

Chemical Variability and Biological Activities of Volatile Oils from *Hyptis suaveolens* (L.) Poit.

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Summary

Hyptis suaveolens (L.) Poit. belongs to the *Lamiaceae* family and is widely used in folk medicine in various countries. The essential oils from *H. suaveolens* have been extensively investigated and are mainly composed of monoterpenes and sesquiterpenes, although significant diterpene content has been reported in recent studies. The survey of the literature concerning *H. suaveolens* essential oils revealed a high level of chemical variability in terms of quantity and composition that is commonly observed for volatile oils from other plant species. However, few researchers have dealt with the reasons for such chemical variability. Our research group has been investigating the relationships between growing conditions of the plants and the *H. suaveolens* (L.) Poit. essential oil composition. The results of these investigations have led to some advances in the characterization and knowledge of *H. suaveolens* chemotypes from Brazil. Nevertheless, since this species presents high level of genetic polymorphism and allows it to adapt to the alterations in environmental features resulting in interpopulational and intrapopulational variability in the volatile oil chemical compositions. Consequently, biochemical assays on the biosynthetic pathway are required in order to detect the molecular mechanisms involved in inducing differential terpenoid biosynthesis within *H. suaveolens*. These are some of the challenges which require resolution leading to an understanding of the complex secondary metabolism of this species, thereby making possible the volatile oil chemical standardization seeking productivity and phytotherapy.

Key words

Hyptis suaveolens (L.) Poit., essential oil, chemical variability

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Introduction

The *Lamiaceae* family comprises 250 genus and approximately 6970 species. The members of the family are spread all over the world, especially in the tropic and subtropical regions. The family grows abundantly in the Mediterranean areas, where it is possible to find the vast majority of the genus constituents. Many species of the *Lamiaceae* family are used in cooking due to the flavour and taste. Besides that, the members of the family are a resource of aromatic essential oils as well as ornamental plants. Phytochemical investigations conducted within *Lamiaceae* species have identified several classes of secondary metabolites derived from the acetate and shikimate pathways as well as small molecules from mixed biosynthesis (Falcão and Menezes, 2003).

The genus *Hyptis*, belonging to the *Lamiaceae* family, consists of approximately 400 species that occur naturally in tropical and subtropical regions: Nigeria, Thailand, India and the southern part of the United States to Argentina. A broad spectrum of pharmacological activities including tumorigenic, anti-fertility and mycotoxic has been reported. In addition, ethnopharmacological reports of empirical therapeutic usefulness have also been documented (Falcão and Menezes, 2003). More than 25 species of the *Hyptis* genus have been subjected to chemical investigations, resulting in the isolation of several classes of compounds (Silva et al., 2000; Falcão and Menezes, 2003).

Among the several *Hyptis* species, *H. suaveolens* has mostly been investigated. This plant is known in Brazil as bamburral, cheirosa and erva-canudo; in Mexico as chía-de-colima, chía gorda, chía grande, conibare and goyohuali (Wulff, 1973) and in El Salvador as chichinguaste (Grassi et al., 2005). It is an aggressive, annual, weedy species that can reach 2 m in height forming dense clumps along roadsides and in the wet margins of ponds, as well as in over-grazed pastures. Originally native to tropical America, *H. suaveolens* is nowadays considered a weed of worldwide distribution. It is commonly found in places where soils have been profoundly disturbed and it causes severe harvest losses in several crops in Brazil, especially along the Brazilian Southeast region. However, it is mostly confined at altitudes below 500 m (Azevedo et al., 2001, 2002).

Taxonomy and botanical aspects

From the botanical point of view, *H. suaveolens* is an herbaceous plant with opposing crossed leaves and entire blades. The semi-woody quadrangular stems are abundantly branched laterally. The flowers are small and clustered into axillary inflorescences, hermaphrodite, pentamer, strongly zygomorphic and bilabiate (Joly, 1998). Investigations carried out on *H. suaveolens* flowering concluded that this species is a short day plant, with critical photoperiod of approximately 13 h. It also possesses a putative *Antirrhinum* and *Arabidopsis* gene *LEAFY* homolog that finely controls the flowering process, particularly with relation to cellular signalling meristematic proliferation (Martins and Polo, 2009). By *in situ* hybridization experiments, it has been verified that the floral expression pattern of this gene in *H. suaveolens* is similar to that reported in *Antirrhinum* and *Arabidopsis*, although it was also expressed in vegetative apices from plants grown within a natural photoperiod. Nevertheless, under extended natural photoperiod (16 h) this gene is not expressed in the vegetative meristem, in agreement with the flow-

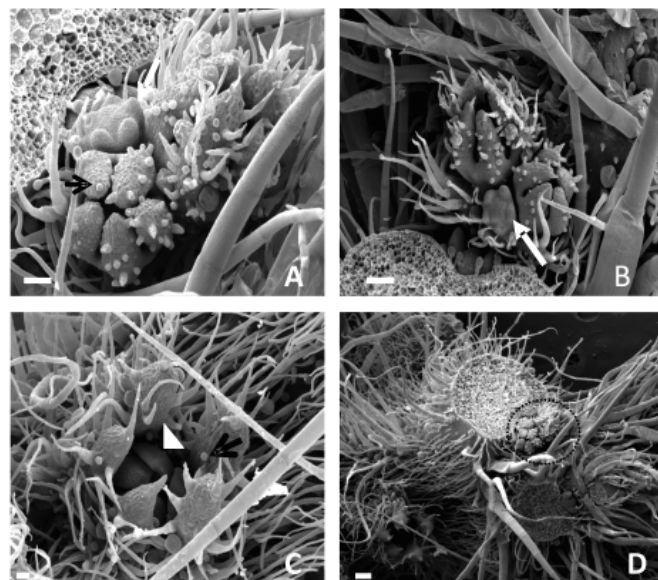


Figure 1 - Scanning electron micrographs (SEM) of *H. suaveolens* floral apices. A and B - initial differentiation of sepals in the floral meristem flanks (white triangle) recovering internal meristematic regions. C - differentiated petals (white triangle) recovering internal meristematic regions. D - the flowers are clustered into axillary inflorescences (circulated). White arrows: sepals origin as five protrusions in the floral meristem flanks. Black arrow: glandular trichomes. Bars: A - 20 μm ; B and C - 30 μm ; D - 100 μm .

ering inhibition under such photoperiodic conditions. Figure 1 shows photomicrographs obtained by scanning electron microscopy, where it can be seen that meristematic differentiation occurs centripetally in the flowers. In this figure, the glandular trichomes can also be observed (Martins and Polo, 2009). These epidermic structures store the essential oils, which contain an aromatic mixture of volatile compounds that imbue a characteristic mint smell to the plant when crushed (Chukwujekwu et al., 2005).

A report on the leaf anatomy of plants from the subtribe *Hypidiinae* (*Lamiaceae*) has been published (Rudall, 1980). A detailed study conducted by Silva (2000) on the anatomy of the aerial parts of *H. suaveolens* has shown the presence of three types of glandular trichomes on the leaves: the peltate glandular trichome, constituted of one basal cell, one unicellular large peduncle and a secretory large round head comprised of four cells; the peltate glandular subsessile trichome, formed by one unicellular base, a very short stalk cell and round head formed by one or two cells; and the capitate glandular trichome, constituted of a stalk formed by two or three cells, a round head formed by one, two or four cells and a multicellular base. Glandular trichomes were also found in the stems. Non-glandular multicellular trichomes were also observed on the leaves of *H. suaveolens* (Silva, 2000).

Phytochemical analysis

The important biological activities associated with *H. suaveolens* (*vide infra*) prompted several research groups to investi-

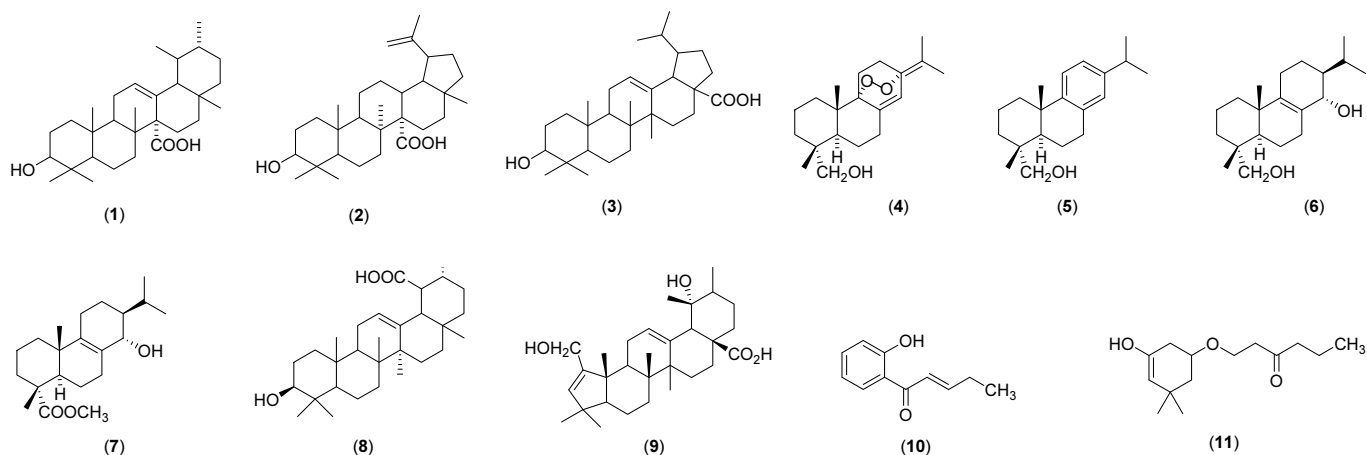


Figure 2 – Structures of some compounds isolated from *H. suaveolens*: peltoboykinoli acid (1); 3 β -hydroxylup-20(29)-en-27-oic acid (2); 3 β -hydroxylup-12-en-28-oic acid (3); 13 α -epi-dioxiabiet-8(14)-en-18-ol (4); dehydroabietinol (5); suavelol (6); methyl suaveolate (7); urs-12-en-3 β -ol-29-oic acid (8); hyptadienic acid (9); (2*E*)-1-(2-hydroxyphenyl)pent-2-en-1-one (10) and 1-[(3-hydroxy-5,5-dimethylcyclohex-3-en-yl)oxy]hexane-3-one (11).

gate the chemical composition of roots, aerial parts and flowers of this species.

It was noticed that the benzene extract of *H. suaveolens* roots prevented the growth of the pathogenic fungus *Helminthosporium oryzae*. A phytochemical investigation of the chemical composition of this extract (Misra et al., 1981) resulted in the isolation of β -sitosterol, oleanolic acid, and the triterpene peltoboykinoli acid (1) (Figure 2). The antifungal activity of the isolated acid was evaluated against *Helminthosporium oryzae*, and the inhibition of mycelial growth was not significant (lower than 10% at 1000 ppm). Other investigations of the roots extracts have led to isolation of the triterpenes 3 β -hydroxylup-20(29)-en-27-oic acid (2) (Misra et al., 1983a) and 3 β -hydroxylup-12-en-28-oic acid (3) (Misra et al., 1983b) (Figure 2).

Bioactivity-guided fractionation of the petroleum ether extract of air-dried leaves of *H. suaveolens* led to the isolation of an abietane-type endoperoxide, 13 α -epi-dioxiabiet-8(14)-en-18-ol (4) (Figure 2). This compound displayed high antiplasmodial activity ($IC_{50} = 0.11 \mu\text{g/mL}$) against a chloroquine-sensitive strain of *Plasmodium falciparum* (Chukwujekwu et al., 2005). It is interesting to notice that compound 4 presented a 70-fold enhancement in terms of antimalarial activity when compared with dehydroabietinol (5), a compound that was also isolated from *H. suaveolens* (Ziegler et al., 2002). This difference was attributed to the presence of the endoperoxide moiety in the structure of compound 4, which is in agreement with several reports in the literature that show the relationship between the antimalarial activity of several synthetic compounds and the presence of endoperoxide functionality in these compounds (Barbosa et al., 1992, 1996; Dond et al., 2005).

Two structurally similar diterpenes, namely suavelol (6) and methyl suaveolate (7), were isolated from the leaves of *H. suaveolens* (Grassi et al., 2006). The compounds were evaluated as inhibitors of croton oil-induced dermatitis of the mouse ear. The experimental results revealed that compounds 6 and 7 possess nearly the same dose-dependent topical anti-inflammatory ac-

tivity. As pointed out by the authors of this investigation, this result explains the rational use of *H. suaveolens* extracts in dermatologic disorders (Grassi et al., 2006).

From the petroleum ether extract of the *H. suaveolens* aerial parts, a pentacyclic triterpene (8) was also isolated (Mukherjee et al., 1984) and the A-ring contracted triterpene (9) was obtained and represents the first example of a compound presenting skeletal type outside the lupine series (Rao et al., 1990). The compounds hentriacontane, friedelin, netricontanone, lupeol acetate and lupeol were isolated from the benzene extract of air-dried and powdered leaves and floral parts of *H. suaveolens* (Saluja and Santani, 1984).

In the study conducted by Raja et al. (2005), the ethyl acetate extract of *H. suaveolens* leaves was fractionated *via* silica gel column chromatography. The fractions obtained were evaluated for antifeedant, oviposition deterrent, ovicidal and larvicidal activity against lepidopteran pests, *Helicoverpa armigera* and *Spodoptera litura*. The maximum antifeedant and ovicidal activity observed during the biological essays were due to compounds (2*E*)-1-(2-hydroxyphenyl)pent-2-en-1-one (10) and 1-[(3-hydroxy-5,5-dimethylcyclohex-3-en-yl)oxy]hexane-3-one (11) (Raja et al., 2005). Although spectroscopic data was not provided, the structure proposed for compound 11 seems to be unreasonable as the six-membered ring was drawn in the enol form of cyclohexanone. In such cases the amount of the enol form in equilibrium with the ketone form is very low (Carey and Sundberg, 2000).

Alongside the studies described above, the essential oils of *H. suaveolens* have also been submitted to several investigations as described herein.

The volatile oils from *Hyptis suaveolens*

The essential oils from *H. suaveolens* have been extensively investigated and are mainly composed of monoterpenes and sesquiterpenes, although significant diterpene proportions have been reported (Martins et al., 2006, 2007). A great variation in

quantity and chemical composition of the volatile oils from *H. suaveolens* is reported in the literature (Table 1), but few researchers have investigated in detail the reasons for such variability (Martins et al., 2006, 2007). In a few cases, inquiries about environmental influences on the chemical composition are made, but in most of the studies the authors attribute the volatile oil chemical variability to the geographic origin or to the genetic charge of the plants investigated. Major compounds identified in volatile oils of *H. suaveolens* from several countries are presented in Table 1.

Three studies carried out on plants of *H. suaveolens* originating from Brazilian Savanna reported a pattern of geographic variation in the essential oil composition. In these studies, it was observed that monoterpene hydrocarbons are mainly produced by plants growing in sampling sites located at higher latitudes and altitudes, whereas sesquiterpenes are produced by plant samples grown at lower ones (Azevedo et al., 2001, 2002; Oliveira et al., 2005).

In contrast to the results obtained with plants from the Brazilian Savanna, the sesquiterpenes were found in minor amounts within the volatile oils of plants from El Salvador and no correlation has been observed between the altitude and the compositional data (Grassi et al., 2005). Furthermore, the essential oils from plants growing at the highest altitudes (above 400 m) were rich in oxygenated monoterpenes (fenchone 15%, and fenchol 8.6%), and the concentration profile of all components was similar to that found in essential oils from plants growing at about 200 m. In El Salvador, the existence of at least three chemotypes was found: the fenchone-fenchol chemotype, the most common and homogenous in the coastal south of El Salvador, the 1,8-cineole chemotype from northern areas of El Salvador and the α -terpinolene from eastern and southeastern El Salvador, also known as 'Oriente' (Grassi et al., 2005). The authors have also observed similarities in the composition between volatile oils from plants collected in different sites and different regions. On the other hand, significant differences in the composition of the essential oils were observed in plants from the same sampling site. The authors concluded that, based on the volatile oil compositions only 56% of the plants could be correctly assigned to their particular geographic collection site. This intrapopulation variability has been rationalized in terms of the accumulation of certain compounds, such as 1,8-cineole (Grassi et al., 2005). Indeed, the results obtained in a rather small country like El Salvador, allows one to question whether the chemical variability in the *H. suaveolens* volatile oils is related exclusively to their geographic origin.

The occurrence of many *H. suaveolens* chemotypes is well documented and some of them are more frequently observed (Table 1). The 1,8-cineole chemotype is the most common (Luz et al., 1984; Lawrence, 1989; Fun and Svendsen, 1990; Mallavarapu et al., 1993; Ahmed, et al., 1994; Azevedo et al., 2001, 2002; Grassi et al., 2005; Oliveira et al., 2005), followed by sabinene (Nayak et al, 1952; Pant et al., 1992; Sidibe et al., 2001; Azevedo et al., 2001, 2002; Eshilokun et al., 2005; Oliveira et al., 2005; Tchoumboungang et al., 2005), β -caryophyllene (Din et al., 1988; Iwu et al., 1990; Azevedo et al., 2002; Malele et al., 2003; Oliveira et al., 2005) and fenchone chemotype (Flores and Medina, 1970; Grassi et al., 2005). The chemical differences that distinguish

the chemotypes are probably related to genotypic features of the plant populations investigated in each case, considering a missing genetic exchange among the plants from different collecting areas. However, it should be taken into account the environmental influences of each geographic area on the expression of the genotypic features that would result in different chemotypes. This fact is more relevant when there is intrapopulation variability in the essential oil composition. Nevertheless, such data is mostly unavailable for *H. suaveolens*, but some efforts were recently started in this direction. For instance, the fenchone-fenchol chemotype from El Salvador (Grassi et al., 2005), typical for south and southeast areas near the Pacific coast, has been cultivated in the northern parts of this country and the essential oil chemical composition has not changed significantly, in comparison with the same plants growing in south areas. This suggests that environmental factors do not play a decisive role in the biosynthesis of the volatile oil constituents (Grassi et al., 2005).

In the study carried out to identify the ground for the variability in essential oil composition of *H. suaveolens*, edaphic factors and growth phases (vegetative, flowering and fruiting stage) have been shown to present a significant correlation with the essential oil composition (Oliveira et al., 2005). The amounts of the sesquiterpenes β -elemene, (*E*)-caryophyllene, germacrene D and bicyclgermacrene in the oils were significantly correlated to aluminium, hydrogen+aluminium content and base saturation. The correlations were determined in fruit samples collected at lower latitudes (mean value S 14°30') in the Brazilian Savanna. The monoterpene hydrocarbons *o*-cymene, limonene and δ -terpinene contents were strongly related to chemical balance in soils (P, Zn, Cu, Mn, base saturation and neutral pH), which is also correlated to the growth phase of the plant (vegetative and flowering sampling) at higher latitudes (mean value S 20°1'). Investigations conducted with other plant species have also shown that the content and composition of volatile oils may change according to the environmental conditions (Economakis et al., 2002; Karioti et al., 2003; Rodrigues et al., 2004).

In order to contribute to further characterization and knowledge of *H. suaveolens* chemotypes, investigation on the influence of growth conditions on their volatile oil content and composition was carried out (Martins et al., 2006, 2007). For such purpose, homozygous plants from an Alfenas (Minas Gerais state, Brazil) lineage were cultivated in the greenhouse under certain growth conditions: the amounts of macronutrients nitrogen, phosphorus and potassium and the photoperiod. The influence of plant age on the volatile oils composition was also evaluated. The highest contents of volatile oils were found in the treatments where the plants were cultivated under nitrogen deficiency (0.33-0.41% w/w) (Martins et al, 2007) or nitrogen, phosphorus and potassium deficiency (0.41-0.45% w/w) (Martins et al., 2006). These results, in both cases, were observed during the second harvest (135-day-old plants) and regardless of the photoperiod condition (natural photoperiod and natural photoperiod extended for 4 h by a 40 W glowing lamp). In general, 135-day-old plants showed higher contents of the essential oils in comparison to 60-day-old plants (mean values 0.31% and 0.18% w/w, respectively).

In terms of essential oil composition, the oxygenated sesquiterpenes (16.85-50.88%) and sesquiterpene hydrocarbons (5.11-27.03%) were the most detected groups in the *H. suaveolens*

Table 1. Selected *H. suaveolens* essential oil characteristics

Geographic origin	Major components	Main terpenoid classes	Comment	Reference
Alfnas (Brazil), plants grown in greenhouse	Spathulenol (3.48-24.70%) Globulol (4.14-14.85%) Dehydroabietol (2.23-16.80%)	OS (16.85-50.88%) SH (5.11-27.03%) OD (3.92-27.22%)	Intrapopulation chemical variability according to nitrogen, phosphorus and potassium availability, plant age and photoperiod.	Martins et al., 2007
Alfnas (Brazil), plants grown in agronomic field	(<i>E</i>)-Caryophyllene (16.93-34.75%) -Elemene (11.21-24.31%) Caryophyllene oxide (2.96-15.62%) Germacrene D (0.96-14.47%)	SH (40.27-66.70%) OS (11.62-39.16%)	Strong compositional differences when compared to plants cultivated in greenhouse and influences of fertilization, interspecific competition and plant age.	Martins et al., 2006
Nigeria	Sabinene (13.2%-30.0%) α -Pinene (1.8%-13.6%) <i>p</i> -Cymene (1.2%-11.7%) Terpinen-4-ol (9.8%-11.4%)	MH (53.9-56.3%) OM(17.7-23.2%)	Chemical variability according to collecting site.	Eshilokun et al., 2005
El Salvador	1,8-Cineole (0%-50.6%) Fenchone (0%-37.4%) Fenchol (0%-22.5%) α -Terpinolene (0%-35.8%)	MH (35.4-54.8%) OM(10.3-39.7%)	Chemical variability according to collecting site and intrapopulation chemical polymorphism.	Grassi et al., 2005
Brazilian Savanna	Limonene (0.37%-35.73%) Sabinene (1.22%-30.98%) 1,8-Cineole (2.49%-27.65%)	MH (2.16-68.57%) SH (5.08-52.02%)	Pattern of geographic variation in the essential oil composition and influences of the plant growth phases and edaphic factors.	Oliveira et al., 2005
Tanzania	β -Caryophyllene (26.0%) β -Elemene (10.4%) trans- α -Bergamotene (7.7%)	SH (58.8%) MH (13.5%)	Just one vegetal source. The oil displayed significant antimicrobial activity against <i>Mucor sp.</i> The authors claimed that the plant under investigation could be a new chemotype.	Malele et al., 2003
Brazilian Savanna	1,8-Cineole (1.08%-27.65%) Spathulenol (9.25%-22.44%) (<i>E</i>)-Caryophyllene (0.74%-19.16%)	OS (7.42-54.78%) MH (17.18-38.93%)	Pattern of geographic variation in the essential oil composition.	Azevedo et al., 2002
Brazilian Savanna	Sabinene (2.93%-31.13%) <i>p</i> -mentha-2,4(8)-diene (0.19%-20.87%) β -Phellandrene (0.92%-18.17%)	MH (36.89-73.40%)	Pattern of geographic variation in the essential oil composition.	Azevedo et al., 2001
Nigeria (Ibadan)	Sabinene (16.5%) trans- α -Bergamotene (13.6%) Terpinen-4-ol (9.6%) α -Pinene (8.5%) β -Caryophyllene (6.2%) Caryophyllene oxide (4.5%)	Not reported	The antimicrobial activity of the essential oil is reported.	Asekun et al., 1999
Viçosa (Brazil), plants grown in greenhouse	β -Caryophyllene (10.39%) and spathulenol (13.30%) – leaves and stems; 1,8-cineole (27.47%) – inflorescence	Monoterpenes (42.1%) Sesquiterpenes (36.8%)	Specimens of <i>H. suaveolens</i> (L.) Poit were cultivated in green house in order to assess the composition of the essential oil produced by this plant. Essential oils from leaves, stems and inflorescences were analyzed.	Silva et al., 2003
Australia	1,8-cineole (32%) β -caryophyllene (29%)	Monoterpenes (49.38%) Sesquiterpenes (36.5%)	The chemical composition of the <i>H. suaveolens</i> essential oil was also compared with <i>H. mutabilis</i> and <i>H. emoryi</i> . In all cases, 1,8-cineole and β -caryophyllene were the major components. Enormous differences in the concentration levels of these two major components were found. This significant variation of the major components allowed an easy differentiation among these three species.	Peerzada, 1997
Nghe, a province in Vietnam	Eugenol (68.2%) Germacrene D (11.0%)	Monoterpenes (11.5%) Sesquiterpenes (20.5%)	The paper reports the chemical composition from a new chemotype. Eugenol (68.2%) was also found in the volatile oil chemical composition.	Le et al., 1996
Guinea-Bissau	β -caryophyllene (42.4%) Bergamotene (14.6%) Terpinolene (8.1%)	Monoterpenes (16.4%) Sesquiterpenes (65.2%)	Volatile compounds were collected by solid phase microextraction (SPME).	Jaenson et al., 2006
South India	Citronellyl acetate (22.3%) Piperitone oxide (7.8%) Pipritenone oxide (6.4%) Piperitone (4.1%) Geranyl acetate (3.5%) Isobornyl acetate (2.8%) β -caryophyllene (20.3%) β -bourbonene(3.2%)	Monoterpenes (46.9%) Sesquiterpenes (23.5%)	The essential oil chemical composition of <i>H. capitata</i> was also determined. Piperitone oxide, citronellyl acetate, geranyl acetate and β -caryophyllene form the common constituents among the species investigated.	Thoppil and Jose, 1995
India (Bangalore and Hyderabad)	Sabine (15.05% - Bangalore; 9.55% - Hyderabad) 1,8-cineole (31.51% - Bangalore; 35.30% - Hyderabad) Linalool (12.51% - Bangalore; 1.34% -Hyderabad) β -caryophyllene (10.11% - Bangalore; 0.29% - Hyderabad)	Bangalore (Monoterpenes - 74.84; Sesquiterpenes -12.24%) Hyderabad (Monoterpenes – 66.12%; Sesquiterpenes – 13.28%)	Essential oils produced by wild plants growing at two different locations (Bangalore and Hyderabad)	Mallavarapu et al., 1993
India (Gorakhpur)	Guaia-1(2)-7-dien-10-ol (0.10%) Guaia-1(2)-11-dien-10-ol (6.6%) Guaia-1(5)-en-11-ol (0.18%)	Not reported	Complete characterization of the compounds by IR, NMR and MS. No further details about the chemical composition of the essential oil are provided.	Singh and Upadhyay, 1994

OS = Oxigenated sesquiterpenes; MH = Monoterpenes hydrocarbons; OM = Oxygenated monoterpenes; SH = Sesquiterpene hydrocarbons

volatile oils from Alfenas (Minas Gerais state, Brazil), whereas monoterpenes were present in smaller amounts (3.65-11.88%). The plants from Alfenas could be classified as a spathulenol chemotype due to its high contents (3.48-24.70%). Dehydroabietol (2.23-16.80%), globulol (4.14-14.85%), α -cadinol (2.81-8.05%) and α -trans-bergamotene (0.43-6.45%) were also found in significant amounts in these volatile oils.

In the same experiment a significant increase in the oxygenated monoterpenes content of 60-day-old plants grown under potassium deficiency and natural photoperiod was observed, when compared to the plants harvested at 135 days.

The major component, spathulenol, showed a significant percentage change according to growth conditions. The observed decrease in spathulenol production under some experimental conditions may be related to phosphorus and nitrogen deficiencies and/or potassium availability during juvenile phases of the plant development, and it may also be related to the photoperiod. Furthermore, reciprocal proportions of spathulenol and α -trans-bergamotene were observed between 60-day-old and 135-day-old plants cultivated under nitrogen, phosphorus and potassium deficiency, regardless of the photoperiod condition, with increase of the α -trans-bergamotene contents in 60-day-old plants and decrease of the spathulenol amounts. In 135-day-old plants, an inversion in the relative amounts of these compounds was observed. This finding suggests that in *H. suaveolens* spathulenol and α -trans-bergamotene are reciprocally altered as consequence of plant age, taking into consideration the macronutrients deficiency. Indeed, 60-day-old plants cultivated under nitrogen, phosphorus and potassium deficiency, regardless of the photoperiod condition, presented significantly different essential oil composition in comparison to plants cultivated in different growth conditions, showing the sensitivity of *H. suaveolens* to nutritional deficiency at earlier stages of plant development.

In 60-day-old plants grown under phosphorus deficiency and longer photoperiod, an increase in the sesquiterpene hydrocarbon amounts and a decrease in the oxygenated sesquiterpenes, were observed when compared to 135-day-old plants. Similarly, 60-day-old plants grown under potassium deficiency and natural photoperiod had an increase in the sesquiterpene hydrocarbon amounts and a concomitant decrease in the oxygenated diterpenes, when compared to 135-day-old plants. The oxygenated diterpenes content increased in 135-day-old plants, mainly in those grown under potassium deficiency and Alfenas natural photoperiod (12 h 55 min \pm 2 min), or in nitrogen, phosphorus and potassium deficiency, regardless of the photoperiod condition.

These reciprocal changes among the amounts of the main terpenoid classes have suggested a possible pattern of oxidative variation in essential oil of *H. suaveolens* influenced by plant age and geographical environments. As a general trend, it has been observed that the amounts of more highly oxidized mono and sesquiterpenes increase as the plants growing sites moves from the central Brazilian plateau to near the Amazonian region (Oliveira et al., 2005). A similar trend in the oxidative gradient for other terpenes has been described by Kaplan et al (1991).

Due to the observed changes in the composition of *H. suaveolens* volatile oils as a result of nutritional deficiency at different development stages, an assay was carried out with the same homozygous plant lineage used by Martins et al. (2006, 2007)

cultivated under even more hostile conditions, such as nitrogen, phosphorus and potassium absence and interspecific competition with other weed species (unpublished data). It was determined that the volatile oils from *H. suaveolens* plants cultivated under natural conditions in the field (agronomic area) differed markedly from the oils produced by plants grown in a greenhouse. The major components of the *H. suaveolens* essential oil produced by plants cultivated in the agronomic area were (*E*)-caryophyllene (mean 24.81%) and β -elemene (mean 20.64%). The highest content of (*E*)-caryophyllene (34.75%) was determined in 75-day-old plants cultivated competitively in the presence of weed species *Emilia sonchifolia* (L.) DC. ex DC., *Bidens pilosa* L., *Phyllanthus niruri* L., *Commelina robusta* Kunth, *Brachiaria plantaginea* (Link) Hitchc. and *Digitaria horizontalis* Willd., at a high density of 50 plants *per m*², and also in fertilized soil with mineral NPK source. It was noted that (*E*)-caryophyllene content decreased from 34.75% in 75-day-old-plants to 16.93% in 120-day-old plants growing under the conditions described above. In contrast, germacrene D contents increased from 1.13% in 75-day-old plants to 14.23% in 120-day-old plants, when the plants were cultivated in fertilized soil and in the presence of weed species. The volatile oils isolated from 75-day-old plants grown in non-fertilized soil and absent of any weed species presented the maximum content of β -elemene (24.31%), yet quantified in samples from the Alfenas plant lineage. The sesquiterpenes caryophyllene oxide and viridiflorol content was significant (15.62% and 9.71% respectively) within the volatile oils from 120-day-old plants cultivated in non-fertilized soils, containing approximately 50 weed specimens *per m*². These percentages were lower in 75-day-old plants cultivated under the same conditions (caryophyllene oxide: 2.96%; viridiflorol: 1.56%). The terpenes globulol and dehydroabietol were absent in the essential oil from *H. suaveolens* cultivated in agronomic areas, whereas spathulenol was produced in small amounts (mean 3.51%) in all the treatments carried out in open field, contrasting strongly with the results obtained in greenhouse cultivation.

As a result of this experiment, it was noted that the sesquiterpene hydrocarbons achieved abundant amounts (mean 64.85%), corresponding to the major group of compounds in the volatile oils produced in all treatments. Again, these data are opposed to those obtained from plants cultivated in the greenhouse, where the oxygenated sesquiterpenes were predominant (16.85%-50.88%). The oxygenated sesquiterpenes were only produced in quantities (39.16%) comparable to non-oxygenated ones (40.27%) in 120-day-old plants grown under drastic conditions of cultivation, in non-fertilized soil and in the presence of weedy species. By analyzing the experiments carried out with the same *H. suaveolens* homozygous chemotype in the greenhouse and in an agronomical field, it can be stated that the essential oil composition is fully influenced by the growing conditions and that the genotypic features of the plants from each geographic area is not the single factor decisive to terpenoid biosynthesis in *H. suaveolens*. Indeed, the differences in essential oil compositions described in several studies must be interpreted as result of interaction between the environmental features such as nutritional availability and chemical balance in soils, photoperiod, sunbeam intensity and quality, interspecific competition, watery regimen as well as factors inherent to plants, mainly the genetic charge and the development stage (Silva et al., 2000; Azevedo et

al., 2001, 2002; Grassi et al., 2005; Oliveira et al., 2005; Martins et al., 2006, 2007; Martins and Polo, 2009).

The complex *H. suaveolens* ecophysiology and terpenoid biosynthesis pathways

From the data discussed so far, it is believed that qualitative or quantitative changes in biosynthesis pathways of compounds present in the volatile oils from *H. suaveolens* are related to adaptive responses linked to environmental pressures. These molecular responses are rapid and occur simultaneously to alterations of external conditions in order to allow the successful adaptation of *H. suaveolens*, resulting in its invasive and strong colonizing potential. To strengthen the above observation, some key molecular data that have been useful in the explanation of *H. suaveolens* floral induction have been discussed (Martins and Polo, 2009). A putative *H. suaveolens* gene LEAFY homolog showed an expression pattern similar to that reported in *Antirrhinum* and *Arabidopsis*. However, the expression of this gene is modified quantitatively according to the photoperiod. The LFY transcripts are strongly decreased in the vegetative meristem from *H. suaveolens* cultivated at length day conditions (16 h), resulting in floral inhibition whereas the LFY expression pattern in the vegetative meristem is increased in plants maintained under photoperiods lower than 13 h of light, culminating in the floral determination. This molecular response to environmental oscillations verified in *H. suaveolens* flowering regulation is also reflected in terpenoid biosynthesis. Conversely, studies dealing with biochemical approaches on the terpene biosynthetic pathway or with functional and expressive pattern of terpenes cyclase are absent.

Other *H. suaveolens* physiological behaviours have been investigated, such as the biochemical and developmental responses to high concentrations of aluminium (Pillay, 2006a, 2006b). Soil aluminium application alters the plant mineral element composition, causing an increase in Mn content in leaves and roots; Fe, Cu, and Zn contents in roots; K, Ca, Mg and N contents in leaves (Pillay, 2006a); aluminium content in different plant organs, with maximum aluminium concentrations in roots (Pillay, 2006a); and also alters the growth and pigment composition, presenting shoot and root dry weight reductions of 53% and 24%, respectively, accompanied by decrease in both chlorophylls a and b, carotene and xanthophyll contents (Pillay, 2006b). All these studies, revealing changes in the *H. suaveolens* features, are valuable and will be useful to clarify the complex ecophysiology of this specie, which was evidenced for the first time in 1970s by seed germination polymorphism and differential rate of seedling growth (Wulff, 1973).

Biological activities of *H. suaveolens*

In the course of the phytochemical analysis description, some biological activities associated with organic extracts of *H. suaveolens* or with compounds isolated from this plant were briefly described. Some of the biological properties of the essential oils derived from *H. suaveolens* were briefly mentioned in Table 1. In fact, this species displayed a broad range of biological properties. Some of these factors will be discussed in more detail subsequently.

Antimicrobial activity is an important activity associated with *H. suaveolens*. For example, the essential oils from *H. suaveolens* grown in Ibadan (Nigeria) displayed significant inhibitory activity against two gram-positive and four gram-negative bacteria at 5 mg mL⁻¹ concentration. Activity against the fungus *Candida albicans* was also observed (Asekun et al., 1999). In another report, the essential oil from Tanzania showed strong antifungal activity against *Mucor sp* and *Fusarium moniliforme* (Malele et al, 2003). From *H. suaveolens* collected in India, Sharma and Tripathi (2008) obtained an essential oil composed of 1,8-cineole (44.4%), α -pinene (11.7%), (*E*)-caryophyllene (10.0%), camphene (5.7%) and β -myrcene (5.3%). This oil was active against the pathogen *Fusarium oxysporum* f. sp. *gladioli* (Sharma and Tripathi, 2008) and the oil treatment caused a decrease of conidiation and anomalies in the hyphae such as a decrease in the diameter of hyphae and granulation of cytoplasm (Tripathi et al., 2009).

The secondary metabolites known as aflatoxins are potent toxins, carcinogenic, mutagenic, immunosuppressive, and teratogenic agents produced by *Aspergillus flavus* and *A. parasiticus*. Eighteen different types of aflatoxins have been identified, and aflatoxin B₁ corresponds to the one produced most abundantly and the most toxic. Besides their effect on the health of humans and animals exposed to them, aflatoxins can also cause impact on agricultural economy due to the loss in crop production and the time and cost involved in monitoring and decontaminating efforts imposed by regulatory guidelines. In the search for naturally available materials to control aflatoxin production from *A. flavus* in soybean seeds, leaf powder of *H. suaveolens* was tested. The inhibitory activity displayed by the leaf powder corresponded to 84.85% (at 5% w/w concentration) and 78.85% (at 10% w/w concentration). The inhibitory activity showed by the fungicide Captan, which has been considered a potent agent to control aflatoxin production, corresponded to 97.57% (at 1.5% w/w concentration) and 98.54% inhibition (at 2.0 % w/w concentration) (Krishnamurthy and Shashikala, 2006).

The essential oil from *H. suaveolens* exhibited pronounced antifungal activity (94% inhibition of growth) against the toxigenic strain Saktiman 3NSt of *A. flavus* at 1 μ L mL⁻¹ (Jaya et al., 2011). The major components of essential oil were: precocene I (23.02%), (*E*)-caryophyllene (8.66%), sabinene (9.19%), caryophyllene oxide (9.53%), bergamotene (6.02%), phenanthrene (3.96%) and limonene (2.47%). The essential oil was found efficacious in arresting the aflatoxin B₁ production by the *A. flavus* at 0.08 to 0.25 μ L mL⁻¹ and completely inhibited the production at 0.5 μ L mL⁻¹ (Jaya et al., 2011). Another *H. suaveolens* essential oil sample was characterized by the components 1,8-cineole (47.64%), γ -ellene (8.15%), β -pynene (6.55%), δ -3-carene (5.16%), (*E*)-cariophyllene (4.69%) and germacrene (4.86%) (Moreira et al., 2009). The essential oil was active against *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus* and *A. niger*. Also, at 10 and 20 μ L mL⁻¹, the oil was able to cause morphological changes in *A. flavus* such as decreased conidiation, leakage of cytoplasm, loss of pigmentation and disrupted cell structure suggesting fungal wall degeneration (Moreira et al., 2009).

In East and West Africa, plants of the *Hyptis* genus are commonly utilized against mosquitoes (Pålsson and Jaenson, 1999). In Guinea Bissau (West Africa), it was demonstrated that smoul-

dering leaves of *H. suaveolens* show repellent activity beyond 80%, whereas for fresh leaves the repellent activity was greater than 70% (Pålsson and Jaenson, 1999).

Interviews conducted with people living in 27 villages in Kenya (sub-locations of Rusinga Island and Rambira location) revealed that direct burning of plants was the most common application method for repelling host-seeking mosquitoes in the surveyed communities. In experiments carried out in Kenya, direct burning of *H. suaveolens* was associated with 49.2% of repellence activity. During the experiments, an alternative method of application known as thermal expulsion was developed. For the other plants tested during the experiments, it was demonstrated that this alternative methodology is generally superior to direct burning except for *H. suaveolens* (Seyoum et al., 2002). Ethyl acetate extract of *H. suaveolens* from Guinea-Bissau significantly reduced probing activity of *Aedes aegypti* (L) (Jaenson et al., 2006).

Cowpea (*Vigna unguiculata* /L./ Walp.) is an important crop in tropic and subtropical regions. Cowpea contains a high level of proteins and in Africa they are commonly used in the human diet. In addition, the green part can be used as a vegetable or as fodder for cattle. The larvae of *Callosobruchus maculatus* (Fabr.) cause high losses during the storage of the cowpea seeds. In the absence of control strategies, storage of cowpea beans can become problematic since *C. maculatus* population increases rapidly over successive generations (Sanon et al., 2006). Since synthetic insecticides are unaffordable for subsistence farmers in Africa, they have been utilizing insecticidal plants as an alternative to control *C. maculatus*. In this regard, *H. suaveolens* has been submitted to several investigations to verify its usefulness as an alternative to control *C. maculatus* (Kéïta et al., 2000; Boeke et al., 2004; Ilboudo et al., 2010).

The essential oils of *H. suaveolens* from markets in Conakry (Guinea Bissau) were evaluated against *C. maculatus* adults. Less than 20% mortality of the adult insects was observed after 48 hours of utilizing 40 L of essential oil over 10 insects. A combination of 0.5 g of aromatized powder with essential oils in stock formulation (1 g of kaolin powder + 150 µL of essential oil) was employed to evaluate the effect of dust on adult longevity and egg laying and significant effects were absent with regard to the parameters investigated. Aromatized kaolin powder was also used to assess the effect on egg hatching and adult emergence. Consequently, it was found that *H. suaveolens* exerted great ovicidal activity and also had a significant effect on adult emergence (Kéïta et al., 2000).

Plant powders can have a protective effect on cowpea beans based on several mechanisms. In addition to direct toxic effects, the plant material may produce odours that repel or confuse the adult beetle of *C. maculatus*, which could prevent invasion or cause emigration from the treated stock. The repellence activity of *H. suaveolens* powder on female beetles of *C. maculatus* was investigated and the behaviour of the insects when they were exposed to treated and untreated beans in linear olfactometer was evaluated. Although there are reports on the use of this plant to protect stored cowpea against *C. maculatus*, under laboratory experimental conditions *H. suaveolens* proved very attractive to the insects. Moreover, the great majority of eggs developed into adults, than on untreated beans. These findings were ascribed

to the low dose utilized for the repellence activity experiment (Boeke et al., 2004).

The parasitoid *Dinarmus basalis* is considered as a promising tool to control *C. maculatus*. The former limits the population increase of the latter at the onset of storage, which reduces seed losses. As a consequence, *D. basalis* could be used for biological control. Since *H. suaveolens* has been utilized by farmers in Africa to aid cowpea storage, researchers have become interested in verifying the effect of sublethal concentrations on *D. basalis*. Olfactory investigations revealed that the lethal subdoses of the volatiles emitted by crushed leaves of *H. suaveolens* and the essential oils were repellent for naive females of *D. basalis*. The authors of this investigation described that these females were able to move in a three-dimensional maze and avoid the host patches associated with *H. suaveolens*. Their reproductive activity was reduced in such patches. No repellence or partial repellence was observed for females which had been exposed to sublethal doses of *H. suaveolens* volatiles during their postembryonic development (Sanon et al., 2006).

Other biological activities of *H. suaveolens* described in the literature are related to its various applications in folk medicine. The plant has been associated with antiseptic (Rojas et al., 1992), anticarcinogenic (Kingston et al., 1979), antibacterial (Fun and Svendsen, 1990; Rojas et al., 1992), anticonvulsant (Akah and Nwanbie, 1993), anti-edematogenic, and anti-nociceptive activities (Bispo et al., 2001; Santos et al., 2007). There are reports on the use of the plant as a skin disinfectant and as a carminative (Grassi et al., 2005). In addition, *H. suaveolens* seeds are utilized traditionally in the treatment of gastrointestinal disorders (Grassi et al., 2005), respiratory tract infections (Iwu, 1993), gall bladder infections (Malele et al., 2003), colds, pain, fever, cramps, skin diseases (Iwu, 1993), indigestion, nausea and flatulence (Fun and Svendsen, 1990). *H. suaveolens* is also associated with curious applications, as in bathing and hair care (Guzman, 1975) and as an appetizing agent (Malele et al., 2003). The employment of *H. suaveolens* as a nematicide (Babu and Sukul, 1990) and a larvicide (Noegroho and Srimulyani, 1997) is also known.

The phytotoxic activity of *H. suaveolens* has been shown on the germination of sorghum (*Sorghum vulgare* Pers.), lettuce (*Lactuca sativa* L.) and radish (*Raphanus sativus* L.) (Rodrigues et al., 2012). Seeds of sorghum, lettuce and radish were sown in sterilized or unsterilized substrates consisting of sand, soil and organic fertilizer containing leaves of *H. suaveolens*. The germination speed index (GSI) and percentage of germination (G%) were calculated to access the allelopathic effects of the *H. suaveolens* leaves. The results showed that sorghum and lettuce were more susceptible to the allelopathic potential of *H. suaveolens*, while for radishes a beneficial effect was observed. Between treatments, the sterilized and unsterilized substrate showed differences suggesting that the breakdown of the plant compounds by microorganisms may be required to enhance the allelopathic effect of *H. suaveolens* leaves.

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