

# Chemistry and Biological Activities of Essential Oils from *Melaleuca* L. Species

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## Summary

Essential oils from species *Melaleuca* genus, especially *M. alternifolia* (Maiden & Betche) Cheel, have been widely used worldwide in various industries. This review is a contribution to *Melaleuca* knowledge and describes five important essential oil-producing species and two subspecies of *Melaleuca* in terms of their essential oil chemical composition, medicinal applications, and leaf morphoanatomy. Some relationships between essential oil composition of these species and important biological activities are presented. Useful parameters for the certification of the essential oils are also highlighted.

## Key words

*Melaleuca*, Myrtaceae, volatile oils, biological activities, leaf morphoanatomy

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## Introduction

### The Myrtaceae family

Myrtaceae family, included in the Myrtales Order (sensu APG, 2003), has about 130 genera and approximately 3800–5800 species of predominantly tropical and subtropical distribution, being concentrated in the Neotropics and Australia (Wilson et al., 2001). In Australia members of the family are distributed in warm tropics and temperate Australia (Simpson, 2006).

Traditionally, Myrtaceae was classified into two subfamilies (with several tribes): Leptospermoideae, with fruits usually a capsule and leaves spiral or opposite, and Myrtoideae, with fleshy fruits and leaves always opposite. However, based on phylogenetic studies (based upon cpDNA *matK* sequences) family Myrtaceae was subdivided into two new subfamilies circumscription: Myrtoideae, with 15 tribes and Psiloxylloideae (the new subfamily) with only two tribes (Wilson et al., 2005). Therefore, all *Melaleuca* representatives are currently included in the subfamily Myrtoideae, in the Melaleuceae tribe.

Within the Myrtales order, the Myrtaceae family stands out for its economic importance. It includes important timber trees, especially *Eucalyptus* spp., oils (e.g., *Eucalyptus* spp., *Melaleuca* spp.), and cultivated ornamentals such as *Callistemon* (bottlebrush), *Chamaelucium* (wax-flower), *Eucalyptus* spp., *Leptospermum* (tea tree), and *Myrtus* (myrtle) (Simpson, 2006). The fleshy-fruited species include many economically important food plants, agricultural crops, and ornamentals, including the Mediterranean genus *Myrtus* (myrtle), spices such as clove (*Syzygium aromaticum* /L./ Merr. & L.M. Perry), all spice (*Pimenta dioica* /L./ Merr.), and bay rum (*Pimenta racemosa* / Mill./ J. W. Moore), and the fruits of *Psidium* (guavas), *Myrciaria*, *Eugenia*, *Syzygium*, *Plinia* and *Luma* (Reynertson et al., 2008).

The Myrtaceae family is known to possess leaves with high concentrations of terpenes and considerable qualitative and quantitative variation in the types of terpenes, according to taxonomic identity and population and individual levels (Keszei et al., 2008). These variations have pharmacological potential and many industrial applications. Among the pharmacognostic studies developed on the family, the following stand out: phytotherapeutic potential of leaves of *Pepper pseudocaryophyllus*, species occurring in the Brazilian cerrado (Paula et al., 2005); antidiabetic properties of *Syzygium alternifolium*, *Eugenia jambola* and *Eugenia puniceifolia* (Sridhar et al., 2005; Brunetti et al., 2006). Besides, edible fruits in the 14 Myrtaceae species are a rich source of biologically active phenolic compounds (Reynertson et al., 2008). Several species have foliar volatile oil used industrially, eg *Eucalyptus*, *Leptospermum*, and specially *Melaleuca* and related genera.

### The *Melaleuca* genus

*Melaleuca* L. is an aromatic and medicinal plant genus, best known for the production of medicinal essential oils. The *Melaleuca* species are generally found in open forest, woodland or shrubland, particularly along water courses and the edges of swamps (Sciarrone et al., 2010). The *Melaleuca* genus belongs to the Melaleuceae tribe, subfamily Myrtoideae (Wilson et al., 2001), and predominantly occurs in Australia. It comprises approximately 230 species of worldwide occurrence (Craven and Lepschi, 1999), with 220 species endemic to Australia and Tasmania, but

also occurring in Indonesia and New Papua Guinea (Craven, 1999). The genus was recognized in 1767 by Linnaeus, with a single species called *M. leucadendra*. In 1873–1867, Bentham published the classic *Flora Australiensis* describing 97 *Melaleuca* species in seven series and creating an artificial group. Following this publication, several other species were reported in the genus. Since the 1960s, several investigations enumerating all the species occurring in Australia have been carried out (Craven, 1999).

The *Melaleuca* genus is characterized as possessing tree or shrub individuals; leaves spiral, decussate or ternate, small to medium-size, the venation pinnate to parallel; flowers in spikes or clusters or sometimes solitary, the basic floral unit being a monad, dyad or triad; sepals 5, rarely 0; petals 5; hypanthium fused to the ovary in the proximal region only; stamens few to numerous; the filaments fused for part of their length into 5 bundles, the anthers dorsifixed, rarely basifixed, and versatile, with two parallel cells that open via longitudinal slits; ovary 3-celled, the ovules few to numerous; fruit a capsule, with an usually woody to subwoody fruiting hypanthium; seeds with a thin testa, generally obovoid-oblong to obovoid, unwinged, cotyledons planoconvex to obovulate (Craven, 1999).

The genus *Melaleuca* also contains hundreds of individual species with a myriad of oil constituents present in the leaf (Brophy and Doran, 2004). Volatile compounds of great economic importance can be found in the species of this genus. Moreover, leaves and stem of several *Melaleuca* species are source of essential oils with strong aroma for medicinal application, with potential use for cancer treatment (Bagg et al., 2006; Garozzo et al., 2011). Commercially useful essential oils are sourced from the broadleaved *M. quinquenervia* (niaouli oil) and *M. cajuputi* (cajuput oil) and the small-leaved *M. alternifolia*-*M. linariifolia* complex (Southwell and Lowe, 1999).

Numerous *Melaleuca* species are naturally found in poorly drained or seasonally flooded regions and in acid soils. They have been utilized for reforestation in Vietnam, in areas deemed unsuitable for agriculture, as well as areas marked by seasonal flooding, acidic soils or a high incidence of fires during the dry season. Because of these characteristics, some *Melaleuca* species are considered as invasive plants (Doran and Gunn, 1994). However, just because of the characteristics mentioned above, species of *Melaleuca* are cultivated in various parts of the world, for commercial purposes, given the importance of its essential oils, which are used in various industries, as discussed below. Southwell and Russell (2002) call attention to the fact that a successful commercialization of essential oils of *Melaleuca* species (eg *M. alternifolia*), depends on choosing the right chemical variety for plantation establishment.

## Chemical composition and medicinal use

### *Melaleuca alternifolia*

*Melaleuca alternifolia* (Maiden & Betche) Cheel is described as one of the most important essential oil-producing species. The trees or shrubs of *M. alternifolia* can reach 2 to 30 m in height, and they grow in riparian watercourses and swamps (Lee et al., 2002). The volatile oil constituents of *M. alternifolia* and closely related species are the compounds responsible for the commercial development of *Melaleuca* as a medicinal and aromatic plant (Southwell and Lowe, 1999). Its oil has strong

**Table 1.** Percentage of the major components in the volatile oil of five *M. alternifolia* chemotypes

| Compound                   | Percentage                   |
|----------------------------|------------------------------|
| 1,8-cineole                | > 8<br>30-45<br>50-64        |
| Terpinolene: terpinen-4-ol | 28-57 :1-2<br>10-18 : 15- 20 |

antimicrobial activity and low toxicity. It has deep penetrating power, eliminating subcutaneous infections and promoting fast healing (Carson et al., 2006; Hammer et al., 2006). *M. alternifolia* essential oil has been thoroughly studied and produced on a commercial scale, with Australia being one of its largest producers. It is obtained by leaf steam distillation and is known as Tea Tree Oil (TTO). The main components of *M. alternifolia* essential oil are the monoterpenes terpinen-4-ol, 1,8-cineole and terpinolene (Shelton et al., 2004). The TTO contains about 100 compounds, of these, terpinen-4-ol is the major antimicrobial component, causing structural damages in cell walls and membranes of bacteria and fungi and disrupting cell integrity (Halcon, 2004). The broad-spectrum antimicrobial activity of TTO is mainly attributed to terpinen-4-ol and 1,8-cineole, major components of the oil, and includes antibacterial, antifungal, antiviral, antiprotozoal and antimycoplasmal activities, all promoting TTO as therapeutic agent (Furneri et al., 2006). Five chemotypes were described according to the concentrations of 1,8-cineole and terpinolene/terpinen-4-ol ratio (Penfold et al., 1948; Butcher and Doran, 1994; Keszei et al., 2010) (Table 1). The antiseptic activity attributed to *M. alternifolia* oil depends on the levels of terpinen-4-ol (Shelton et al., 2004).

The compound 1,8-cineole has been reported to cause skin irritation even in quantities lower than 10%. As a consequence, oils with levels of 1,8-cineole below 7% are preferred for cosmetics production (Caboi et al., 2002). These results guided the determination of the International Standard Organization (ISO 4370), establishing for the commercial oil of *M. alternifolia* minimum terpinen-4-ol levels of 30% and maximum 1,8-cineole levels of 15% (ISO, 1996).

Studies carried out in Brazil described chemotypes with low concentrations of 1,8-cineole (1.8-3.5%) even in specimens of *M. alternifolia* subjected to different levels of water stress (Silva et al., 2002). In another investigation, the variations in the concentrations of main components of essential oil samples from different harvest times were determined. Low concentration of 1,8-cineole were also found (0.3 to 1.3%), and greater quantities of terpinen-4-ol (49.8 to 53.5%) (Silva et al., 2003).

Some terpenes present in TTO can alter cell permeability by their insertion between the fatty acid chains of the lipidic bilayer of biological membranes, changing their nature, structure and function (Sikkema et al., 1995). Such alterations increase the fluidity, interfering with the membrane permeability (Bard et al., 1988), which leads to loss of cytoplasmic contents (Carson et al., 2002). TTO can also indirectly affect the cell respiration, as it causes damage to mitochondria membranes (Cox et al., 2000). The ultra-structural analysis of bacteria treated with TTO showed damages to the plasmatic membrane that can be recognized by

**Table 2.** Uses and activities described for Tea Tree Oil (TTO)

| Use  | Reference   |
|--|---|
| Treatment of human melanoma  | Calabrini et al., 2004; Giordani et al., 2006   |
| Anti-inflammatory activity   | Brand et al., 2001; Caldefie-Chezet et al., 2006                                      |
| Antimicrobial activity   | Silva et al., 2003; Carson et al., 2006; D'Arrigo et al., 2010; Mondello et al., 2003 |
| Fungicide  | Oliva et al., 2003; Bagg et al., 2006; Hammer et al., 1998                            |
| Acaricide  | Iori et al., 2005   |
| Insecticide  | Callander and James, 2012   |
| Treatment of insect bites and skin infections                              | Budhiraja et al., 1999  |
| Treatment of subcutaneous infections caused by fungi                       | Nielsen e Nielsen, 2006; Wendy et al., 2007   |
| Activity against herpes simplex virus (HSV), causal agent of labial herpes | Carson et al., 1998   |
| Cosmetic industry  | Riedl, 1997   |
| Dandruff treatment   | Satchell et al., 2002   |
| Treatment of oral and genital candidiasis                                  | Jandourek et al., 1998; Mondello et al., 2003   |
| Bacterial respiration inhibitor  | Cox et al., 2000  |
| Antiviral  | Schnitzler et al., 2001; Minami et al., 2003  |
| Treatment of acne  | Carson et al., 1998   |
| Treatment of methicillin -resistant <i>Staphylococcus aureus</i>           | Caelli et al., 2000   |
| Healing activity   | Boland et al., 1984   |
| Activity against protozoa  | Mikus et al., 2000  |

the alterations in the mesosome and loss of cytoplasmic content (Gustafson et al., 1998).

Some sesquiterpenes can also inhibit the action of mitochondrial ATPase in yeasts, adversely affecting respiration (Lunde et al., 2000). However, the different components of *M. alternifolia* essential oils have different mechanisms of fungicidal action yet to be elucidated (Hammer et al., 2004). Studies have reinforced the hypothesis that TTO also acts on the plasmatic membrane inducing potassium loss and inhibiting respiration (Cox et al., 2000). Loss of intracellular material, inability to maintain homeostasis and inhibition of respiration following treatment with TTO is consistent with the mechanism of action that involves the loss of membrane integrity and function (Giordani et al., 2006). Because of the efficiency of this mechanism at the cellular level, TTO represents itself as an effective alternative for treating several diseases, in addition to its usefulness by hygiene and a sepsis related industries (Table 2).

### *Melaleuca armillaris*

*Melaleuca armillaris* (Sol. ex Gaertn.) Sm. is the most widely cultivated species of the genus *Melaleuca*. It is commonly known as the Bracelet Honey Myrtle and grows into large spreading shrub or small tree (Hayouni et al., 2008). The trees of this species, which can reach up to 5 m in height, grow in rocky and very shallow soils with low water retention capacity (Doran, 1994). As a consequence, this species exhibits good tolerance to drought. Chemical investigations of *M. armillaris* are scarce, particu-

**Table 3.** Main components of the volatile oils of *M. armillaris* from different origin

| Origin  | 1,8-cineole (%) | terpinene-4-ol (%) |
|---|-----------------|--------------------|
| Brazil (Silva, 2007)                                  | 80.2            | 1.0                |
| Australia (Aboutabl et al., 1991; Farag et al., 2004) | 33.9-43.7       | 18.7               |
| Egypt (Aboutabl et al., 1991)                         | 33.7            | 24.8               |
| Tunisia (Hayouni et al., 2008)                        | 68.9%           | -                  |

larly on essential oil chemical composition. Despite the few reports on the essential oil chemical composition of *M. armillaris* (Table 3), it can be seen that 1,8-cineole corresponds to the major component, with concentrations varying from 33.7% to 80.2%.

The chemical variability observed in Table 3 can be the result of plant environmental conditions. Factors such as temperature, relative humidity, total duration of sunlight exposition and wind regime, among others greatly affect the production and the chemical composition of essential oils (Simões and Sptizer, 2000).

A study conducted with samples of essential oils extracted from 42 Australian plant species identified only six with potent insecticide activity and among them *M. armillaris*. The observed insecticidal activity was attributed to 1,8-cineole, present in the essential oil with 42.7% (Lee et al., 2004). Besides the insecticidal action, this compound also displays anti-inflammatory activity for being likely to inhibit the cyclooxygenase pathway, preventing prostanoid synthesis and consequently reducing symptoms of inflammatory diseases (Dewhirst, 1980). In Germany, 1,8-cineole was registered and licensed as a medicinal product and it is sold in the form of 100 mg capsules for treatment of acute and chronic bronchitis, sinusitis and respiratory infections (Juergens et al., 2003). This terpene has therefore great therapeutic potential for treating respiratory and inflammatory diseases (Juergens et al., 2004). It is also present in the essential oil of leaves of several *Eucalyptus* species (Myrtaceae), *Eucalyptus citriodora* (55%), *Eucalyptus globulus* (71%), *Eucalyptus punctata* (66%), *Eucalyptus maculata* (51%), *Eucalyptus globules* subsp. *maidenii* (70%) and *Eucalyptus smithii* (84%) (Chalchat et al., 1997). The presence of high concentrations of 1,8-cineole in volatile oils of *Melaleuca* species suggests that they can be used as an alternative source of this compound.

#### *Melaleuca ericifolia*

*Melaleuca ericifolia* Sm. is mainly native to Australia and coastal areas of Tasmania, occurring in flooded areas, showing tolerance to this condition (Chalchat et al., 1997). It can reach up to 20 m in height, forming a dense crown. The linear leaves are acicular, and dark-green. The literature reports the occurrence of three chemotypes in this species based on the proportions of 1,8-cineole, linalol and methyleugenol (Table 4).

The *M. ericifolia* essential oil has shown to possess antimicrobial, antifungal and antiviral activities, as well as antioxidant properties (Farag et al., 2004). The leaf extracts of this species showed bactericidal activity against gram-positive and gram-negative bacteria, e.g. *Staphylococcus aureus* (Hussein et al., 2007). These results indicate the pharmacological potential

**Table 4.** Main components of the volatile oils of *M. ericifolia* from different origin

|                                    | 1,8-cineole | linalool | methyleugenol |
|------------------------------------|-------------|----------|---------------|
| Brazil (Silva et al., 2007)        | 79.5        | 60       | 96.8          |
| Australia (Brophy and Doran, 2004) | 34.5        | -        | -             |

of this species. However, further studies are necessary to find the occurrence of other chemotypes and their possible pharmacological uses.

#### *Melaleuca cajuputi*, subspecies *cajuputi* and subspecies *platyphylla*

*M. cajuputi* subspecies *cajuputi* is found in Northern Australia, Papua-New Guinea, Indonesia, Thailand and Vietnam (Doran, 1999) while *M. cajuputi* subspecies *platyphylla* Barlow occurs in northern Queensland, southwestern Papua-New Guinea and southeastern Irian Jaya. Trees of these species can typically reach up to 25 m in height. Depending on the situation, however, they can reach 40 m in height or even can be found as shrubs. They grow in marshy soils, drain lines and in seasonally flooded soils, but they can also occur in dry, rocky and infertile soils (Turnbull, 1986). This species is well adapted to flooded areas, tolerating even salt-water flooding. They have been utilized as ornamental and for beekeeping (Penfold, 1948).

*M. cajuputi* subspecies *cajuputi* is the main source of cajuput oil, which is widely used in the folk medicine of Southeastern Asia (Doran, 1999). Three chemotypes were identified for this species coming from Indonesia and Japan. One chemotype presents high concentration (50-70%) of 1,8-cineole, the other shows low concentration (31%) of it, and another chemotype does not show the presence of this terpene (Sakasegawa, 2003). In Brazil, a chemotype with concentration of 1,8-cineol was indentified (44%) (Silva et al., 2007). The economic value of this essential oil is directly related to the levels of this terpene. In Indonesia, oils with concentration of 1,8-cineole above 55% are considered of first quality, and bellow this value, they are considered standard oils (Sakasegawa, 2003). Cajuput oil is used for easing headache, toothache, rheumatism, convulsions and as insect repellent (Ogata, 1969).

In Brazil, a comparative study carried out on the essential oil chemical composition of *M. cajuputi* subsp. *cajuputi* and subsp. *platyphylla* indicated that the monoterpene 1,8-cineole is the major component in both subspecies, followed by the oxygenated monoterpene terpineol (22.6%) in subspecies *cajuputi* and citronelol (15.2%) in subspecies *platyphylla*. The volatile oils of subspecies *cajuputi* also presented pinene (2.8%) and viridiflorol (13.3%) (Silva et al., 2007). Analyses of essential oil profiles are used as a tool for biosystematic and chemotaxonomic studies. Studies on the chemical composition of essential oils of five *Hypericum* (Clusiaceae) species native to Greece, proposed a phylogenetic reconstruction that confirms the existent taxonomic divisions for this genus (Panos et al., 2005). Another study carried out with five species of the genus *Psidia* (Asteraceae) with a very complex taxonomy based mainly on morphological traits,



provided important information on the composition of the essential oil, which can be used as a chemotaxonomic tool for characterizing some *Psidia* species (Gauvin et al., 2005). However, regarding the aforementioned subspecies of *Melaleuca*, a thorough study using populations from different locations, as well as the use of several samples from different conditions is required before making any inference on the use of essential oil profiles as a taxonomic tool for confirmation of their degree of kinship.

### *Melaleuca leucadendra*

*Melaleuca leucadendra* (L.) L. species are distributed between northern Australia and southern New Guinea, also occurring in Indonesia. The trees, which can reach 22 m to 40 m in height and diameter up to 1.5 m, grow in plain lands along rivers, coasts or seasonal swamps, in loamy and sandy soils (Boland et al., 1984). They are tolerant to acid, infertile and marshy soils. Formation of adventitious roots is observed when they grow in flooded areas. The trees also present good fire tolerance (Turner et al., 1984).

Four chemotypes were reported for *M. leucadendra*. One contains 1,8-cineole (64.3%) as the main component (Aboutabl et al., 1991); two are characterized by high methyleugenol and *E*-methylisoeugenol concentrations (up to 99% and 88% respectively) (Brophy, 1988); and the last shows viridiflorol (28.2%) and 1,8-cineole (21.3%) as essential oil major components. In Brazil, the occurrence of a chemotype with high concentrations of methyleugenol (96.6%) was reported (Silva et al., 2007). Because of the high concentration of this compound, when compared to other species such as *Ocimum selloi* (65.5%) (Martins et al., 1997), *Ocimum gratissimum* (46.8%) (Vostrowsky et al., 1990) and *Thapsia maxima* (59.6%) (Avato et al., 1991), this chemotype can be considered a promising source of methyleugenol. This compound has a slight eugenol aroma with many industrial applications in perfume composition and as a food aromatizer (Guenther, 1972). The important biological properties reported for this compound is the capacity to attract fruit fly males, genus *Bractocera* (Diptera: Tephritidae), therefore showing potential for use in biological control as bait (Shelly, 2001). It is also used for medicinal purposes, having anticonvulsant, anaesthetic, analgesic and muscle-relaxing properties (Dallmeier et al., 1981). It can be, however, cytotoxic and genotoxic as well (Burkey et al., 2000). Further studies are needed to establish the risk-benefit ratio for the use of this oil.

The 1,8-cineole-rich chemotype can have the same pharmacological applications previously discussed for *Melaleuca* species rich in this compound.

### *Melaleuca quinquenervia*

*M. quinquenervia* (Cav.) S. T. Blake is known as a weed. It was introduced to southern Florida largely from Australia early in the 20th century and it has become one of the world's worst woody weeds (Padovan et al., 2010). This species is well adapted to marshy soils, also occurring in sandy soils that can become black in color due to the presence of organic material. Its trees can reach 8 to 12 m in height. *M. quinquenervia* grows from the western coast to northern Australia, and is likewise native to New Caledonia, New Guinea and Indonesia (Boland et al., 1984). A number of chemotypes are described for this species occurring in Madagascar, Australia, New Guinea, New Caledonia

**Table 5.** Origin and major components of the volatile oils of *M. quinquenervia* chemotypes. Bold numbers in parenthesis correspond to different chemotypes described in the literature

| Origin   | Chemotypes  |
|--|---|
| Madagascar (Ramanoelina et al., 2008)                        | (1): 37% 1,8-cineole; (2): 20% viridiflorol and 5% terpinolene; (3): viridiflorol (48%) (4): ( <i>E</i> -nerolidol (87%)  |
| Australia and New Guinea (Wheeler et al., 2007)              | (5): <i>E</i> -nerolidol (74-95%) and linalol (14-30%), (6): 1,8-cineole (10-75%), viridiflorol (13-66%), $\alpha$ -terpineol (0.5-14%) and <i>E</i> -caryophyllene (0.5-28%), in varying proportions |
| New Caledonia (Trilles et al., 2006)                         | (7): 1,8-cineole (up to 76%); (8): derived from terpinene; (9): derived from $\alpha$ -pinene and viridiflorol  |
| Brazil (Silva et al., 2007)                                  | (10): viridiflorol (71%)  |
| Florida, the Caribbean and Hawaii (Ramanoelina et al., 2008) | (11): ( <i>E</i> -nerolidol and viridiflorol  |

and Brazil, mostly based on the proportions of 1,8-cineole, viridiflorol and *E*-nerolidol (Table 5).

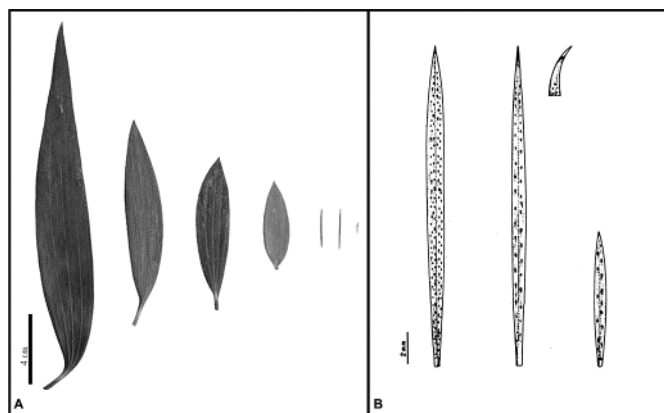
*M. quinquenervia* can be a source of 1,8-cineole-rich essential oil called Niaouli oil, which is used in pharmaceutical preparations for the relief of coughs and colds, rheumatism and neuralgia and in aromatherapy (Elliot and Jones, 1993). The compounds *E*-nerolidol and linalol have widespread use in the perfume industry (Ireland et al., 2002). Besides, linalol has been tested as an acaricide (Prates et al., 1998), bactericide and fungicide (Belaiche et al., 1996). It has been successfully used in medicine as a sedative (Sugawara et al., 1998). *Oxyops vitiosa* larvae fed with leaves of an *E*-nerolidol-rich chemotype had increased mortality and decreased biomass gains, demonstrating its possible insecticide effect (Dray et al., 2004). There are studies reported in the literature on the moderate fungicidal activity of this compound (Gijsen et al., 1992).

## Leaf morphoanatomy

### Morphological description

On this topic some data obtained from morphoanatomical studies carried out in Brazil with seven species produced on a commercial scale are presented (Silva, 2007).

The *Melaleuca* species described in this paper present alternate leaves showing entire margin and parallelodromous venation (Figure 1A), characteristics that were already reported in the literature on the genus (Boland et al., 1994). Leaves of *M. alternifolia*, *M. armillaris* and *M. ericifolia* are sessile and linear with acute apex and base. They can be differentiated by the size and distribution of the glands. These characteristics are useful for identifying sterile material, and can be seen even in the herborized material contributing to quality control of oil extraction and correct species identification, particularly in the form of leaf fragments (Figure 1B). The other species are petiolated with coriaceous leaves. *M. leucadendra* presents adult leaves more or less lanceolate, with five longitudinal veins; *M. quinquenervia* has lanceolate to oblanceolate hard leaves, with five longitudinal veins (rarely 3 or 6), with other less distinct veins



**Figure 1.** A: General aspects of leaves of *Melaleuca* species. From left to right: *M. leucadendra*; *M. quinquenervia*; *M. cajuputi*, subspecies *cajuputi*; *M. cajuputi*, subspecies *platyphylla*; *M. alternifolia*; *M. armillaris* and *M. ericifolia*. B: Camera lucida drawing of leaves of *M. alternifolia*; *M. armillaris* and *M. ericifolia* (respectively, from left to right). Notice the gland distribution pattern: random in *M. alternifolia* and linear in the other species. Also notice the curved apex, typical to *M. armillaris* and the reduced leaf size of *M. ericifolia* compared with the other species.

and little visible glands. *M. cajuputi* subsp. *cajuputi* has adult leaves with tector trichomes and 3-5 veins. Leaves of *M. cajuputi* subsp. *platyphylla* have similar characteristics of subspecies *cajuputi*, however with a short petiole, which is also found in *M. dealbata*, *M. leucadendra* and *M. preissiana* (Boland et al., 1994).

In a recent investigation the anatomical characterization of colleters representative of Myrtoideae, including several *Melaleuca* species have been described. In this study three new types of colleters with potential application in studies of phylogenetic relationships within the Myrtaceae were identified (Silva et al., 2012).

### Anatomical description

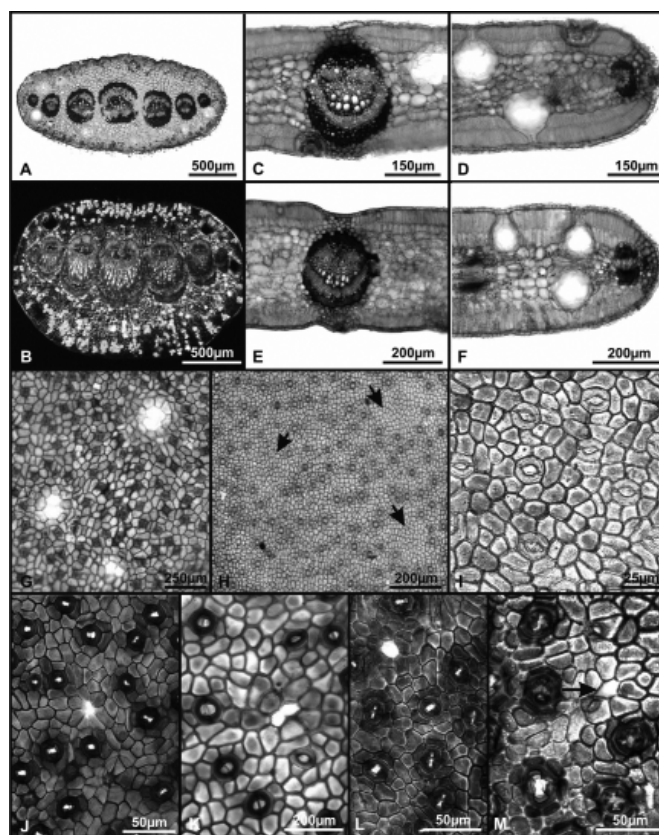
**Petiole.** The petiole is anatomically similar in all the studied petiolated species (Table 6). The perivascular fiber sheath surrounding the petiole, of variable thickness, depending on the species, has been reported in the Myrtaceae family (Solereder, 1908; Howard, 1979). In these species, the petiole presents no distinctive character for species delimitation. It can be used, however, as a unifying character for the *Melaleuca* genus.

**Leaf blade.** The characteristics observed in the analysis of leaf blade cross-sections and front views are summarized in Table 7 and 8 respectively.

**Stomata.** Anomocytic or paracytic stomata are present on both leaf epidermal surfaces of the Myrtaceae family (Solereder, 1908; Metcalfe and Chalk, 1979). However other stoma types such as anemostaurocytic, paracytic and cyclocytic can be present, but some authors do not consider them as a good diagnostic character for some genera of the Myrtaceae family (Fontenelle et al., 1994; Arruda et al., 1994). In these species, the occurrence of anomocytic stomata is a constant character and can be considered a unifying element for the genus *Melaleuca*.

**Table 6.** Petiole characteristics of *M. cajuputi* subsp. *cajuputi*; *M. cajuputi* subsp. *platyphylla*; *M. leucadendra*; *M. quinquenervia*

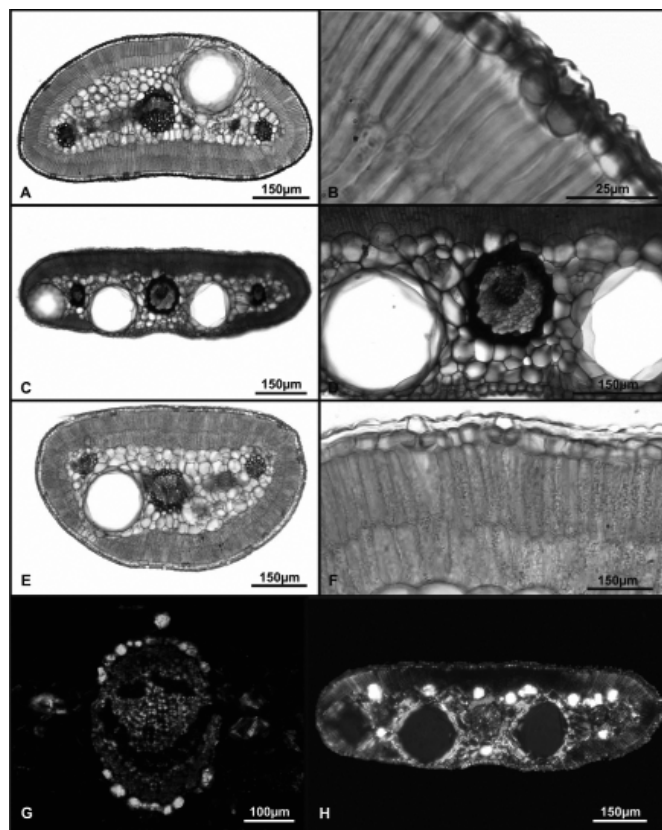
| Structure           | Characteristic   |
|---------------------|--|
| Epidermis           | Uniseriate   |
| Cortical parenchyma | Occurrence of secretory cavities and crystal idioblasts containing druses (Figure 2, A and B).   |
| Vascular system     | Consisting of a set of approximately seven bicollateral vascular bundles with smaller caliber in the extremities. The bundles are partially surrounded by dense fibers forming a cap in the adaxial surface (Figure 2, A). |



**Figure 2.** A-B: petiole cross-sections. A: *M. cajuputi* subsp. *cajuputi*.; B: *M. leucadendra* (polarized light showing crystal idioblasts containing druses). C-F: leaf blade cross-sections of *Melaleuca* species. C-D: *M. cajuputi* subsp. *cajuputi*; E-F: *M. leucadendra*. G-M: Epidermal dissociation showing epidermal cell wall thickening with straight to sinuate outline and the covering cells of the secretory cavity (arrows). G: *M. alternifolia*; H: *M. armillaris*; I: *M. ericifolia*; J: *M. cajuputi* subsp. *cajuputi*; K: *M. leucadendra*; L: *M. cajuputi* subsp. *platyphylla*; M: *M. quinquenervia*.

**Mesophyll arrangement.** The leaves of the seven *Melaleuca* species are isobilateral with compact arrangement. These characteristics are usually found in vertical leaves, common in the Myrtaceae family (Fontenelle et al., 1994). Compact mesophyll

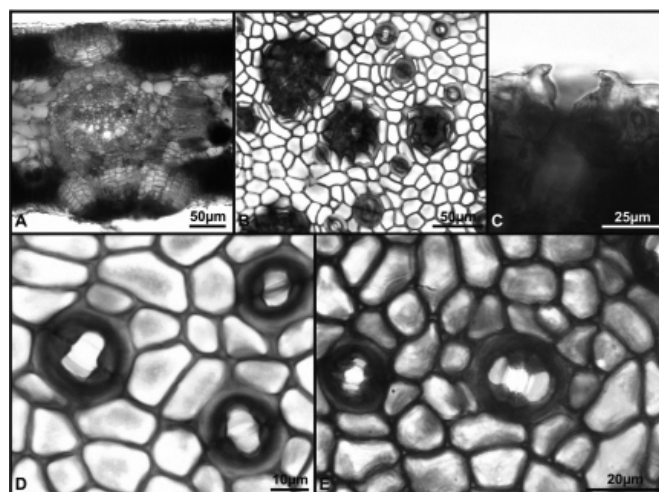




**Figure 3.** A-F: leaf cross-sections of *Melaleuca* species. A-B: *M. ericifolia*; C-D: *M. alternifolia*; E-F: *M. armillaris*. G-H: cross-sections under polarized light showing crystal idioblasts containing druses, monocrystals and crystal sheath surrounding vascular bundles. D: *M. cajuputi* subsp. *platyphylla*. E: *M. alternifolia*.

and reduced intercellular space volume are xeromorphic characteristics that can reflect xeric environmental conditions (Fahn and Cutler, 1992). Compaction of mesophyll can be a structural reinforcement, since the photosynthetic performance is not always higher in cells of the palisade parenchyma. Because of this mechanical strength, when the volume of spongy parenchyma is smaller than the palisade parenchyma, the stiffness of leaves may be less impacted by tissue dehydration (James et al., 1999). Another factor to be considered is the excess luminosity that plants are exposed to in tropical regions. Some authors attribute the presence of xeromorphic character in tropical plants to this factor (Dias et al., 2007). The presence of a thicker cuticle, thicker palisade parenchyma and more compact spongy parenchyma, and a higher degree of lignified structures in the mesophyll, as was found in the studied species, is reported in the literature as a response to the excess luminosity (Boeger et al., 2003).

**Secretory cavities.** The leaf secretory system of these species consists of numerous cavities near the epidermis and in the interface between palisade and spongy parenchyma (Figures 2 and 3). The secretory cavities in Myrtaceae have schizogenous origin (Solereider, 1908; Fahn, 1979); however, they can be schizolysig-



**Figure 4.** A-B: lenticel-like structures in leaf mesophyll of *Melaleuca*. A: *Melaleuca quinquenervia* (cross-sections). B: *M. leucadendra* (epidermal dissociation). C-E: detail of the thick cuticle forming an epistomatic ridge. C: *M. leucadendra*: (cross section). Front view: D: *M. quinquenervia* (front view). E: *M. cajuputi* subsp. *platyphylla* (front view).

enous as observed in *M. alternifolia* (List et al., 1995). The type and the position of secretory cavities in leaves, as well as the type of secretory epithelium were used to study the taxonomic relations of the order Myrtales. In this study, the occurrence of cavities in the 14 studied families of this order was rare, being found only in the families Myrtaceae and Psiloxilaceae and characterized by the presence of a secretory epithelium with a single cell layer (Keating et al., 1984).

**Vascular system.** Vascular bundles are bicollateral in all the species. The presence of bicollateral vascular bundles surrounded by fibers and parenchyma cells was already reported in the Myrtaceae family (Solereider, 1908). *M. alternifolia* and *M. ericifolia* showed distinct midrib only in the cross section, being identified by the medium trace of larger caliber (Figure 3, A, C, D, E). There is a tendency for replacing the midrib model by the alternate arrangement of ribs with larger and smaller caliber in the other species.

The midrib may be not well defined in individuals belonging to the order Myrtales (Keating et al., 1984). However, there is no reference to species lacking the midrib, suggesting for the Myrtaceae, the hypothesis of the midrib pattern specialization that could be confirmed by a deeper analysis of other species in the family. In general, this hypothesis suggests that a semicircular trace could have evolved in two directions: i) reduction of a wide trace to a narrow arc of conducting tissue; ii) the change from a recurved trace to an adaxially flattened trace (Fahn, 1990). This second tendency is found in *M. ericifolia* and *M. alternifolia*, in which we observe an adaxially recurved trace, and flattened trace with absence of midrib and an alternate bundle pattern of larger and smaller caliber in the other species (Figure 2, C-F and Figure 3, A, C, D and E).

**Crystal idioblasts.** Crystal idioblasts were lacking only in *M. ericifolia*. The other species showed idioblasts containing druses and/or monocrystals (Table 7). The type and distribution of crystals can constitute diagnostic characters for taxonomic studies (Metcalf and Chalk, 1975), being commonly found in the Myrtaceae family (Dietz et al., 1988).

**Leaf margin.** The sinuate leaf margin with interrupted palisade parenchyma, which is replaced by a group of collenchyma cells (Figure 2, C-F) as described for the genus *Melaleuca* (Dietz et al., 1988), seems not to be a universal characteristic, since it was not found in *M. alternifolia*, *M. armillaris* and *M. ericifolia*. The epidermal cells from this region are slightly conical and anticlinally elongated, with the cuticle thicker than the leaf surface, which is accentuated in *M. quinquenervia*.

**Lenticel-like structures.** The anatomical study on the leaves of individuals of these five species and two subspecies grown in Brazil on a commercial scale in non-flooded areas, showed the

presence of suberized areas in the leaf epidermis. Lenticels usually occur on the periderm of stems, roots and fruits (Mauseth et al., 1988) and are related with the aeration of the internal tissues (Metcalf and Chalk, 1950). In the leaves, however, the presence of such structures is considered rare. Similar structures were reported in representatives of Myrtaceae (Neish et al., 1995), such as *Eucalyptus incrassata* and *E. laevopinea* (Morretes et al., 1985), and *Tripodanthus acutifolius* of the Loranthaceae family (Larson et al., 1989). Warts of suberized tissues were reported in leaves of *Eucalyptus calophylla*, *E. globulus*, *E. gunii*, *E. megacarpa*, *E. oblique*, *E. siderophloia*, and *Acmena floribunda* (Solleder, 1908). There is no consensus in relation to the proper denomination used to describe the suberized areas present in the leaf blade (Larson et al., 1989), especially because these structures, although similar to lenticels, occur in the epidermis. Up to now, the probable function of lenticel-like structures in the leaves is unknown. Several authors affirm that in plants of flooded areas, besides stomata closure (Marschner, 1995), there is production of

**Table 7.** Characteristics of leaf cross sections of *Melaleuca* species

| Characteristic        | Occurrence   | Leaf blade - cross section  |  |
|-----------------------|--|---|--|
|                       |  | Description   |  |
| Stomata distribution  | All species  | Amphistomatic. Except for <i>M. alternifolia</i> , all the other species showed thick cuticular ridge covering the guard cells and forming an epistomatic chamber over the external atrium of the ostiole (Figure 3, B and F; Figure 4, C-E). |  |
| Type of stomata       | All species  | Anomocytic (Figure 2, I-M).   |  |
| Epidermis             | All species  | Uniseriate (Figure 2, C-F).   |  |
| Mesophyll arrangement | All species  | Compact isobilateral, spongy parenchyma presents variable number of cell layers (4-8) (Figure 2, C-F).  |  |
| Vascular system       | All species  | Bicollateral vascular bundles in all species ((Figure 2, C-F; Figure 3, D).   |  |
|                       | <i>M. alternifolia</i> , <i>M. armillaris</i> and <i>M. ericifolia</i>   | Occurrence of midrib of larger caliber between two ribs of smaller caliber, showing the same midrib structural organization (Figure 3, A, C and E).   |  |
|                       | Other species  | Alternated arrangement between ribs of larger and smaller calibers  |  |
| Crystal idioblasts    | <i>M. ericifolia</i>   | Lacking   |  |
|                       | <i>M. alternifolia</i>   | Containing druses   |  |
|                       | <i>M. armillaris</i> and <i>M. leucadendra</i>   | Containing druses and monocrystals.   |  |
|                       | <i>M. cajuputi cajuputi</i> , <i>M. cajuputi platyphylla</i> and <i>M. quinquenervia</i>                         | Containing monocrystals   |  |
|                       | <i>M. cajuputi cajuputi</i> , <i>M. cajuputi platyphylla</i> , <i>M. quinquenervia</i> and <i>M. leucadendra</i> | Druses involving the fiber sheath that surrounds the vascular bundles (Figure 3, G).  |  |
| Leaf margin           | All species, except for <i>M. alternifolia</i> , <i>M. armillaris</i> and <i>M. ericifolia</i> .                 | Sinuate with interrupted palisade parenchyma replaced by a group of collenchyma cells (Figure 2, C-F).  |  |

**Table 8.** Leaf characteristics of *Melaleuca* species, in front view

| Characteristic                        | Occurrence   | Leaf blade – front view   |  |
|---------------------------------------|--|---|--|
|                                       |  | Description   |  |
| Covering cells of secretory cavities  | <i>M. alternifolia</i> and <i>M. leucadendra</i>   | Occurring in pairs  |  |
|                                       | <i>M. cajuputi</i> subsp. <i>cajuputi</i> , <i>M. cajuputi</i> subsp. <i>platyphylla</i> and <i>M. quinquenervia</i>                         | Occurring separately  |  |
|                                       | <i>M. ericifolia</i> and <i>M. armillaris</i>  | Covering cells not present in the cavities, but instead distinct regions on the abaxial surface of the epidermis without the occurrence of stomata (Figure 2, H and I). |  |
| Cuticular ridge covering guards cells | <i>M. alternifolia</i>   | Lacking   |  |
|                                       | Other species  | Thick, forming an epistomatic chamber over the external atrium of the ostiole (Figure 3, B and G; Figure 4, C-E).   |  |
| Epidermal alterations                 | <i>M. cajuputi</i> subsp. <i>cajuputi</i> , <i>M. cajuputi</i> subsp. <i>platyphylla</i> , <i>M. quinquenervia</i> and <i>M. leucadendra</i> | Petioled species showed structures similar to lenticels on both epidermal surfaces (Figure 4, A and B).   |  |



**Table 9.** Occurrence of *Melaleuca* species in their natural habitat

| Species   | Soil Type  | Reference                               |
|---|--|---|
| <i>M. quinquenervia</i>   | Better development in marshy soils, but can occur in sandy soils.  | Boland et al., 1984                     |
| <i>M. alternifolia</i>  | Riparian zones of freshwater and swamps.   | Lee et al., 2002                        |
| <i>M. cajuputi</i> , subsp. <i>cajuputi</i> and subsp. <i>platyphylla</i> | Marshy soils, drainage lines and in flooded soils for six or more months of the year. It can also occur in areas of dry, rocky and infertile soil. Adapted to flooded areas, tolerating even saltwater flooding. | Turnbull, 1986; Doran e Gunn, 1994      |
| <i>M. ericifolia</i>  | Flooded areas, tolerance to flooding.  | Ladiges et al., 1981                    |
| <i>M. armillaris</i>  | Rocky and very shallow soils, with small water retention capacity, high tolerance to flooding.   | Doran e Gum, 1994                       |
| <i>M. leucadendra</i>   | River plains, coasts or seasonal swamps, in clay, sandy soils, tolerance to acid, infertile and marshy soils, adventitious root in flooded areas, good tolerance to fire.  | (Boland et al., 1984; Doran e Gum, 1994 |

phytotoxic compounds that accumulate in the leaves, stems and roots (Kelsey, 1996). Evidence indicates that such compounds are quickly transported by the plants to sites with higher oxygen rates where they are then metabolized (Kelsey, 1996). The lenticel hypertrophy of stems and roots could, therefore, aid in eliminating potentially toxic compounds in plants under conditions of root anaerobiosis (Dickison, 2000).

A large comparative study of *Melaleuca* species occurring in their natural environment and other dry soil environments is necessary to establish a correlation between these structures and genetic and/or environmental factors.

#### Xeromorphic characters

Despite the fact that the studied species naturally occur in flooded environments (Table 9), several xeromorphic characters were observed: leaf reduction in *M. alternifolia*, *M. ericifolia* and *M. armillaris*. The formation of small linear leaves is associated with reduction of the leaf surface, which contributes to minimization of water loss (Larcher, 2000). All the species presented the following characteristics: i) compact isobilateral mesophyll, with two layers of palisade parenchyma; ii) thick cuticle constituting a barrier against excessive water loss, protection against microorganism invasion and UV radiation (Larcher, 2000); iii) presence of epistomatic ridges. Stomata in xerophytes are frequently sunken in crypts or depressions or are surrounded by cuticular projections or small waxy sticks for protection against the excessive water and gas losses (Turner, 1995; Larcher, 2000); iv) sclerenchyma tissue associated with vascular bundles. In xerophytes there is an increase in the proportion of mechanical strength tissues and lignified walls. This can be related to the deficiency of some nutrients in the soil (Larcher, 2000).

Plants growing in soils with low water retention and low nutrients have leaves with xeromorphic characteristics, even if they belong to tropical forest environments (Cao, 2000). These species are classified as sclerophyte, a controversial term, meaning "hard leaves" (Edwards, 2000). This concept is, however, applied more when relating sclerophytes with seasonal water shortages, low nutrient levels in the soil, defences against herbivores or protection mechanisms of leaf longevity (Vahl, 1991).

Despite the increase in nutrient availability in flooded areas, the formation of short-chain organic acids (Agostinetto, 2001) damages the root system (Camargo, 2001), which impairs nutrient absorption. One can then suppose that the xeromorphic characters of *Melaleuca* species could be the result of poor nutrient absorption leading to scleromorphism. On the other hand, such characteristics can also be a response of the plant to non-flood-

ed environments, invalidating the hypothesis of low degree of genetic plasticity in these species. As it is not always possible to distinguish whether the xeromorphic characters are hereditary or plant responses to environmental factors, and since we have not found any study in the literature on the anatomy of these species occurring in their natural environment until now, the suggestions posed in this work could only be confirmed by comparative studies of *Melaleuca* species occurring in their natural environment and in other areas.

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