for ability to inhibit NO production. Seven sesquiterpene lactones (costunolide, dehydrocostus lactone, eremanthine, zaluzanin C, magnolialide, santamarine and spirafolide) potently inhibited LPS-induced NO production (IC50 values of 1.2–3.8 μM). Other sesquiterpene constituents also showed inhibitory activity (IC50 < or = > 21 μM). α-Methylene-γ-butyrolactone also showed inhibitory activity (IC50 = 9.6 μM), while mokko lactone and watsonol A, reductants of the α-methylene-γ-butyrolactone moiety by $NaBH_4$ or DIBAL, and a 2-mercaptoethanol adduct of dehydrocostus lactone, showed little activity (IC50 < or = > 18 μM). These results indicated that the α-methylene–γ-butyrolactone moiety was important for activity. Furthermore, costunolide and dehydrocostus lactone inhibited inducible nitric oxide synthase (iNOS) induction in accordance.

Almost complete inhibition of 3-nitrotyrosine formation (91%) was achieved with the essential oil obtained from the leaves of *L. nobilis* (at 300 μg/ml). 1,8-Cineole (eucalyptol), accounting for 50% of this essential oil, was inactive in this model, thus evidencing a major role for the minor volatile compounds present in the leaves (Matsuda *et al.*, 2000; Chericoni *et al.*, 2005).

Antifungal activity

The potential of bay leaf essential oils against species belonging to *Eurotium*, *Aspergillus* and *Penicillium* genus has been demonstrated (Geeta and Reddy, 1990; Guynot *et al.*, 2003). Biological assays showed that fungitoxicity against *Fusarium moniliforme* (*Gibberella fujikuroi*), *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phytophthora capsici* was due to different concentrations of the phenolic fraction in the essential oils (Muller Riebau *et al.*, 1995; Pandey, 1997; Pandey and Dubey, 1997).

Anticonvulsant

The leaf essential oil of *L. nobilis*, which has been used as an antiepileptic remedy in Iranian traditional medicine, was evaluated for anticonvulsant activity against experimental seizures (Sayyah *et al.*, 2002). The essential oil protected mice against tonic seizures induced by maximal electroshock and especially by pentylenetetrazole. Components responsible for this effect may be methyleugenol, eugenol and pinene present in the essential oil. At anticonvulsant doses, the essential oil produced sedation and motor impairment. This effect seems to be related in part to cineol, eugenol and methyleugenol (Sayyah *et al.*, 2002).

Insecticidal

Essential oils from laurel were evaluated for fumigant toxicity against all developmental stages of the confused flour beetle (*Tribolium confusum*). GC-MS analysis showed that 1,8-cineole was the major component of the essential oils. The vapours of laurel essential oil were toxic to all the stages of *T. confusum* (Isikber *et al.*, 2006). Repellency and toxicity of essential oil from *L. nobilis* (Lauraceae) against the rust-red flour beetle (*T. castaneum* Herbst) were also reported by Andronikashvili and Reichmuth (2003). The toxicity of ethanol extracts from *L. nobilis* on the large diamondback moth, *Plutella xylostella*, was 55% (Erturk *et al.*, 2004).

The behavioural responses of adult female western flower thrips, *Frankliniella occidentalis*, to volatiles from meadowsweet (*Filipendula ulmaria*), bay laurel and sage (*Salvia officinalis*) were investigated in laboratory bioassays by Chermenskaya *et al.* (2001). Volatiles collected by entrainment of a solvent extract of *F. ulmaria* were more attractive than was the original extract. *F. occidentalis* also was attracted significantly to volatiles from *L. nobilis* and *S. officinalis*. Analysis by gas chromatography and mass spectrometry identified 1,8-cineole (eucalyptol) as one of the main volatile components of all three plant species. In coupled gas chromatography–electroantennography studies with *F. ulmaria*, both 1,8-cineole and methyl salicylate elicited responses from *F. occidentalis*. Eucarvone was identified as the major component of *F. ulmaria* volatiles, but showed no electrophysiological activity. The behavioural responses of thrips to a range of concentrations of 1,8-cineole and methyl salicylate were tested using a modified Pettersson 'star' olfactometer. 1,8-Cineole showed some attractant activity for the thrips at 0.01 mg, but methyl salicylate was repellent at all the concentrations tested.

The bruchid, *Acanthoscelides obtectus*, is one of the most damaging pests of kidney beans (*Phaseolus vulgaris*) worldwide. However, aromatic plants from the families Lamiaceae, Lauraceae, Myrtaceae and Poaceae can protect *P. vulgaris* by a direct or delayed insecticidal effect, through increased adult mortality and inhibition of reproduction (both oviposition and adult emergence). The insecticidal effect is due to the presence of factors other than those in the essential oils as there is no significant difference between the efficacy of distilled and intact plant extracts. Inhibition of reproduction is particularly important. The results suggest that lipid, as well as non-lipid allelochemicals, such as phenolics, or non-protein amino acids or flavonoids may be involved in the toxicity of extracts of aromatic plants to *A. obtectus* (Regnault Roger and Hamraoui, 1995; Mackeen *et al.*, 1997).

24.5. International Standards

Tables 24.3 and 24.4 describe the physical and chemical specifications for whole bay leaves and ground leaves. The minimum volatile oil content required for whole leaves is 1.5% and 1.0% for ground leaves.

24.6. Conclusion

The bay leaf belongs to the family Lauraceae and is one of the most popular culinary spices in the West. The bay leaf has been used as

Table 24.3. Whole bay (laurel leaves): chemical and physical specifications.

Specification	Suggested limits
ASTA cleanliness specifications	
Whole dead insects, by count	2
Mammalian excreta (mg/lb)	1
Other excreta (mg/lb)	10.0
Mould, % by weight	2.00
Insect defiled/infested, % by weight	2.50
Extraneous, % by weight	0.50
Detectable action levels	
Mouldy pieces by weight (av. %)	5
Insect-infested pieces by weight (av. %)	5
Mammalian excreta, after processing (mg/lb, av.)	1
Volatile oil (% min.)	1.5
Moisture (% max.)	9.0
Ash (% max.)	4.0
Acid-insoluble ash (% max.)	0.8

Source: Tainter and Grenis (1993).

Table 24.4. Ground bay: chemical and physical specifications.

Specification	Suggested limits
Detectable action levels	None
Volatile oil (% min.)	1.0
Moisture (% max.)	9.0
Total ash (% max.)	4.0
Acid-insoluble ash (% max.)	0.8
Military specifications	
Volatile oil (ml/100 g) (% min.)	1.0
Moisture (% max.)	7.0
Total ash (% max.)	4.5
Acid-insoluble ash (% max.)	0.5
Granulation (% min. through a USS No. 30)	95
Bulk index (mg/100 g)	220

Source: Tainter and Grenis (1993).

a herbal medicine and has pharmaceutical activity which includes antibacterial, antifungal, antidiabetic and anti-inflammatory effects. In fresh bay leaves, 1,8 cineole is the major aroma constituent. Other compounds of interest are α-terpinyl acetate, sabinene, α-pinene, β-pinene, β-elemene, α-terpineol, linalool and eugenol. The flowers and fruits also possess aroma. Season of harvest and time of harvest influence the aroma constituents. Considering the wide-ranging medicinal property, bay leaves need more attention in future.

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