Chapter 4

The Expression of Biodiversity in the Secondary Metabolites of Aromatic Plants and Flowers Growing in Colombia

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Additional information is available at the end of the chapter

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Abstract

A network of research groups has carried out a bioprospective study of Colombia's vegetal biodiversity, with focus on aromatic plants. This chapter presents results on the chromatographic analysis of flower fragrances and essential oils obtained from vegetal material collected in botanical expeditions to various Colombian regions. Essential oils and flower fragrances are composed of volatile substances that differ greatly in polarity, functional groups, and relative amounts. The study of these complex mixtures requires special sampling and analysis techniques, described in this chapter. The large chemical diversity of the essential oil and flower fragrance constituents is a formidable characterization challenge. Typically, the number of essential oil components surpassed 50. It was rare to find an essential oil composition in which a single substance was present with a relative amount above 50%.

Keywords: essential oil, flower scent, gas chromatography, HS-SPME, tropical

1. Introduction

The Research Center for Agroindustrialization of Aromatic and Medicinal Tropical Vegetal Species, CENIVAM, is a multidisciplinary research network of groups from Colombian public and private universities that joined efforts to study Colombia's agricultural biodiversity, with focus on aromatic plants. Under permit from Colombia's Environment Ministry, botanical expeditions were organized to obtain vegetal material from different regions in the country. A primary taxonomical identification made by the researchers in the field was subsequently replaced by the assessment made at Colombia's National Herbarium, where exsiccatae of all studied materials have been deposited. The vegetal material was dried, chopped, and either distilled or macerated,

to obtain essential oils and extracts, respectively. Samples of these secondary metabolites were sent to several collaborating groups for the characterization of their biological activity. High-resolution chromatographic and mass spectrometric techniques were utilized in the chemical characterization of the essential oils and extracts. The combined knowledge of chemical composition and biological activity serves as the basis for the sustainable use of the biodiversity in the development of new consumer products for the cosmetics, hygiene, food, and pharmaceutical industries. Pilot essential oil production units have been implemented in some municipalities (Socorro, Sucre, and Barbosa) in the state of Santander. Farmers associations have been trained on good agricultural practices, post-harvest treatment of the vegetal material, and operation of stills designed at Universidad Industrial de Santander, UIS, for the rural essential oil extraction under either hydrodistillation or steam distillation. Thanks to these pilot units, farmers have begun production of essential oils of *Cymbopogon nardus*, *C. martinii*, and *Lippia origanoides*. New developments have started to extend the cultivation and essential oil production to additional species, such as *Cananga odorata*, *Pogostemon cablin*, *Vanilla planifolia*, *Lippia alba*, and *Rosmarinus officinalis*.

This chapter presents results from essential oil and flower fragrance analysis. Flowers maintained in CENIVAM's experimental garden were sampled both in vivo and in vitro to characterize their volatile compounds. The complex combination of volatile compounds emitted by flowers depends on the plant species, its habitat, phenological state, propagation strategy, time of day, circadian rhythm, climate, and many more variables.

2. Study of tropical flowers volatile compounds

The study of natural products includes a very interesting area: isolation and analysis of floral fragrances, which can be monitored both *in vivo* and *ex vivo* [1]. The study of volatile secondary metabolites, emanated from flowers, is important in many areas of the biological and chemical sciences, in agriculture, for the pest control, in the study of plant-insect interactions, allelopathy, in analytical sciences (sample preparation and chromatography), in the pharmaceutical, in perfumes and cosmetics, flavors and fragrances industries, among others.

Floral fragrances are complex mixtures, product of the metabolism of a flowering plant; they are composed of hundreds of molecules of different biochemical origin, with different physicochemical characteristics (polarity, volatility, and solubility); they contain various functional groups (hydrocarbons, alcohols, aldehydes, ketones, acids, esters, ethers, etc.), and can be found in diverse concentrations (from parts per trillion, ppt, to parts per million, ppm). They are predominantly lipophilic substances, with molecular weight less than 300 Da, non-polar or moderately polar, and with high vapor pressure. The human nose can be more sensitive than a chromatographic detection system, to some floral fragrance substances, present at the trace level; therefore, it is necessary to carry out the extraction and concentration processes of the floral fragrance in such a way that its components are detectable and can be identified. This constitutes a very big analytical challenge. Nowadays, this challenge is solved by applying different strategies: headspace extraction techniques (*headspace*), distillation methods, extractive solvents, and an active surface, *i.e.*, adsorption/thermal desorption processes using adsorbents with different physical characteristics [2, 3].

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For the instrumental analysis of volatile fractions and extracts, gas chromatography (GC) is used in one-dimensional (1D) or two-dimensional (2D) versions (GCxGC), in capillary columns of different polarities, using universal detection systems (flame ionization detector (FID); mass selective detector (MSD), in *full scan* mode), or selective detection, e.g., selective nitrogen and phosphorus detector (NPD), flame photometric detector(FPD), to register nitrogenous or sulfur compounds, respectively, and very specific detection systems such as electroantennography, electronic nose, and so on [4]. High-resolution mass spectrometry detection systems (HR-TOF, Orbitrap) are nowadays an excellent alternative for exact mass determination (elemental composition analysis), selective and very sensitive detection of secondary metabolites in complex floral scent mixtures.

Many and very diverse compounds have been detected and identified in floral fragrances. More than 1700 have been recorded in a diverse group of flowers studied [5]. The main families of chemical compounds found in floral scents include hydrocarbons (saturated, cyclic and olefinic); terpenes, basically, monoterpenoids and some sesquiterpenoids; benzenoids and phenylpropanoids, the oxygenated compounds of mixed nature, *e.g.,* alcohols, aldehydes, ethers, esters (fatty acid derivatives), and substances that contain heteroatoms, such as sulfur or nitrogen.

The basic biological function of the floral fragrance is to promote or facilitate cross-pollination, which is a vital process in the life cycle of most angiosperm plants. The knowledge of the floral fragrance chemical composition is important to understand plant-insect interaction, the chemical strategies not only to attract the pollinators but also to deter the herbivores and to face the pathogens, to adapt to different abiotic stresses; to study the biochemical pathways of secondary metabolism in a plant, its adaptability and biological evolution. Also, it is of practical interest to know the floral composition as a source of inspiration to create new fragrances and odorous mixtures, which are used in the cosmetics, perfumes, personal hygiene products, or aromatherapy industries.

The floral fragrances of diverse plants, despite of having a different smell, could contain many common compounds. Among these, the terpenoids are a large group: monoterpene (ocimenes, phellandrenes, carenes, terpinenes, limonene, and *p*-cymene), sesquiterpenes (caryophyllenes, farnesenes, bisabolenes, cadinenes, cubebenes, elemenes, germacrenes, and their structural isomers), and their oxygenated analogues (caryophyllene oxide, farnesol alcohols, nerolidol, and their esters), and some irregular terpenes. Among the most frequent oxygenated monoterpenes one can find alcohols: linalool, geraniol, nerol, and their acetates; ketones: carvone, menthone, verbenone; and aldehydes: citral (geranial and neral), and their oxides. Another family in the floral fragrance is made up of hydrocarbons, aliphatics, C_1-C_{30} (more frequently, C_{13}-C_{21} hydrocarbons, and olefinics, including some cycloparaffins). These substances, together with the fatty acids, C_{12}-C_{22}, are part of the wax protective layer that lines the petals of many flowers, a lot of fatty acid derivatives (alcohols, ketones, ethers, esters, and lactones) could predominate in the floral scents of some flowers. A distinctive odoriferous note in the floral fragrance is due to the presence in its mixtures of compounds that contain sulfur or nitrogen atoms, probably originating from the metabolism of amino acids; among these volatile secondary metabolites, there are compounds with nitro group, indoles, oximes, nitriles, anthranilates, and sulfides, among the most common.

We studied the chemical composition of volatile fractions of 30 tropical plants; their floral scents were monitored by *in vivo* solid-phase micro-extraction (SPME), exposing the fiber, coated with Carboxen/poly(dimethylsiloxane) (CAR/PDMS) or Carboxen/divinylbenzene/poly(dimethylsiloxane) (CAR/DVB/PDMS) to the flower, mostly at its anthesis stage, for 20–30 minutes. The on-fiber collected volatiles immediately were desorbed into the injection port of a gas chromatograph coupled to mass spectrometer (GC-MS). **Table 1** contains the results of these analyses; the presence of diverse groups (monoterpenoids, sesquiterpenoids, benzenoids, oxygenated compounds, and fatty acid derivatives, as well as sulfur- and nitrogen-containing compounds, very characteristic for some plants (*Cananga odorata, Sansevieria guineensis, Erythroxylum coca, Moringa oleifera, Stapelia gigantea*) can be observed. The plants have been cultivated in the experimental plots of the Research Center CENIVAM (Bucaramanga, Colombia). Although diverse volatile fractions may contain common compounds, they are mostly different in their chemical composition.

2.1. Methods for floral fragrance isolation

Before proceeding to collect the volatile flowers, it is important to establish if their monitoring will be done *in vivo* (in the field) or *ex vivo*. The experimental setup for each purpose will be different. Some extraction techniques are not applicable in the field for *in vivo* flower monitoring. It is also important to have prior knowledge about the concentration of volatiles that the flower emits since the extraction and concentration system used will depend on the volatile fraction quantity. The bouquet of volatile substances produced by the flowers can have from 1 to more than 100 compounds but generally contains 20–60 different substances. The concentration of volatiles emitted ranges from low picogram levels to more than 30 μg/L [6].

Some methods of collecting floral volatiles can have an automated design that allows monitoring for 24 hours or longer periods. However, most extraction techniques make a momentary capture, a "snapshot" of the floral volatiles emitted [7]. The extraction methods of the flower volatile secondary metabolites can be divided into three large categories, namely: (I) Headspace techniques (*headspace*, HS) in static or dynamic modes; (II) Distillation techniques, among them steam distillation, water-steam distillation, hydro-distillation, hydro-distillation assisted by microwave radiation, and (III) Extractive techniques, using solvents of different nature, *e.g.*, fats (maceration, *enfleurage*, obtaining ointments), non-polar solvents (hydrocarbons, obtaining concretes), polar solvents (alcohols, obtaining absolutes), and supercritical fluids (mainly, CO_2). Headspace techniques are used in two different "formats": *static headspace* (S-HS) and *dynamic headspace*, *e.g.*, purge and trap (P&T). Today, the most popular technique for the analysis of floral fragrances is solid-phase micro-extraction (SPME), operated in the *headspace* mode (HS-SPME). This method of extraction on a polymeric adsorbent, which covers a fused silica fiber combines the high selectivity of extraction, which is achieved with the choice of the polymer, its chemical nature and thickness, and the optimization of sampling conditions (volumes of the material and headspace, temperatures, pre-equilibrium, and fiber exposure times, sample agitation modes, additives, etc.), with the concentration of analytes on the fiber. The extraction and simultaneous concentration of the sample are processes that distinguish the SPME technique and make it very advantageous compared to other methods [8].

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Species	Family	Chemical composition
Thunbergia grandiflora	Acanthaceae	**Monoterpenoids**: *trans*-β-ocimene, linalool. **Benzenoids**: methyl salicylate. **Oxygenated compounds**: acetaldehyde, 3-octanone, 1-octen-3-one, 3-octanol, 1-octen-3-ol, lauryl acetate. **Sulfur and nitrogen compounds:** dimethyl sulfide, benzyl nitrile. **Hydrocarbons:** *n*-pentadecane.
Cananga odorata	Annonaceae	**Monoterpenoids:** α-pinene, β-pinene, β-myrcene, limonene, 1,8-cineole, *trans*-β-ocimene, terpinolene, *allo*-ocimene, linalool, α-terpineol, geranyl acetate, geraniol, nerol. **Sesquiterpenoids:** α-cubebene, α-ylangene, α-copaene, β-copaene, *trans*-β-elemene, *trans*-β-caryophyllene, *cis*-muurola-3,5-diene, *trans*-muurola-3,5-diene, α-humulene, α-muurolene, γ-muurolene, germacrene D, α-farnesene, α-cadinene, γ-cadinene, δ-cadinene, cadina-1,4-diene, calamenene. **Benzenoids:** *p*-methyl anisole, benzaldehyde, methyl benzoate, ethyl benzoate, methyl salicylate, 3,4-dimethoxy-toluene, 2-ethyl phenyl acetate, anethole, benzyl acetate, *p*-cresol, cinnamyl acetate, methyl isoeugenol. **Oxygenated compounds (fatty acid derivatives):** 3-methyl 3-butenyl acetate, 3-methyl 2-butenyl acetate, hexyl acetate, *cis*-3-hexenyl acetate. **Sulfur- and nitrogen-containing compounds:** phenyl acetonitrile, 4-methyl benzaldoxime, 2-phenyl-1-nitroethane, benzyl nitrile, methyl anthranilate, indole.
Aristolochia ringens	Aristolochiaceae	**Sesquiterpenoids:** α-farnesene. **Benzenoids:** benzaldehyde, methyl benzoate, methyl salicylate, benzyl acetate, benzyl alcohol, 3-phenyl propyl acetate, benzyl isovalerate, eugenol, methyl eugenol, *trans*-cinnamaldehyde, methyl anisate, methyl *trans*-cinnamate, benzyl tiglate, cinnamyl acetate, benzyl benzoate, benzyl salicylate. **Oxygenated compounds (fatty acid derivatives):** methyl acetate, ethyl acetate, 2-isopentyl acetate, penten-1-yl acetate, 3-hexenyl acetate, hexyl acetate, heptyl acetate, octyl acetate, nonyl acetate, decyl acetate, decenyl acetate, undecyl acetate, lauryl acetate, dodecenyl acetate, tetradecenyl acetate, hexanol, heptanol, octanol, 1-octen-3-ol, nonanol, decenol, dodecenol, octanal, nonanal, decanal, dodecanal, tridecanal, pentadecanal, 2-pentyl furan, methyl decanoate, methyl myristate. **Sulfur- and nitrogen-containing compounds:** dimethyl sulfide, dimethyl pyrazine, methoxy-dimethyl pyrazine. **Hydrocarbons:** heptadiene, tetradecadiene, methyl tridecane, *n*-pentadecane.
Sansevieria guineensis	Asparagaceae	**Monoterpenoids:** limonene, 1,8-cineole, carvone, α-terpineol. **Sesquiterpenoids:** *cis, cis*-farnesol. **Benzenoids:** benzyl alcohol, methyl benzoate, ethyl benzoate, benzyl salicylate, eugenol, methyl eugenol, methyl isoeugenol, benzyl benzoate. **Oxygenated compounds (fatty acid derivatives):** 2-methyl-butan-1-ol, tridecanal, pentadecanal. **Sulfur- and nitrogen-containing compounds:** methyl anthranilate.

Species	Family	Chemical composition
Polianthes tuberosa	Asparagaceae	**Monoterpenoids:** limonene, *cis*-limonene oxide, *trans*-limonene oxide, linalool, nerolidol.
		Benzenoids: 2-phenyl ethanol, 3-hexenyl benzoate.
		Sulfur- and nitrogen-containing compounds: benzyl nitrile.
Plumeria rubra	Apocynaceae	**Monoterpenoids:** limonene, *cis*-limonene oxide, *trans*-limonene oxide, linalool.
		Sesquiterpenoids: nerolidol.
		Benzenoids: 2-phenyl ethanol, 3-hexenyl benzoate.
		Sulfur- and nitrogen-containing compounds: benzyl nitrile.
Stapelia gigantea	Apocynaceae	**Monoterpenoids:** α-pinene, β-pinene, α-phellandrene, Δ^3-carene, β-phellandrene, *trans*-β-ocimene, *cis*-β-ocimene, *allo*-ocimene, *neo-allo*-ocimene, 1,3,8-*p*-menthatriene, *trans, trans*-2,6-dimethyl-1,3,5,7-octatetraene, *p*-cymenene, 2,6-dimethyl-1,3,5,7-octatetraene.
		Benzenoids: anisole, methoxy benzene.
		Oxygenated compounds (fatty acid derivatives): butanoic acid, pentanoic acid, 3-methyl hexanoic acid.
		Sulfur- and nitrogen-containing compounds: dimethyl disulfide, dimethyl trisulfide, methoxy phenyl oxime.
Veitchia merrillii	Arecaceae	**Monoterpenoids:** limonene. 1,8-cineole, *trans*-β-ocimene, *cis*-β-ocimene, linalool oxide, linalool.
		Benzenoids: methyl salicylate,2-phenyl ethanol.
Cannabis indica	Cannabaceae	**Monoterpenoids:** α-pinene, α-thujene, camphene, β-pinene, Δ^3-carene, β-myrcene, α-phellandrene, β-phellandrene, α-terpinene, limonene, *cis*-β-ocimene, γ-terpinene, *trans*-β-ocimene, *allo*-ocimene, terpinolene, *p*-cymenene, linalool, limonen-4-ol, fenchol, α-terpineol.
		Sesquiterpenoids: α-ylangene, *cis*-α-bergamotene, α-santalene, *trans*-β-bergamotene, *trans*-β-caryophyllene, *allo*-aromadendrene, *cis*-β-farnesene, α-humulene, α-selinene, β-selinene, γ-selinene, α-bulnesene, valencene, bicyclogermacrene, *trans, trans*-α-farnesene, δ-amorphene, selina-3,7(11)-diene.
Ipomoea horsfalliae	Convolvulaceae	**Sesquiterpenoids:** α-cubebene, β- cubebene, α-copaene, β-copaene, *trans*-β-elemene, *trans*-β-caryophyllene, *trans*-muurola-3,5-diene, *trans*-muuro-4 (14), 5-diene, γ-muurolene, germacrene D, dauca-5,8-diene, α-selinene, β-selinene, bicyclogermacrene, δ-cadinene, γ-cadinene, *cis*-calamenene, caryophyllene oxide.
		Benzenoids: 2-phenyl ethanol.
		Oxygenated compounds (fatty acid derivatives): acetaldehyde, butanal, 2-propenal, 2-methyl butanal, 3-methyl butanal, ethanol, 1-penten-3-one, hexanal, *cis*-2-penten-1-al, 1-penten-3-ol, 3-methyl-1-butanol, 2-penthyl furane, pentanol, 3-octanone, 1-octen-3-one, *cis*-2-pentenol, hexanol, *cis*-3-hexenol.
		Sulfur- and nitrogen-containing compounds: dimethyl sulfide.

Species	Family	Chemical composition
Erythroxylum novogranatense	Erythroxylaceae	**Monoterpenoids:** β-myrcene, α-phellandrene, α-pinene, Δ^3-carene, β-phellandrene, *trans*-β-ocimene, *cis*-β-ocimene, *allo*-ocimene, *neo-allo*-ocimene, 1,3,8-*p*-menthatriene, linalool, *cis*-linalool oxide, linalool. **Sesquiterpenoids:** α-farnesene. **Benzenoids:** benzaldehyde, methyl benzoate, benzeneacetaldehyde, methyl salicylate, ethyl salicylate, benzyl alcohol, 2-phenyl ethanol. **Oxygenated compounds (fatty acid derivatives):** propan-2-one, 2-methyl furane, butanal, propanol, butan-2-one, 2-methyl butanal, 3-methyl butanal, ethanol, 3-buten-2-one, pentanal, hexanal, 1-penten-3-ol, *cis*-2-pentenol, 2-*trans*-hexanal, heptanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 3-*cis*-hexenol, nonanol, bovolide. **Sulfur- and nitrogen-containing compounds:** dimethyl sulfide, 2-methyl butane nitrile, 3-methyl butane nitrile, 1-methyl-1H-pyrrole, 2-phenyl nitroethane, ecgonidine methyl ester, quinoline, benzene acetonitrile, 6-methyl-2-pyridinecarboxaldehyde.
Brownea macrophylla	Fabaceae	**Oxygenated compounds (fatty acid derivatives):** hexanol, 2-heptanone, 2-heptanol, 2-nonanol, 3-octanol.
Perilla frutescens	Lamiaceae	**Monoterpenoids:** perillene, linalool, perilla aldehyde. **Sesquiterpenoids:** α-cedrene, *trans*-α-bergamotene, *trans*-β-elemene, β-cedrene, *trans*-β-caryophyllene, α-humulene, *trans*-β-farnesene, α-himachalene, α-selinene, β-selinene, *trans*, *trans*-α-farnesene, cuparene. **Benzenoids:** benzaldehyde, methyl salicylate. **Oxygenated compounds (fatty acid derivatives):** *trans*-2-hexenal, *cis*-3-hexenol, 3-octanol, *trans*-2-hexen-1-ol, 1-octen-3-ol.
Plectranthus amboinicus	Lamiaceae	**Monoterpenoids:** Δ^3-carene, γ-terpinene, *p*-cymene, carvacrol. **Sesquiterpenoids:** *trans*-α-bergamotene, *trans*-β-caryophyllene, α-humulene. **Oxygenated compounds (fatty acid derivatives):** 1-octen-3-ol.
Persea americana	Lauraceae	**Monoterpenoids:** α-pinene, α-thujene, β-pinene, β-phellandrene, β-myrcene, limonene, 1,8-cineole, 4,8-dimethyl-1,3,7-nonatiene, *trans*-linalool oxide, *cis*-linalool oxide, linalool. **Sesquiterpenoids:** *trans*-β-caryophyllene. **Benzenoids:** benzeneacetaldehyde. **Oxygenated compounds (fatty acid derivatives):** acetaldehyde, butan-2-one, ethanol, pentan-3-one, 1-penten-3-ol, hexanal, 4-pentenal, 2-pentenal, 3-hexenal, 1-penten-3-ol, 3-methyl-1-butanol, *trans*-2-hexenal, hexanol, *cis*-3-hexenol, *trans*, *trans*-2,4-hexadienal, *trans*-2-hexen-1-ol. **Sulfur- and nitrogen-containing compounds:** benzeneacetonitrile, indole. **Hydrocarbons:** tridecane.

Species	Family	Chemical composition
Gossypium barbadense	Malvaceae	**Monoterpenoids:** α-pinene, camphene, β-pinene, sabinene, β-myrcene, α-phellandrene, β-phellandrene, α-terpinene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, γ-terpinene, *p*-cymene, *allo*-ocimene, *neo-allo*-ocimene, 1,3,8-*p*-menthatriene, bornyl acetate. **Sesquiterpenoids:** α-cubebene, α-copaene, *trans*-β-caryophyllene, aromadendrene, γ-gurjunene, α-bulnesene, α-humulene, γ-muurolene, viridiflorene, α-muurolene, bicyclogermacrene, γ-cadinene, *cis*-calamenene. **Oxygenated compounds (fatty acid derivatives):** acetaldehyde, ethanol, 1-penten-3-ol. **Sulfur- and nitrogen-containing compounds:** dimethyl sulfide.
Medinilla myriantha	Melastomataceae	**Monoterpenoids:** italicene, *cis*-thujopsene. **Sesquiterpenoids:** β-cedrene. **Benzenoids:** *p*-methyl anisole, benzaldehyde. **Oxygenated compounds (fatty acid derivatives):** hexanal, heptanal, 3-octanone, hexyl acetate, octanal, 1-octen-3-one, *cis*-3-hexenyl acetate, 6-methyl-5-heptene-2-one, hexanol, *cis*-3-hexenol, 3-octanol, nonanal, 1-octen-3-ol, heptanol, octanol, nonanol, *cis*-3-nonen-1-ol, 2-undecanone.
Moringa oleifera	Moringaceae	**Monoterpenoids:** α-pinene, camphene, 4,8-dimethyl-1,7-nonadiene, β-pinene, sabinene, β-myrcene, α-phellandrene, α-terpinene, 2,3-dehydro-1,8-cineole, limonene, β-phellandrene, 1,8-cineole, *cis*-β-ocimene, γ-terpinene, *trans*-β-ocimene, *p*-cymene, terpinolene, *p*-cymenene, thujone, *cis*-sabinene hydrate, *cis*-linalool oxide, terpinene-4-ol, linalool, *trans*-sabinene hydrate, α-terpineol, citronellol, nerol, *trans*-β-ionone. **Sesquiterpenoids:** *cis*-β-farnesene, α-farnesene, *trans*-nerolidol. **Benzenoids:** benzaldehyde, methyl benzoate, benzyl acetate, benzyl alcohol, *cis*-methyl cinnamate, *trans*-methyl cinnamate, *trans*-ethyl cinnamate, *trans*-cinnamyl acetate. **Oxygenated compounds (fatty acid derivatives):** acetaldehyde, ethanol, 2-ethyl furane, pentan-3-one, 1-penten-3-one, butyl acetate, hexanal, 3-methyl-2-butenal, 3-buten-3-ol, 3-methyl-butan-1-ol, *trans*-2-hexenal, 2-penten-1-yl acetate, 2-pentyl furane, octan-3-one, *n*-hexyl acetate, octanal, *cis*-3-hexenyl acetate, *trans*-3-hexenyl acetate, 6-methyl-5-penten-2-one, heptyl acetate, 2-heptenyl acetate, 1-octen-3-ol, heptanol, octanal, octanol, nonyl acetate, 2,6-nonadienal, nonanol, 2-nonen-1-ol, 2,6-nonadien-1-ol, lauryl acetate. **Sulfur- and nitrogen-containing compounds:** carbonyl sulfide, carbon disulfide, dimethyl sulfide, *sec*-butyl nitrile, 3-methyl butanenitrile, 2-propyl iso-thiocyanate, 1-butyl iso-thiocyanate, 2-butyl iso-thiocyanate, benzeneacetonitrile, benzylnitrile, benzyl iso-thiocyanate.
Cattleya mendelii	Orchidaceae	**Monoterpenoids:** α-pinene, β-myrcene, limonene, *cis*-β-ocimene, *trans*-β-ocimene *p*-cymene, linalool oxide, linalool, terpinene-4-ol. **Sesquiterpenoids:** α-cubebene, β-cubebene, α-copaene, aromadendrene, α-muurolene, γ-muurolene, germacrene D, *trans, trans*-α-farnesene, γ-cadinene, *cis*-nerolidol. **Benzenoids:** benzaldehyde, benzyl acetate, methyl salicylate, 2-phenyl ethanol. **Oxygenated compounds (fatty acid derivatives):** 3-octanone, isopentyl acetate, isopentyl butyrate.

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Species	Family	Chemical composition
Cattleya trianae	Orchidaceae	**Sesquiterpenoids:** β-bourbonene, *trans*-β-caryophyllene.
		Benzenoids: benzaldehyde, methyl benzoate, methyl salicylate, 2-phenyl ethanol, benzyl alcohol, methyl cinnamate.
		Oxygenated compounds (fatty acid derivatives): nonanal, octanal.
Vanilla planifolia	Orchidaceae	**Monoterpenoids:** α-pinene, β-pinene, β-myrcene, limonene, 1,8-cineole, *cis*-β-ocimene, *trans*-β-ocimene, *p*-cymene, terpinolene, *allo*-ocimene, 1,3,8-*p*-menthatriene, perillene, *cis*-limonene oxide, *trans*-limonene oxide, *cis*-salvene, *p*-cymenene, *cis-epoxy*-ocimene, myrtenal, terpinene-4-ol, *cis*-dihydrocarvone, *trans*-dihydrocarvone, limonen-4-ol, piperitone, borneol, 2,6-dimethyl-1,5,7-octatriene, carvone, dihydrocarveol, nerol, geraniol, *cis*-dihydrocarvone oxide, *trans*-dihydrocarvone oxide,
		cis-carveol, *trans*-carveol, carvacrol,
		Sesquiterpenoids: α-patchoulene, *trans*-salvene, guaiacol.
		Benzenoids: 2-phenyl ethanol, 4-methyl phenol.
		Oxygenated compounds (fatty acid derivatives): 1-(furan-2-yl) pentan-2-one, butanol, hexanol, octanol, nonanol.
		Sulfur- and nitrogen-containing compounds: methoxy phenyl oxime. **Hydrocarbons:** *trans*-2,4-undecadiene.
Vanilla pompona	Orchidaceae	**Monoterpenoids:** *cis*-geranyl acetone.
		Benzenoids: 4-methyl phenol.
		Oxygenated compounds (fatty acid derivatives): *cis*-3,7-dimethyl octa-2,6-dienal, nonanal, 6-methyl-5-hepten-2-one, hexanol, *cis*-3-hexen-1-ol, 2-ethylhexan-1-ol.
		Sulfur- and nitrogen-containing compounds: methoxy phenyl oxime. **Hydrocarbons:** *trans*-2-methyl-2-pentene.
Passiflora edulis	Passifloraceae	**Monoterpenoids:** *cis*-β-ocimene, *trans*-β-ocimene, *p*-cymene,
		cis-4,8-dimethyl-1,3,7-nonatriene, 1,3,8-p-menthatriene, *p*-cymenene, *trans, trans*-2,6-dimethyl-1,3,5,7-octatetraene.
		Sesquiterpenoids: β-gurjunene, *trans*-β-caryophyllene, aristolene, farnesol, *cis*-calamenene.
		Benzenoids: benzaldehyde, 1-methoxy 4-methyl benzene, anisole,
		4-ethyl resorcinol, 2-methoxy-4-methyl-1-(1-methylethyl) benzene, methyl benzoate, 3-hexen-1-yl benzoate, 1,2-dimethoxy benzene, 1,4-dimethoxy benzene, methyl salicylate, *p*-methoxy phenethyl alcohol, 3,5-dimethoxy toluene, 2-methoxy phenol, butyl benzoate, benzyl alcohol, 2-phenyl ethanol, methyl eugenol, anisaldehyde, methyl 2-methoxybenzoate, methyl 4-methoxybenzoate, *cis*-3-hexenyl benzoate, 1,2,4-trimethoxy benzene, benzyl tiglate, 1,3,5-trimethoxy benzene, eugenol,
		3,4-dimethoxyphenol, *p*-anisyl alcohol, 2-(4-methoxyphenyl) ethanol,
		4-methoxy phenol, benzyl benzoate.
		Oxygenated compounds (fatty acid derivatives): propanal,
		2-propanone, 2-hydroxy acetic acid, propanol, 2-butanone, 1-penten-3-one, *trans*-2-pentenal, *trans*-2-hexenal, 3-hydroxy-2-butanone, hexanol, *cis*-3-hexenol, *trans*-2-hexen-1-ol, acetic acid, *trans, trans*-2,4-heptadienal, octanol, dodecyl acetate, 2-hexyl hexanoic acid, benzyl *trans*-2-butenoate.
		Sulfur- and nitrogen-containing compounds: indole.

Species	Family	Chemical composition
Gardenia augusta	Rubiaceae	**Monoterpenoids:** β-myrcene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, *allo*-ocimene, *p*-cymene, linalool oxide, linalool, nerol, geraniol, cyperene. **Sesquiterpenoids:** α-*cis*-bergamotene, β-elemene, β-copaene, *trans*-β-caryophyllene, *cis*-muurola-3,5-diene, α-humulene, β-farnesene, germacrene D, α-muurolene, α-selinene, α-farnesene, α-cadinene, δ-cadinene, γ-cadinene, *cis*-calamenene, *epi*-α-muurolol, α-cadinol. **Benzenoids:** benzaldehyde, methyl salicylate, 2-phenyl ethanol. **Oxygenated compounds (fatty acid derivatives):** *trans*-2-hexenal, 2-pentyl furane, hexanol, *cis*-3-hexenol, 2-hexen-1-ol, 2-octenal, *trans*-2-octen-1-ol, *cis*-3-nonen-1-ol. **Sulfur- and nitrogen-containing compounds:** benzyl nitrile, indole. **Hydrocarbons:** tridecane, tetradecane, hexadecane.
Coffea arabica	Rubiaceae	**Monoterpenoids:** α-pinene, β-myrcene, limonene, 1,8-cineole, *cis*-β-ocimene, γ-terpinene, *trans*-β-ocimene, *p*-cymene, *p*-cymen-8-ol, perillene, *p*-cymenene, linalool oxide, nerol oxide, linalool, neral. **Benzenoids:** methyl benzoate, phenyl acetaldehyde, ethyl benzoate. **Oxygenated compounds (fatty acid derivatives):** propanal, 2-methyl propanal, 2-propenal, propanol, 2-butanone, 2-methyl-butenal, 3-methyl butanal, 2-pentanal, 3-pentanone, 1-penten-3-one, hexanal, *tert*-butyl alcohol, *trans*-2-methyl-2-butenal, *cis*-2-penten-1-al, 2-methyl-2-hexenone, heptanal, 2-methyl-1-butanol, 2-hexenal, 2-pentyl furane, 2-hexanol, 2-octanone, *cis*-2-penten-1-ol, *trans*-2-heptenal, 5-methyl-2-hexanol, 6-methyl-5-hepten-2-one, heptanol, 2,4-heptadienal, decanal, 2-nonenal, octanol, nonanol. **Sulfur- and nitrogen-containing compounds:** dimethyl sulfide, *o*-methyl oxime 2-propanone, 2-methyl butyl nitrile, 3-methyl butyl aldoxime. **Hydrocarbons:** tridecane, tetradecane, pentadecane, 1-pentadecene, 7-pentadecene, hexadecane, 3-hexadecene, 7-hexadecene, heptadecane.
Posoqueria longiflora	Rubiaceae	**Monoterpenoids:** *trans*-β-ocimene, γ-terpinene, *allo*-ocimene, linalool. **Sesquiterpenoids:** α-cubebene, β-bourbonene, β-copaene, *trans*-β-elemene, *trans*-β-caryophyllene, caryophyllene oxide, α-humulene, α-amorphene, germacrene D, α-muurolene, *trans*, *trans*-α-farnesene, γ-cadinene, cubebene, *cis*-calamene. **Benzenoids:** benzaldehyde, methyl benzoate, phenyl acetaldehyde, ethyl benzoate, benzyl acetate, methyl salicylate, benzyl alcohol, benzyl isovalerate, 2-phenyl ethanol, isopentyl benzoate, 2-methoxy-phenol, *trans*-methyl cinnamate, *cis*-3-hexyl benzoate, eugenol. **Oxygenated compounds (fatty acid derivatives):** isopentyl acetate, 1-penten-3-ol, 4-pentenyl acetate, 2-pentyl furane, 3-methyl-2-butenyl acetate, hexyl acetate, *cis*-3-hexyl acetate, *trans*-2-heptanal, *trans*-2-hexenyl acetate, hexanol, *cis*-3-hexenol, nonanal, *trans*-2-octenal, 1-octen-3-ol, octyl acetate, *trans, trans*-2,4-heptadienal, octanol, nonyl acetate, nonanol, *cis*-3-nonen-1-ol. **Sulfur- and nitrogen-containing compounds:** *cis*-3-methyl butyl aldoxime, *trans*-3-methyl butyl aldoxime, benzyl nitrile. **Hydrocarbons:** dodecane.

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Species	Family	Chemical composition
Brugmansia suaveolens	Solanaceae	**Monoterpenoids:** α-thujene, α-pinene, 6-methyl hept-5-en-2-one, sabinene, β-pinene, β-myrcene, α-terpinene, limonene, 1,8-cineole, *cis*-β-ocimene, *trans*-β-ocimene, *p*-cymene, α-terpinolene, *allo*-ocimene, citronellal, *p*-cymenene, *cis*-sabinene hydrate, linalool, terpinen-4-ol, citronellol, neral, geraniol, geranial. **Sesquiterpenoids:** *trans*-β-caryophyllene, *trans*-β-farnesene, α-terpineol, *trans, trans*-farnesol, farnesal, *trans*-nerolidol. **Benzenoids:** benzaldehyde, methyl benzoate, methyl salicylate, benzyl alcohol, 2-phenyl ethanol, 4-methoxy benzaldehyde, benzyl benzoate, benzyl salicylate. **Oxygenated compounds (fatty acid derivatives):** hexanal, *cis*-3-hexen-1-ol, hexanol, nonanal, decanal. **Sulfur- and nitrogen-containing compounds:** Indole. **Hydrocarbons:** *n*-dodecane, *n*-pentadecane.
Datura metel	Solanaceae	**Monoterpenoids:** linalool. **Oxygenated compounds (fatty acid derivatives):** 3-pentanone, 1-penten-3-ol, 3-methyl-1-butanol, 2-pentyl furane, hexanol, *cis*-3-hexenol, 4-methyl hexanol.
Petrea volubilis	Verbenaceae	**Monoterpenoids:** β-myrcene, Δ^3-carene, limonene, *cis*-β-ocimene, *trans*-β-ocimene, *p*-cymene, γ-terpinolene, 4,8-dimethyl-1,3,7-nonatriene, *allo*-ocimene, linalool, α-terpineol, nerol, geraniol, geranyl acetate. **Sesquiterpenoids:** α-copaene, *trans*-β-caryophyllene, farnesol, *trans, trans*-α-farnesene. **Benzenoids:** benzaldehyde, methyl salicylate, 2-phenyl ethanol, *cis*-3-hexenyl benzoate. **Oxygenated compounds (fatty acid derivatives):** *trans*-2-hexenal, 3-octanone, octanal, 1-octen-3-one, *cis*-2-penten-1-ol, hexanol, 3-penten-2-ol, *cis*-3-hexenol, 3-octanol, *trans*-2-hexen-1-ol, hexyl 2-methyl butanoate, 1-octen-3-ol, *cis*-3-hexenyl butanoate, *cis*-3-hexenyl 2-methyl butanoate, *cis*-3-hexenyl pentanoate, octanol, 1-nonen-3-ol, nonanol, *cis*-3-hexenyl angelate, *cis*-3-nonen-1-ol. **Hydrocarbons:** 3-methyl pentadecane.
Hedychium coronarium	Zingiberaceae	**Monoterpenoids:** α-pinene, β-pinene, β-myrcene, 1,8-cineole, *cis*-β-ocimene, *trans*-β-ocimene, *allo*-ocimene, γ-terpinene, terpinolene, linalool, *trans-p*-2-menthen-1-ol, terpinene-4-ol, α-terpineol, *cis*-jasmone. **Sesquiterpenoids:** *trans*-β-caryophyllene, caryophyllene oxide, *trans, trans*-α-farnesene, *trans, trans*-farnesol. **Benzenoids:** methyl benzoate, 2-methylbutyl benzoate, benzyl benzoate. **Oxygenated compounds (fatty acid derivatives):** *cis*-3-hexenol. **Sulfur- and nitrogen-containing compounds:** 1-nitro-2-methyl butane, *cis*-2-methyl butyl aldoxime, *trans*-2-methyl butyl aldoxime, 3-methyl butyl aldoxime, *trans*-3-methyl butyl aldoxime, phenyl acetonitrile, indole. **Hydrocarbons:** nonane.

Table 1. Volatile compounds isolated by ***in vivo*** HS-SPME from 30 tropical flowers, grown in Colombia.

The headspace methods provide information on the chemical composition of the volatile fractions; distillation techniques, on essential oils, distillates or condensates while extractive methods (solvents, supercritical CO_2), on the chemical composition of mixtures that may include substances of low-volatility, and higher molecular mass (> 400 Da), which in general are called extracts. The compositions of these mixtures can be differentiated not only quantitatively but also qualitatively. As mentioned above, in condensates and extracts will prevail "heavier" compounds, fatty acids, long-chain paraffinic hydrocarbons, their alcohols or aldehydes while in the volatile fractions, low-molecular-weight compounds are found, which eventually can "scape" during the distillation, in the depressurization stage (SFE-CO_2) or during the concentration of the extracts.

The chemical composition of the volatile fraction of flowers depends both on intrinsic (genetic) factors of the species and on extrinsic, environmental factors [9]. The habitat, the environment where the plant grows, the conditions (temperature, humidity, light, type of soil, micronutrients, etc.) in which floral secondary metabolites are monitored, will affect the qualitative and quantitative composition of the volatile fraction emitted and collected. For this reason, it is very important during the collection of floral volatiles to maintain control, continually monitoring conditions. Many external factors will affect the production of flower volatiles. These include changes in temperature, humidity, increase or decrease in light energy, among others. Some stress conditions (water, light, and nutrition) can notably alter the generation of floral volatiles or even suppress their production [10].

Some aspects of the study of floral fragrances should contemplate the state of development of the flower [11]. The flowers of the ylang-ylang tree (*Cananga odorata* Hook Fil. and Thomson, genuine form, Annonaceae family) are important raw material for obtaining essential and absolute oils, which are valuable ingredients in many perfumes, soaps, shampoos, and lotions. In the tree, the flower remains several weeks while it matures, it starts as a very small, green flower, which then increases in size, staying several days green, and then turns yellow, large, with brown spots. In the same tree, it is common to find flowers in different degrees of maturation, along with the fruits that carry seeds, through which this plant species spread. In the ylang-ylang tree, the composition of flower volatiles varies markedly with its state of maturity. In small, green flowers, 10 times fewer components are recorded, than in a yellow, mature flower. In the mature, yellow and fully developed ylang-ylang flowers, 16–4 times more light-oxygenated substances are found (*p*-methylanisole, benzyl alcohol, 1,8-cineole, methyl and ethyl benzoate and salicylate, linalool, nerol, geraniol, benzyl acetate, anethole, cinnamyl acetate, and others) and heavier oxygenated substances (sesquiterpenols, farnesal, farnesol, nerolidol and their acetates, cedrol, benzyl benzoate and benzyl salicylate, others), than in green, small flowers that begin their development. The nitrogen-containing compounds, phenyl acetonitrile, 4-methylbenzaldoxime, indole, 2-phenyl-1-nitroethane, and methyl anthranilate, only appear in mature, large and yellow flowers [12].

The composition of the secondary metabolites in the floral emission also varies according to the part of the flower from which the volatiles are extracted. In the petals of the ylang-ylang flowers, oxygenated compounds (oxygenated monoterpenes, benzenoids, and phenylpropanoids) prevail while in the ovaries (central part of the flower, small and compact) monoterpene and sesquiterpene hydrocarbons abound [13]. The relative percentage composition

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of the families of compounds present in the ylang-ylang flowers depends on the extraction method: steam distillation or simultaneous solvent distillation-extraction (SDE) allow mixtures of secondary metabolites to be obtained, rich in light oxygenated compounds (50–60%), and in heavy oxygenated compounds (18–20%) while extraction with supercritical fluid, SFE-CO_2, isolates extracts, rich in aliphatic hydrocarbons (Cn > 20) and terpenes, nitrogen-containing compounds, and even some fatty acids (C_{14}-C_{18}).

The profile of volatile compounds emitted by the flower also depends on the time of day; the insects that pollinate it can be diurnal or nocturnal, and from this, the kinetics of emanation of fragrant compounds and the type of volatile emitted by the flower will also depend, which vary, for most of the flowers, with the time of the day (circadian rhythm), and according to the biological function they fulfill. For example, in ylang-ylang flowers, the amount of nitrogenous substances changes during the day: it is maximum at dawn, decreases afternoon, and increases again in the afternoon and evening hours.

The flower fragrance of *Brugmansia suaveolens* (Solanaceae family) follows a clear circadian rhythm: the emission of volatiles increases in the evening and reaches its maximum at nine o'clock at night; then, the volatile emission slowly begins to decrease; in the morning and during the day, the flowers almost do not smell, although they attract massively the bees. It is interesting to note that some flowers change their fragrance after they have been pollinated; this happens with the flowers of some orchids (*Ophrys sphegodes*) [14].

Notorious changes can be observed (**Figure 1**) in *Vanilla pompona* (Orchidaceae family) volatile fraction isolated by HS-SPME from flowers after their pollination.

In carrion flower *Stapelia gigantea* (Apocynaceae family), which emitted fetid, nasty, and badly smelling volatiles (dimethyl disulfide, dimethyl trisulfide, butanoic acid, 3-methyl butanoic acid, hexanoic acid), the number of volatiles diminished after the oviposition of the green bottle fly (*Lucilia sericata*) had occurred (**Figure 2**).

Distinct parts of the flower fulfill different biological roles in it; for example, to protect from herbivores or to attract pollinators, to call for natural enemies or to increase or diminish

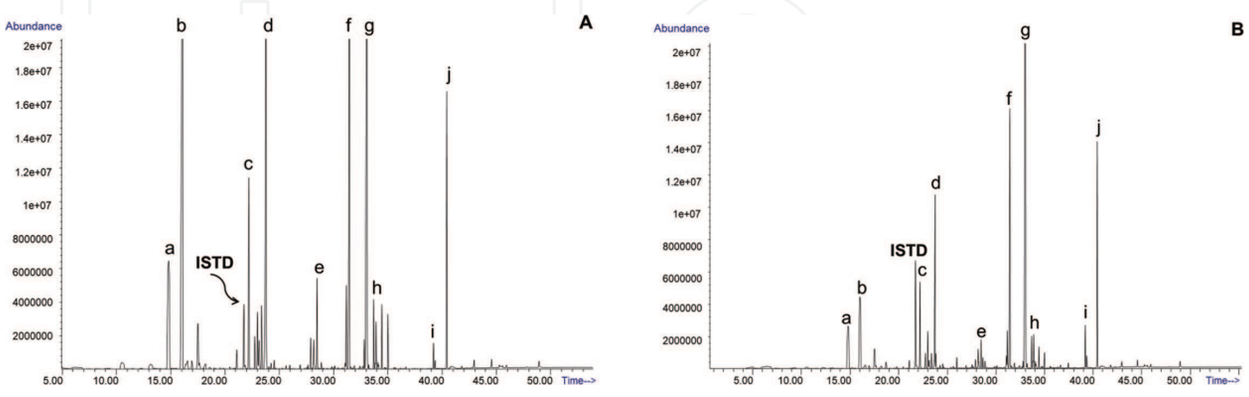

Figure 1. Chromatographic profiles (GC–MS, EI, 70 eV, DB-WAX column, 60 m) of *Vanilla planifolia* flower volatiles, isolated by *in vivo* HS-SPME (CAR/PDMS) at: (**A**) 7 a.m. before pollination and (**B**) 7 p.m. after pollination. Main compounds found in vanilla floral scent: (**a**) β-myrcene; (**b**) limonene; (**c**) *trans*-epoxy myrcene; (**d**) *trans*-limonene oxide; (**e**) *trans*-dihydrocarvone; (**f**) carvone; (**g**) *trans*-carvone oxide; (**h**) *trans*-carveol; (**i**) *p*-methyl phenol; and (**j**) *diepoxy* limonene. Internal standard (ISTD) – *n*-tetradecane.

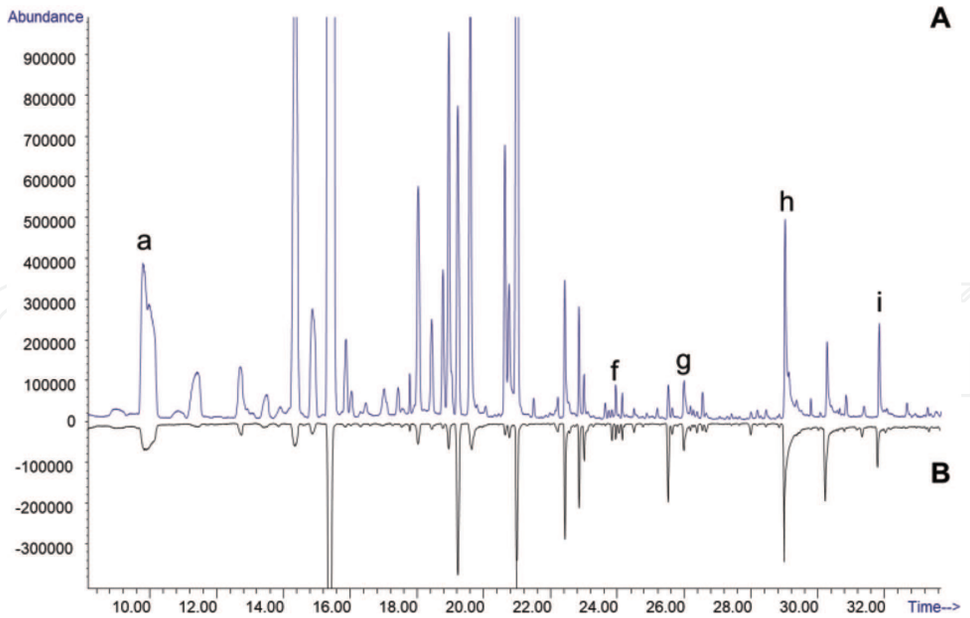

Figure 2. Chromatographic profiles (GC–MS, EI, 70 eV, DB-WAX column, 60 m) of volatiles isolated from *Stapelia gigantea* carrion flower, by *in vivo* HS-SPME (CAR/PDMS): (**A**) during oviposition and (**B**) after oviposition of the green bottle fly *Lucilia sericata* (Insecta: Diptera: Calliphoridae). Main compounds found in carrion flower odor are as follows: (**a**) dimethyl disulfide; (**b**) (3*E*-, 5*E*-)-2,6-dimethyl-1,3,5,7-octatetraene; (**c**) *trans*-β-ocimene; (**d**) dimethyl trisulfide; (**e**) 2,6-dimethyl-1,3,5,7-octatetraene (isomer); (**f**) butanoic acid; (**g**) 3-methyl butanoic acid; (**h**) methoxy phenyl oxime; and (**i**) hexanoic acid.

flower temperature or transpiration. **Figure 3** shows chromatographic profiles (HS-SPME/GC/MS) of volatiles emitted from distinct parts of passion fruit (*Passiflora edulis*) flower, where volatile compounds differ qualitatively or quantitatively; some of these volatile metabolites are unique to each part of the flower.

2.2. Chromatographic analysis of floral fragrances

The substances that make up the volatile fraction isolated from flowers are of low-molecular-weight (<300 Da) and are mixtures of components with different polarity and concentration. Thanks to the volatile nature of these compounds, their analysis is done by gas chromatography (GC). Due to the complexity of some mixtures of volatiles isolated from flowers and the presence in them of isomeric substances (geometrical, positional, stereoisomers), it is recommended to make their analysis in capillary fused-silica columns, preferably long, of 50–60 m, with internal diameters (DI) of 0.25, 0.22, or 0.20 mm. The smaller internal diameters, although they allow to increase the resolution, eventually, can also compromise the sensitivity. Columns with the thickness of the stationary phase (d_f) equal to or greater than 0.25 μm are used, so that the shape of peaks, their separation and the sensitivity, necessary for their reproducible detection, are adequate.

Generally, for the injection of the sample (T° of the injector, usually, of 230–250°C), the split ratio of 1:30 can be used, but when the concentrations of some components of interest are low, it is convenient to inject in splitless mode. When the splitless injection mode is used, to decrease the "dispersion" or the widening of the peaks of very volatile substances, the

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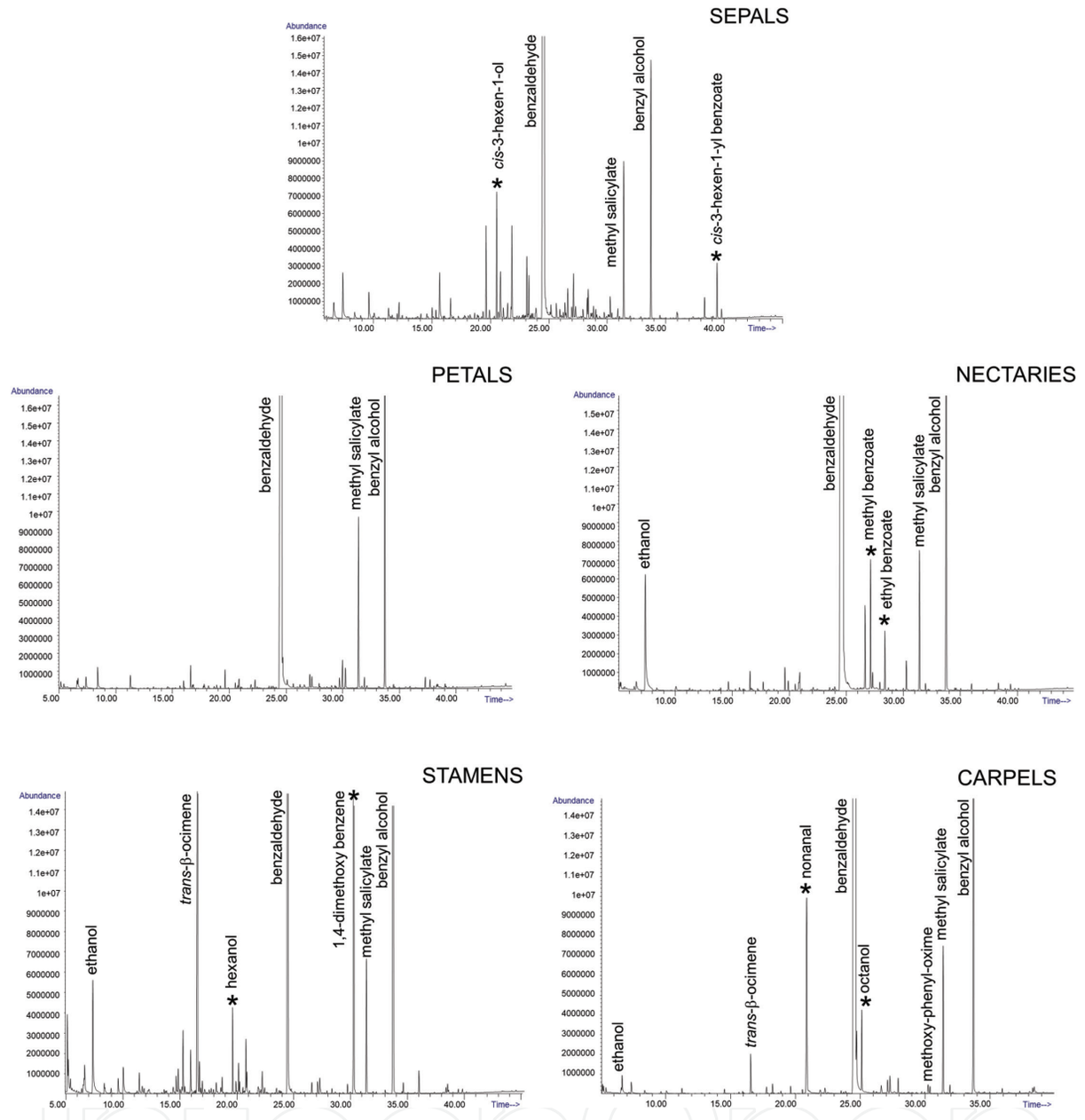

Figure 3. Chromatographic profiles (GC–MS, EI, 70 eV, DB-WAX column, 60 m) of volatiles, obtained by HS-SPME (CAR/PDMS) from distinct parts of passion fruit flower (*Passiflora edulis*). Each part of the flower (sepals, petals, nectaries, stamens or carpels) possesses a "diagnostic", unique volatile compounds (*), found only in this part of the flower.

injection can be done in the *pulsed splitless* mode; it is when the inlet pressure of the carrier gas, during the transfer of the sample by the liner, increases by 2–3 times. Considering the presence of some thermolabile substances (esthers, oximes), the *on-column* injection or the temperature-programmed injection (PTV, programmed-temperature vaporizer), could be a suitable alternative.

For the analysis of the volatile fractions, the initial column temperature of 35–50°C would be advisable; the nature of the sample (volatile compounds) does not require that the final temperature of the column be high; 200–250°C will be sufficient to elute the most retained

components. The heating speed of the column is a function of its length: the longer it is, the slower the column must be heated, 3–4°C/min, but if shorter columns are used, *e.g.,* 30 m, one could increase the temperature of the column more rapidly, at a rate of 5–10°C/min. Of course, the process of programming the temperature in the column is optimizable, depending on the complexity of the mixture of substances to be analyzed (number of components, isomerism or structural similarity), its nature (polarity, molecular weight), the column dimensions (L, DI), the type of the stationary phase (polarity), and its thickness (d_f).

For the analysis of the floral volatile fraction, two columns are used in combination: one with the polar stationary phase, poly(ethylene glycol) (e.g., INNOWAX, DB-WAX, HP-20 M, and others) and the other, with the non-polar stationary phase, poly (dimethyl siloxane) (HP-1, Ultra 1, DB-1, BP-1, and others) or 5% -phenyl poly (dimethyl siloxane) (HP-5, DB-5, Ultra 2, CPSil 5, BP-5, and others).

Enantioselective gas chromatography takes advantage of the fact that the enantiomers have different retention times when compounds that can form adducts are inserted in the stationary phase whose stability is a function of the three-dimensional (3D) form of the analyte. The cyclodextrins with their cone geometry with cavity of different size have turned out to be very effective chiral agents, constituting inclusion complexes that allow the discrimination of isomers according to their shape. The fragrances of jasmine (*Jasminum grandiflorum*) and other flowers (*Osmanthus fragrans*, *Boronia megastigma*) contain a mixture of methyl jasmonate stereoisomers. Wilfred König reported the separation of all isomers by means of preparative gas chromatography in which he used columns packed with cyclodextrins [15]. This allowed to confirm the estimate made by Acree and Barnard, that the methyl (+)-*epi*-jasmonate isomer has an odor threshold about 500 times lower than that of the major isomer, methyl (−)-jasmonate [16].

The aldehydes and lilac alcohols are oxygenated monoterpenes found in plant species of many families. Lamiaceae (*Origanum vulgare)* [17], Orchidaceae (*Platanthera* sp.) [18], Rosaceae (*Prunus padus)* [19], and Rubiaceae (*Cephalanthus occidentalis*) are some examples of these families. Each of the lilac molecules has three chiral carbons, which gives rise to eight stereoisomers of the aldehyde and eight stereoisomers of the lilac alcohol. Dötterl and colleagues [20] managed to separate all the isomers of the aldehyde and seven isomers of the lilac alcohol, by means of a two-dimensional gas chromatography system in which a 30 m-capillary column with stationary phase of 5%-phenyl poly (dimethyl siloxane) was bound by means of a T-valve to a mass selective detector, and another capillary column of 30 m with a stationary phase formed by phenyl-poly (dimethyl siloxane) (70%) and a cyclodextrin derivative (30%). Each column had an independent oven. This system was modified to convert it into micropreparative chromatography. The output of the second column was connected to a flow divider that allowed to directing a part of the effluent toward an FID, and the other part toward a stirring bar covered with PDMS, to absorb the separated analytes. This modification allowed to collecting the isomers that were then used in electroantennography experiments in which antennas of different insects were used to examine if there was any selective response for any of the isomers. It was found that the antennae of the *Hadena bicruris* moth responded to the eight isomers of aldehyde lilac, but they were more sensitive to some isomers than to others.

The most common detection system for comparative analysis and for the quantification of compounds in the volatile fractions isolated from flowers is the flame ionization detector

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(FID), a simple, robust system with an acceptable sensitivity, and a wide dynamic range. Selective detectors, such as the nitrogen and phosphorus detector (NPD) and the flame photometric detector (FPD), are very useful tools for the selective detection of nitrogenous and sulfur compounds, very common in floral fragrances.

The most important and widely used detection system in the analysis of volatile mixtures is the mass selective detector; its combination with capillary gas chromatography (GC-MS) is a perfect instrument to achieve separation and identification (presumptive or confirmatory) of components present in a mixture. The ionization mode most used for the analysis of volatile substances is the impact with electrons (EI) of 70 eV-energy. The EI mass spectra contain a lot of information because in the spectrum signals of numerous ionized fragments appear, which form a unique combination that allows to differentiate one molecule from the other, even if they are isomers.

The mass (*m*/*z*) of fragments (ions or ion-radicals) and their relative abundances, which make up the fragmentation pattern, are the guide to differentiate the structures. The linear retention indexes (LRI) measured experimentally in the polar and non-polar columns are compared with those recorded in the literature or in databases [21–24]. Probably, the greatest progress in the analysis of complex mixtures, such as essential oils or volatile fractions isolated from flowers, has been done with the introduction of comprehensive chromatography GC × GC [25–27]. The use of two orthogonal columns (non-polar and polar) linked through a modulator, and the use of a low-resolution (quadrupole, linear time-of-flight time, and TOF) or high-resolution (HR TOF) mass spectrometers, allow to have a complete picture on the number, and quantity of components in a mixture since some analytes can co-elute in one of the columns typically used in one-dimensional chromatography; but the use of two orthogonal

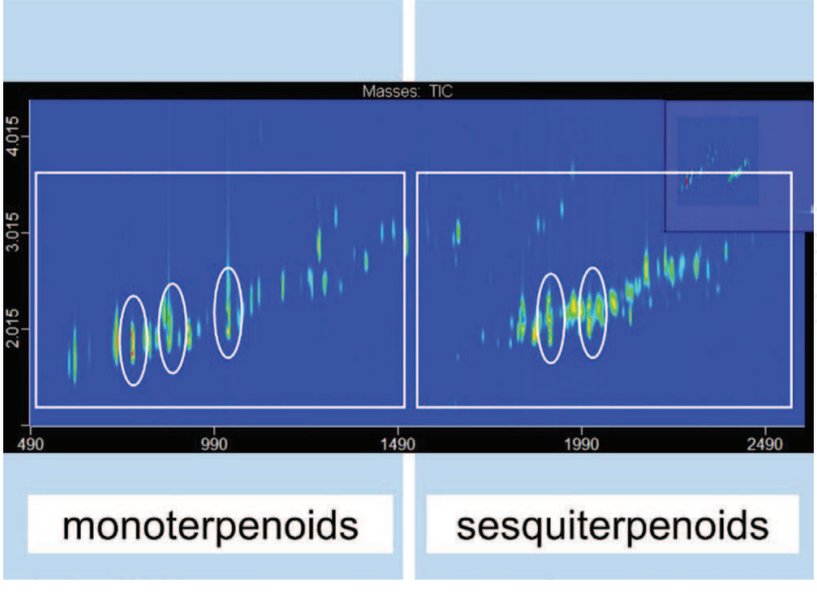

Figure 4. GCxGC chromatogram (TIC) of *Cannabis indica* female flower volatiles, obtained with high-resolution time-of-flight analyzer (HRTOF-MS) and cryogenic dual jet/loop modulator.1D – First column: Rxi-5MS, 30 m, L, 250 μm, DI, 0,25 μm, d_f. 2D – Second column: Rxi-17Sil MS, 2 m, L, 250 μm, DI, 0,25 μm, d_f. Modulation time: 5 s. Two groups of terpenoids, *that is*, monoterpenoids and sesquiterpenoids are clearly distinguished in the chromatogram. Compounds co-eluted in the 1D column are separated in the 2D column (areas of co-eluted peaks are encircled).

columns avoids this problem. In addition, GCxGC allows "classifying" the substances by families, such as can be observed in **Figure 4**, which shows two groups of substances, monoterpenes and sesquiterpenes, in the scent emitted by *Cannabis* female flowers.

3. Essential oil composition

Essential oils were obtained by steam distillation. High-resolution gas chromatography coupled to mass spectrometry was used for component identification. Relative amounts of essential oil constituents were calculated from the peak areas of chromatograms obtained with gas chromatography with flame ionization detection. Linear retention indices were determined on polar (Carbowax) and non-polar (DB-5) capillary chromatographic columns. Tentative compound identification was based on the comparison of retention indices with published values, and the comparison of mass spectra with those of databases [21–24]. **Table 2** presents the main constituents found in the gas chromatographic analysis of essential oils isolated from plant material collected in botanical expeditions carried out by CENIVAM.

Species	Composition
Aristolochiaceae family	
Aristolochia anguicida.	α-Ylangene (10%), *trans*-β-caryophyllene (27%), bicyclogermacrene (8%), α-humulene (3%), β-farnesene (5%).
Aristolochia ringens	β-Bourbonene (4%), β-elemene (10%), *trans*-β-caryophyllene (15%), *trans*-muurola-4(14),5-diene (17%), curzerene (23%), bicyclogermacrene (9%).
Asteraceae family	
Achyrocline alata	α-Pinene (3%), *p*-cymene (3%), thymol (24%), *trans*-β-caryophyllene (14%), thymyl acetate (2%).
Achyrocline satureioides	α-Pinene (7%), *trans*-β-caryophyllene (25%), γ-muurolene (9%), γ-cadinene (8%), caryophyllene oxide (13%).
Ageratina aff. *Popayanensis*	Thymol (26%), carvacrol (37%), δ-cadinene (1%).
Ambrosia arborescens	Chrysanthenone (14%), β-cubebene (4%), 2-ethyldien-6-methyl-heptadienal (4%), γ-curcumene (19%), *ar*-curcumene (8%), germacrene D (9%).
Ambrosia peruviana	γ-Curcumene (14%), *ar*-curcumene (25%), β-bisabolene (18%), spathulenol (5%), phytol (5%).
Austroeupatorium inulifolium	α-Pinene (2%), *trans*-β-caryophyllene (10%), germacrene D (17%), spathulenol (5%),caryophyllene oxide (4%).
Baccharis cf. nitida	α-Eudesmol (17%), squalene (1%), spathulenol (1%), caryophyllene oxide (1%).
Baccharis decussata	*trans*-β-Caryophyllene (17%), germacrene D (9%), *trans*-nerolidol (10%), premnaspirodiene (6%), γ-amorphene (6%).
Baccharis latifolia	α-Pinene (3%), limonene (8%), kessane (4%), viridiflorol (4%), *cis*-cadin-4-en-7-ol (5%), β-eudesmol (9%).
Baccharis trinervis	*trans*-β-Caryophyllene (20%), *trans*-β-guaieno (19%), viridiflorol (12%), germacrene D (14%), α-humulene (3%).

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Species	Composition
Bidens reptans	*p*-Cymene (3%), β-copaene (3%), germacrene D (3%), caryophyllene oxide (3%), 1-phenyl-hepta-1,3,5-triene (2%).
Calea glomerata	α-Zingiberene (27%), germacrene D (11%), *trans*-β-caryophyllene (7%), *ar*-curcumene (5%), limonene (3%).
Calea prunifolia	1,8-Cineole (4%), borneol (6%), *trans*-β-farnesene (3%), *ar*-curcumene (16%), α-zingiberene (14%).
Calea sessiliflora	α-Zingiberene (35%), germacrene D (17%), *ar*-curcumene (13%), viridiflorol (3%), β-sesquiphellandrene (4%).
Chromolaena pellia	Caryophyllene oxide (5%), β-amyrin (6%), germacrene D (3%), *trans*-β-caryophyllene (1%), squalene (6%).
Condylidium cuatrecasasii	Δ^2-Carene (7%), Δ^3-carene (37%), β-phellandrene (3%), *trans*-β-caryophyllene (7%), liguloxide (3%).
Conyza bonariensis	α-Pinene (8%), β-pinene (7%), α-phellandrene (4%), cyclofenchene (4%), isoelemicin (4%), caryophyllene oxide (5%), 1,3,5-trimethoxy-3-methyl-propenyl benzene (4%).
Ichthyothere terminalis	α-Pinene (4%), sabinene (40%), β-pinene (3%), terpinen-4-ol (7%), *trans*-β-caryophyllene (3%)
Parthenium hysterophorus	*trans*-β-Caryophyllene (12%), limonene (12%), germacrene B (6%), *p*-cymene (6%), caryophyllene oxide (6%).
Simsia fruticulosa	α-Thujene (11%), α-pinene (12%), β-myrcene (4%), α-copaene (4%), spathulenol (4%).
Stevia aff. Lucida	α-Pinene (25%), camphene (16%), β-pinene (11%), α-phellandrene (12%), *p*-cymene (5%), limonene (12%).
Stevia ovata	*trans*-β-Caryophyllene(10%), germacrene D (8%), bicyclogermacrene (5%), *trans*-nerolidol (19%), germacrene D-4-ol (4%), caryophyllene oxide (4%), guaiol (4%).
Tagetes caracasana	*cis*-β-Ocimene (12%), dihydrotagetone (16%), *allo*-ocimene (2%), *cis*-tagetone (58%), *trans*-ocimene (2%).
Tagetes heterocarpha	*cis*-β-Ocimene (3%), dihydrotagetone (13%), *cis*-tagetone (16%), *cis*-β-ocimene (6%), *trans*-ocimene (12%), spathulenol (5%).
Tagetes zipaquirensis	β-Myrcene (5%), *trans*-β-ocimene (12%), dihydrotagetone (42%), 6,7-epoxy myrcene (13%), *trans*-tagetone (3%), *cis*-tagetone (3%).
Verbesina centroboyaca	β-Myrcene (8%), α-humulene (4%), germacrene D (7%), germacrene D-4-ol (4%), hinesol (4%), valerianol (12%).
Wedelia calycina	Germacrene D (15%), β-phellandrene (14%), β-pinene (14%), α-pinene (20%), α-phellandrene (9%).
Boraginaceae family	
Cordia curassavica	α-Copaene (17%), *trans*-β-caryophyllene (22%), germacrene D (18%), *trans*-β-guaiene (8%), α-pinene (6%).
Burseraceae family	
Protium heptaphyllum	*trans*-β-Caryophyllene (20%), α-humulene (9%), γ-cadinene (13%), caryophyllene oxide (20%), germacrene D (3%).
Chloranthaceae family	
Hedyosmum racemosum	α-Pinene (6%), sabinene (21%), β-pinene (9%), 1,8-cineole (6%), *trans*-4-thujanol (5%).

Species	Composition
Euphorbiaceae family	
Croton ferrugineus	*trans*-β-Caryophyllene (37%), dilapiol (23%), germacrene D (13%), *cis*-chrysantenyl acetate (7%), bicyclogermacrene (3%).
Fabaceae family	
Dalea cliffortiana	Methyleugenol (69%), *trans*-β-caryophyllene (15%), α-humulene (1%), germacrene D (5%), caryophyllene oxide (3%).
Chenopodiaceae family	
Chenopodium ambrosioides	α-Terpinene (21%), *p*-cymene (15%), ascaridole (47%), *iso*-ascaridole (12%).
Lamiaceae family	
Eriope crassipes	6-Methyl-5-hepten-2-one (5%), α-cubebene (3%), α-copaene (3%), methyl citronellate (3%), α-muurolene (3%).
Hyptis brachiata	α-Copaene (5%), *trans*-β-caryophyllene (11%), germacrene D (16%), 7-*epi*-α-selinene (5%), caryophyllene oxide (4%).
Hyptis brachiata	Caryophyllene oxide (2%), *trans*-nerolidol (2%), α-humulene (2%), carvacrol (5%).
Hyptis colombiana	Germacrene D (22%), *trans*-β-caryophyllene (34%), caryophyllene oxide (14%), α-copaene (5%), bicyclogermacrene (3%).
Hyptis dilatata	*trans*-β-Caryophyllene (26%), Δ^3-carene (4%), α-gurjunene (10%), camphor (8%), palustrol (9%).
Hyptis mutabilis	β-Elemene (6%), *trans*-β-caryophyllene (6%), germacrene D (12%), curzerene (30%), β-bourbonene (2%).
Hyptis pectinata	β-Bourbonene (8%), *trans*-β-caryophyllene (15%), germacrene D (18%), bicyclogermacrene (7%), caryophyllene oxide (5%).
Hyptis suaveolens	Sabinene (2%), α-phellandrene (2%), fenchone (7%), *trans*-β-caryophyllene (13%), germacrene D (3%).
Lepechinia betonicifolia	α-Pinene (8%), β-pinene (7%), limonene (10%), 1,8-cineole (8%), *trans*-β-caryophyllene (7%), γ-curcumene (7%), α-zingiberene (8%), ledol (8%).
Lepechinia bullata	β-Pinene (13%), α-copaene (4%), *trans*-β-caryophyllene (21%), α-humulene (7%), caryophyllene oxide (17%).
Lepechinia conferta	α-Pinene (5%), β-pinene (10%), *p*-cymene (17%), palustrol (8%), α-cedrene (4%).
Lepechinia salviifolia.	α-Pinene (3%), β-pinene (10%), Δ^3-carene (5%), limonene (21%), *trans*-β-caryophyllene (6%), ledol (4%).
Lepechinia vulcanolica	α-Pinene (8%), 3-octenol (9%), limonene (19%), *trans*-β-caryophyllene (9%), germacrene D (10%), palustrol (7%).
Marsypianthes chamaedrys	α-Copaene (5%), *trans*-β-caryophyllene (4%), spathulenol (6%), caryophyllene oxide (15%), humulene II epoxide (5%).
Mentha suaveolens	*cis*-β-Ocimene (4%), 1-octen-3-yl acetate (4%), piperitenone oxide (52%), nepetalactone (6%), germacrene D (10%).
Minthostachys mollis	Menthone (7%), pulegone (6%), *cis*-piperitone epoxide (30%), piperitenone oxide (26%), *trans*-β-caryophyllene (5%), germacrene D (6%).
Minthostachys septentrionalis	Piperitone epoxide (59%), piperitone oxide (13%), *trans*-β-caryophyllene (9%), germacrene D (1%), α-humulene (2%).

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Species	Composition
Ocimum americanum	Linalool (23%), estragole (63%), *cis*-α-bisabolene (4%).
Ocimum campechianum	Methyleugenol (54%), 1,8-cineole (3%), *trans*-β-caryophyllene (13%), α-humulene (3%), germacrene D (3%).
Ocimum tenuiflorum	Eugenol (22%), β-elemene (23%), *trans*-β-caryophyllene (23%), α-humulene (3%), germacrene D (5%).
Perilla frutescens	Perilla ketone (48%), 1-octen-3-ol (32%), linalool (6%), 3-octanone (5%), 3-octanol (3%).
Plectranthus amboinicus	Carvacrol (13%), *trans*-β-caryophyllene (1%) α-amirine (38%), viminalol (21%), estigmast-4-en-3-one (10%).
Salvia aratocensis	*trans*-β-Caryophyllene (5%), γ-cadinene (7%), 1,10-di-*epi*-cubenol (12%), *epi*-α-cadinol (16%).
Salvia aratocensis subsp. suratensis	*trans*-β-Caryophyllene (5%), *cis*-β-farnesene (2%), γ-cadinene (10%), 1-*epi*-cubenol (16%), *epi*-α-cadinol (22%).
Salvia rubriflora	*trans*-β-Caryophyllene (13%), α-farnesene (9%), spathulenol (8%), caryophyllene oxide (5%).
Salvia sagitatta	*cis*-Pinocamphone (6%), linalool acetate (5%), α-terpinyl acetate (24%), *trans*-β-caryophyllene (6%), palustrol (7%), curzerenone (10%).
Satureja aff. Andrei	Limonene (2%), *p*-mentha-3,8-diene (4%), *cis*-pulegol (6%), pulegone (23%), *trans*-β-caryophyllene (7%).
Myrtaceae family	
Calycolpus moritzianus	1,8-Cineole (20%), α-terpineol (6%), *trans*-β-caryophyllene (8%), guaiol (5%), γ-eudesmol (7%).
Psidium sartorianum	1,8-Cineole (16%), terpinen-4-ol (11%), α-terpineol (13%), *trans*-β-caryophyllene (20%), β-pinene (3%).
Piperaceae family	
Piper auritum	Safrol (9%), myristicin (5%).
Piper bogotense	α-Pinene (9%), α-phellandrene (14%), *p*-cymene (4%), limonene (5%), linalool (5%), *trans*-sesquisabinene hydrate (14%)
Piper bremedeyeri	α-Pinene (20%), β-pinene (32%), limonene (4%), β-elemene (4%), *trans*-β-caryophyllene (6%), germacrene D (4%).
Piper carpunya	*p*-Cymene (11%), 1,8-cineole (11%), safrol (12%), methyleugenol (5%), cumunyl acetate (5%)
Piper cf. divaricatum	α-Pinene (11%), β-pinene (5%), α-phellandrene (6%), 1,8-cineole (18%), linalool (15%), *trans*-β-caryophyllene (8%).
Piper cf. subflavum	Apiol (27%), dillapiol (1%), *trans*-β-caryophyllene (1%), δ-cadinene (1%), *cis*-calamenene (1%).
Piper marginatum	α-Phellandrene (11%), limonene (8%), β-elemene (4%), *trans*-β-caryophyllene (11%), bicyclogermacrene (4%).
Piper medium	β-Phellandrene (22%), germacrene D (12%), *trans*-β-caryophyllene (6%), bicyclogermacrene (4%), *trans*-β-elemene (3%).
Scrophulariaceae family	
Achetaria bicolor	*trans*-Pinocarveol (8%), pinocarvone (6%), α-humulene (18%), humulene II epoxide (5%).
Turneraceae family	
Turnera diffusa	Drima-7,9(11)-diene (23%), valencene (6%), β-selinene (6%), viridiflorene (7%), dihydrokaranone (15%).

Species	Composition
Verbenaceae family	
Dalea coerulea	α-Phellandrene (6%), *p*-cymene (8%), β-phellandrene (13%), piperitone (5%), α-caracolene (7%), spathulenol (5%).
Lantana boyacana	α-Pinene (5%), sabinene (11%), 1,8-cineole (14%), *trans*-β-caryophyllene (9%), α-humulene (6%).
Lantana fucata	β-Phellandrene (4%), *trans*-β-caryophyllene (14%), α-zingiberene (6%), germacrene D (10%), δ-cadinene (3%), caryophyllene oxide (7%).
Lippia alba	Limonene (30%), carvone (50%), piperitone (3%), piperitenone (6%), bicyclosesquiphellandrene (4%).
Lippia americana	*trans*-β-caryophyllene (21%), germacrene D (12%), δ-cadinene (6%), caryophyllene oxide (13%), β-cubebene (5%).
Lippia canescens	*trans*-β-caryophyllene (27%), α-humulene (12%), caryophyllene oxide (8%), limonene (7%), *p*-cymene (6%).
Lippia micromera	*p*-Cymene (13%), γ-terpinene (9%), thymol methyl ether (25%), thymol (27%), γ-terpinene (9%).
Lippia origanoides	γ-Terpinene (6%), *trans*-β-caryophyllene (7%), α-humulene (4%), caryophyllene oxide (2%), *p*-cymene (10%).

Table 2. Main constituents of essential oils of species collected in botanical outings.

4. Biological activity of essential oils

One of the most important research lines of the CENIVAM Project is related to the study of different biological activities of essential oils. Bioactivity assays against *Trypanosoma cruzi* (epimastigotes and amastigotes), *Leishmania chagasi* (promastigotes and amastigotes), Vero cell assays, and THP cells were carried out at the Research Center for Tropical Illnesses, CINTROP. About 48% of the essential oils examined were active against *T. cruzi*. The essential oils were also tested against *L. chagasi* (promastigotes and amastigotes) and 19% were active [28, 29]. The virucidal activity of some essential oils was studied at the CINTROP, against the dengue (serotype 2) [30–33], and yellow fever [31, 34] viruses. Of the essential oils tested, respectively, 83 and 66% were active against this type of virus, constituting this result in a very interesting contribution, especially considering that there is not much data in the literature on the activity of essential oils against this type of virus. The essential oils of two chemotypes of *Lippia origanoides* (Fam. Verbenaceae) were in vitro potent agents against dengue and yellow fever viruses, which warrant the future study of the mechanism of their antiviral action.

Different CENIVAM groups, among them, in the Research Center for Biomolecules, CIBIMOL [35, 36], Environmental and Computational Chemistry of the University of Cartagena [37, 38], Chemistry and Biology of the Universidad del Norte, in Barranquilla [39] measured the antioxidant activity of essential oils by different techniques, e.g., lipid oxidation, measurement of secondary end products of lipoxidation, thiobarbituric acid reactive substances (TBARS) test, and free radical trapping tests (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, ABTS + and 2,2-diphenyl-1-picrylhydrazyl, DPPH). Grosso modo, about 40% of the analyzed samples were active in different tests of antioxidant activity.

The study of the cytotoxic activity (acute toxicity, LC50) of the essential oils was carried out by several groups. Cecilia Mesa et al. examined the activity against *Candida krusei* and *Aspergillus*

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fumigatus [40]. *Lippia origanoides* and *L. alba* have several chemotypes, and these have been the subject of detailed study to check that their cytotoxicity does not prevent their use in some topical pharmaceutical applications [41, 42]. The group of Environmental and Computational Chemistry has used the *Artemia franciscana* assay to test for acute toxicity [43]. More than 30% of all samples analyzed in these trials did not have any degree of toxicity. In the same group, anti-quorum sensing activities, teratogenic and antigenotoxic effects, and the insect repellent activity of essential oils were studied [44, 45].

Insect repellency is an interesting biological activity that leads to rather soon implementation of essential oils as active ingredients of commercial products. Olivero et al. have examined the potential application of essential oils to repel insects of importance to food storage [46–49]. Another application of insect repellence is the prevention of diseases for which *Aedes aegypti* is the vector [50–52]. More than 50% of the tested essential oils and pure terpenes proved to be good insect repellents (56 and 80%, respectively).

The assays of the anti-genotoxic and chemopreventive activity carried out at the CIBIMOL-UIS group demonstrated a DNA protective effect of the essential oils of several chemotypes of *Lippia alba* and *Lippia origanoides* (Fam. Verbenaceae) [53–56].

Several bacterial strains have been employed in assays of essential oil antibacterial activity [57, 58]. Due to their carvacrol and thymol content, *L. origanoides* oils have shown important antibacterial activity [59]. Antimycobacterial activity, which is of interest in tuberculosis research, has received special attention by CENIVAM researchers [60, 61]. It has been determined in oils from the state of Santander [62].

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Conflict of interest

The authors declare that they have no conflict of interest with this chapter contents.

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References

[1] Dudareva NA, Pichersky E, editors. Biology of Floral Scent. Boca Raton, USA: CRC, Taylor & Francis Group; 2006. 346 p

[2] Stashenko EE, Martínez JR. Sampling flower scent for chromatographic analysis. Journal of Separation Science. 2008;**31**:2022-2031. DOI: 10.1002/jssc.200800151

[3] Stashenko EE, Martínez JR. Sampling volatile compounds from natural products with headspace/solid-phase micro-extraction. Journal of Biochemical and Biophysical Methods. 2007;**70**:235-242. DOI: 10.1016/j.jbbm.2006.08.011

[4] Choi H-S. Characterization of *Citrus unshiu* (*C. unshiu* Marcov. Forma *Miyagawa-wase*) blossom aroma by solid-phase microextraction in conjunction with an electronic nose. Journal of Agricultural and Food Chemistry. 2003;**51**:418-423

[5] Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. Diversity and distribution of floral scent. The Botanical Review. 2006;**72**:1-120

[6] Knudsen JT, Gershenzon J. The chemical diversity of floral scent. In: Dudareva NA, Pichersky E, editors. Biology of Floral Scent. Boca Raton, USA: CRC, Taylor & Francis Group; 2006. p. 40

[7] Augusto F, Leite e Lopes A, Zini C. Sampling and sample preparation for analysis of aromas and fragrances. Trends in Analytical Chemistry. 2003;**22**:160-169. DOI: 10.1016/S0165-9936(03)00304-2

[8] Flamini G, Cioni PL, Morelli I. Use of solid-phase micro-extraction as a sampling technique in the determination of volatiles emitted by flowers, isolated flower parts and pollen. Journal of Chromatography. A. 2003;**998**:229-233. DOI: 10.1016 /S0021-9673(03)00641-1

[9] Xiang L, Milc JA, Pecchioni N, Chen L. Genetic aspects of floral fragrance in plants. Biochemistry (Moscow). 2007;**72**:351-358. ISSN 0006_2979

[10] Bernhardt P, Sage T, Weston P, Azuma H, Lam M, Thiem L, Bruhl J. The pollination of *Trimenia moorei* (Trimeniaceae): Floral volatiles, insect/wind pollen vectors and stigmatic self-incompatibility in a basal angiosperm. Annals of Botany. 2003;**92**:445-458. DOI: 10.1093/aob/mcg157

[11] Granero AM, Egea Gonzalez FJ, Guerra Sanz JM, Martínez Vidal JL. Analysis of biogenic volatile organic compounds in zucchini flowers: Identification of scent sources. Journal of Chemical Ecology. 2005;**31**:2309-2322. DOI: 10.1007/s10886-005-7103-2

[12] Stashenko EE, Quiroz N, Martínez JR. HRGC/FID/NPD and HRGC/MSD study of Colombian ylang-ylang (*Cananga odorata*) oils obtained by different extraction techniques. Journal of High Resolution Chromatography. 1996;**19**:353-360

[13] Stashenko EE, Martínez JR, Macku C, Shibamoto T. HRGC and GC-MS analysis of essential oil from Colombian Ylang-Ylang (*Cananga odorata* Hook Fil. et Thomson, *forma genuina*). Journal of High Resolution Chromatography. 1993;**16**:441-444

[14] Schiestl FP, Ayasse M, Paulus HF, Lofstedt C, Hansson BS, Ibarra F, Franche W. Orchid pollination by sexual swindle. Nature. 1999;**399**:421-422

[15] König WA. In: Lough WJ, Wainer IW, editors. Chirality in the Natural World—Odours and Tastes. En: Chirality in Natural and Applied Sciences. Oxford, UK: Blackwell Publishers; 2002. pp. 261-284

[16] Acree TE, Barnard J. The analysis of odour-active volatiles in gas chromatographic effluents. In: Shreier P, editor. Analysis of Volatiles. Berlin, Germany: de Gryter; 1984. pp. 251-267

[17] Andersson S, Nilsson LA, Groth I, Bergström G. Floral scents in butterfly-pollinated plants: Possible convergence in chemical composition. Botanical Journal of the Linnean Society. 2002;**140**:129-153. DOI: 10.1046/j.1095-8339.2002.00068. x

[18] Tollsten L, Bergström G. Fragrance chemotypes of *Platanthera* (Orchidaceae)—The result of adaptation to pollinating moths? Nordic Journal of Botany. 1993;**13**:607-613. DOI: 10.1111/j.1756-1051. 1993.tb00105.x

[19] Surburg H, Güntert M, Schwarze B. Volatile constituents of European bird cherry flowers (*Padus avium* mill.). Journal of Essential Oil Research. 1990;**2**:307-316. DOI: 10.1080/10412905.1990.9697889

[20] Dötterl S, Burkhardt D, Weißbecker B, Jürgens A, Schütz S, Mosandl A. Linalool and lilac aldehyde/alcohol in flower scents. Electrophysiological detection of lilac aldehyde stereoisomers by a moth. Journal of Chromatography A. 2006;**1113**:231-238. DOI: 10.1016/j.chroma.2006.02.011

[21] Davies NW. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. Journal of Chromatography. 1990;**503**:1-24

[22] Adams RP. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectrometry. Carol Stream, IL: Allured Publishing; 2001

[23] Joulain D, Konig WA. The Atlas of Spectral Data of Sesquiterpene Hydrocarbons. Hamburg: E. B. Verlag; 1998

[24] Babushok VI, Zenkevich I. Retention indices for most frequently reported EO compounds in GC. Chromatographia. 2009;**69**(3/4):257-269

[25] Marriott P, Shellie R, Cornwell C. Gas chromatographic technologies for the analysis of essential oils. Journal of Chromatography A. 2001;**936**:1-22

[26] Shellie RA, Marriott PJ. Comprehensive two-dimensional gas chromatography-mass spectrometry analysis of *Pelargonium graveolens* EO using rapid scanning quadrupole mass spectrometry. Analyst. 2003;**128**:879-883

[27] Tranchida PQ, Zoccali M, Franchina FA, Bonaccorsi I, Dugo P, Mondello L. Fast gas chromatography combined with a high-speed triple quadrupole mass spectrometer for the analysis of unknown and target citrus essential oil volatiles. Journal of Separation Science. 2013;**36**:511-516

[28] Escobar P, Leal SM, Herrera LV, Martínez JR, Stashenko EE. Chemical composition and antiprotozoal activities of Colombian Lippia spp. essential oils and their major components. Memórias do Instituto Oswaldo Cruz. 2010;**105**:184-190

[29] Neira LF, Mantilla JC, Stashenko EE, Escobar P. Toxicidad, genotoxicidad y actividad anti-Leishmania de aceites esenciales obtenidos de cuatro quimiotipos del género *Lippia*. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2018; **17**(1):68-83

[30] Yañez-Rueda X, Betancur-Galvis L, Agudelo-Gómez LS, Zapata MB, Correa-Royero J, Mesa-Arango AC, Stashenko EE. Composición química y actividad biológica de aceites esenciales de Calycopus moritzianus recolectado en el Norte de Santander, Colombia. Revista de la Universidad Industrial de Santander. Salud. 2009;**41**:259-267

[31] Meneses R, Torres FA, Stashenko EE, Ocazionez RE. Aceites esenciales de plantas colombianas inactivan el virus del dengue y el virus de la fiebre amarilla. Revista de la Universidad Industrial de Santander. Salud. 2009;**41**:236-243

[32] Ocazionez RE, Meneses R, Torres FA, Stashenko EE. Virucidal activity of Colombian Lippia essential oils on dengue virus replication in vitro. Memórias do Instituto Oswaldo Cruz. 2010;**105**:304-309

[33] Flechas MC, Ocazionez RE, Stashenko EE. Evaluation of in vitro antiviral activity of essential oil compounds against dengue virus. Pharmacognosy Journal. 2018;**10**(1):55-59

[34] Meneses R, Ocazionez RE, Martínez JR, Stashenko EE. Inhibitory of essential oils obtained from plants grown in Colombia on yellow fever virus replication in vitro. Annals of Clinical Microbiology and Antimicrobials. 2009;**8**:1-6

[35] Stashenko EE, Ruiz C, Muñoz A, Castañeda M, Martínez JR. Composition and antioxidant activity of essential oils of Lippia origanoides H.B.K. grown in Colombia. Natural Product Communications. 2008;**3**(4):563-566

[36] Tafurt G, Martínez JR, Stashenko EE. Evaluación de la actividad antioxidante de aceites esenciales en emulsiones degradadas por radiación ultravioleta. Revista Colombiana de Química. 2005;**34**(1):43-55

[37] Olivero J, González T, Guette J, Jaramillo B, Stashenko EE. Chemical composition and antioxidant activity of essential oils isolated from Colombian plants. Revista Brasileira de Farmacognosia. 2010;**20**:568-574

[38] Jaramillo BE, Stashenko EE, Martínez JR. Composición química volátil de Satureja brownei (Sw.) Briq.y determinación de su actividad antioxidante. Revista Cubana de Plantas Medicinales. 2010;**15**:52-63

[39] Munoz A, Kouznetsov VV, Stashenko EE. Composición y capacidad antioxidante in-vitro de aceites esenciales ricos en timol, carvacrol, trans-anetol o estragol. Salud UIS. 2009;**41**:287-294

[40] Correa-Royero J, Tangarife V, Durán C, Stashenko EE, Mesa-Arango A. In vitro antifungal activity and cytotoxic effect of essential oils and extracts of medicinal and aromatic

plants against *Candida krusei* and *Aspergillus fumigatus*. Brazilian Journal of Pharmacognosy. 2009;**20**:734-741

[41] Zapata B, Durán C, Stashenko EE, Correa-Royero J, Betancur-Galvis L. Actividad citotóxica de aceites esenciales de *Lippia origanoides* H.B.K. y componentes mayoritarios. Revista de la Universidad Industrial de Santander. Salud. 2009;**41**:215-222

[42] Mesa-Arango AC, Montiel-Ramos J, Zapata B, Durán C, Betancur-Galvis L, Stashenko EE. Citral an carvone chemotypes from the essential oils of Colombian *Lippia alba* (Mill.) N.E. Brown: Composition, cytotoxicity and antifungal activity. Memorias do Instituto Oswaldo Cruz. 2009;**104**:878-884

[43] Olivero-Verbel J, Güette-Fernandez J, Stashenko EE. Acute toxity against *Artemia franciscana* of essential oils isolated from plants of the genus *Lippia* and *Piper* collected in Colombia. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2009;**8**:419-427

[44] Olivero-Verbel J, Barreto-Maya A, Bertel-Sevilla A, Stashenko E. Composition, anti-quorum sensing and antimicrobial activity of essential oils from *Lippia alba*. Brazilian Journal of Microbiology. 2014;**45**(3):759-767

[45] Jaramillo B, Olivero J, Stashenko E, Wagner-Döbler I, Kunze B. Anti-quorum sensing activity of essential oils from Colombian plants. Natural Product Research. 2012; **26**(12):1075-1086

[46] Olivero-Verbel J, Caballero-Gallardo K, Jaramillo-Colorrado B, Stashenko EE. Actividad repelente de los aceites esenciales de Lippia origanoides, Citrus sinensis y Cymbopogon nardus cultivadas en Colombia frente a Tribolium castaneum, Herbst. Revista Salud UIS. 2009;**41**:244-250

[47] Olivero-Verbel J, Nerio LS, Stashenko EE. Bioactivity against Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) of Cymbopogon citratus and Eucalyptus citriodora essential oils grown in Colombia. Pest Management Science. 2010;**66**:664-668

[48] Nerio LS, Olivero-Verbel J, Stashenko EE. Repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus zeamais* Motschulsky (Coleotera). Journal of Stored Products Research. 2009;**45**:212-214

[49] Caballero K, Olivero J, Stashenko EE. Repellent activity of essential oils and some of their individual constituents against Tribolium castaneum herbst. Journal of Agricultural and Food Chemistry. 2011;**59**:1690-1696

[50] Carreño AL, Vargas LY, Duque JE, Kouznetsov VV. Design, synthesis, acetylcholinesterase inhibition and larvicidal activity of girgensohnine analogs on *Aedes aegypti*, vector of dengue fever. European Journal of Medicinal Chemistry. 2014;**78**:392-400

[51] Castillo R, Stashenko EE, Duque JE. Insecticidal and repellent activity of several plant-derived essential oils against *Aedes aegypti*. Journal of the American Mosquito Control Association. 2017;**33**(1):25-35

[52] Ríos N, Stashenko EE, Duque JE. Evaluation of the insecticidal activity of essential oils and their mixtures against *Aedes aegypti* (Diptera: Culicidae). Revista Brasileira de Entomologia. 2017;**61**:307-311

[53] Vicuña GC, Stashenko EE, Fuentes JL. Chemical composition of the Lippia origanoides essential oils and their antigenotoxicity against bleomycin-induced DNA damage. Fitoterapia. 2009;**81**:343-349

[54] López MA, Stashenko EE, Fuentes JL. Chemical composition and antigenotoxic properties of *Lippia alba* essential oils. Genetics and Molecular Biology. 2011;**34**:479-488

[55] Fuentes JL, Garcia-Forero A, Quintero N, Prada-Medina N, Rey N, Franco DA, Contreras DA, Córdoba Y, Stashenko EE. The SOS Chromotest applied for screening plant antigenotoxic agents against ultraviolet radiation. Photochemical & Photobiological Sciences. 2017;**16**:1424-1434

[56] Quintero N, Córdoba N, Stashenko EE, Fuentes JL. Antigenotoxic effect against ultraviolet radiation-induced DNA damage of the essential oils from Lippia species. Photochemistry and Photobiology. 2017;**93**(4):1063-1072

[57] Pino Benítez N, Stashenko EE. Validación antibiótica de plantas medicinales de noroeste de Colombia contra *Staphylococcus aureus*. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2009;**8**:145-150

[58] Pino Benítez N, Melendez E, Stashenko EE. Composición química y actividad antibacteriana del aceite esencial de hojas de Piper lanceaefolium, planta usada tradicionalmente en Colombia. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas. 2009;**8**:301-304

[59] Sarrazin SLF, da Silva LA, Assunção d, Oliveira RB, Calao VYP, da Silva R, Stashenko EE, Maia JGS, Mourão RHV. Antimicrobial and seasonal evaluation of the Carvacrol-Chemotype oil from Lippia origanoides Kunth. Molecules. 2015;**20**:1860-1871

[60] Bueno J, Coy ED, Stashenko EE. Antimycobacterial natural products – and opportunity for the Colombian biodiversity. Revista Espanola de Quimioterapia. 2011;**24**:175-183

[61] Bueno J, Escobar P, Martínez JR, Leal SM, Stashenko EE. Composition of three essential oils, and their mammalian cell toxicity and antimycobacterial activity against drug resistant tuberculosis and nontuberculosis mycobacteria strains. Natural Product Communications. 2011;**6**:1743-1748

[62] Moreno-Vargas MF, González LA, Martínez JR, Stashenko EE, Ribón W. Evaluation of the antimycobacterial activity of four essential oils derived from endemic plants of Santander-Colombia against mycobacterium tuberculosis. Natural Product Comunications. 2016;**1**(0):1-2