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Chapter

Study of Essential Oils Obtained from Tropical Plants Grown in Colombia

Elena Stashenko and Jairo René Martínez

Abstract

Researchers from several Colombian universities have joined efforts for over 15 years to characterize the composition and biological properties of more than a thousand samples of essential oils (EOs) obtained from aromatic plants collected during at least 30 botanical outings in different regions of Colombia. This chapter presents a brief description of essential oil extraction and chemical characterization techniques, followed by a representative list of references to publications on EO composition obtained from tropical aromatic plants that grow in Colombia. Opportunities for the development of interesting products for the pharmaceutical, cosmetics, hygiene, and food industries are illustrated with a few selected works on the evaluation of cytotoxicity, antioxidant, antiviral, antigenotoxic activities, and repellence of these essential oils.

Keywords: *Lippia*, CENIVAM , antioxidant, antigenotoxic, antiviral, repellence

1. Introduction

Colombia, located at South America's northwest, has coasts on the Caribbean and the Pacific Ocean, extensive prairies and mountains with many forests, wild pastures and cultivated land, rivers, and lakes. The country is rich in many natural resources and water. Contrasting landscapes and varied climatic conditions have made it after Brazil, the second most biodiverse country. This biodiversity includes medicinal and aromatic plants; most native aromatic plants remain unexamined. The aromatic herbs and spices commonly used in everyday life were brought to Colombia by the Spanish conquerors five centuries ago (basil, chamomile, mint, parsley, oregano, rosemary, sage, thyme, etc.); some (citronella, lemongrass, clove, ginger, cinnamon) were introduced later, in the last two centuries.

The extension of land cultivated with medicinal and aromatic plants more than doubled between 2007 (1253 ha) and 2015 (2709 ha) [1]. These plantations are located mainly in the Andean region, some in the Eastern Plains of Colombia. The crop of medicinal and aromatic plants amounted to 16,188 tons in 2015. This vegetal material was used for many applications different from essential oil (EO) extraction, in over 100 companies and 2500 commercial establishments [1]. Aromatic plants are used in Colombia's food industry for beverages, and a portion of the crop is exported in fresh (8288 tons in 2017) [2]. Colombia has currently no commercial enterprise dedicated to the cultivation of aromatic plants destined to produce

essential oils for export or the national market. Brazil, India, China, Indonesia, and the United States are Colombia's main essential oil suppliers. In 2017, the total cost of the country's essential oil imports was 14.289 million dollars, while the country exported just 298 thousand dollars [2]. Since there is no essential oil production, the EO exported amounts corresponded to commercialization of previously imported oils.

The publication of Colombian scientific articles on EO research started in 1974 and grew slowly during the following 30 years (less than three articles per year). The transition point was marked by the creation in 2005 of a network of research groups that joined their expertise around the development of the EOs agroindustry. The Research Center of Excellence for the Agroindustrialization of Aromatic and Tropical Medicinal Species (CENIVAM), under technical and administrative coordination at the Industrial University of Santander (Bucaramanga), has been a leader in aromatic plants and EO studies in Colombia for more than a decade. Over 250 scientific articles comprise the results of its investigations, which have been focused on the multidisciplinary and systematic search of promising native plants and on introduced species such as ylang-ylang, palmarosa, turmeric, patchouli, mints, basils, citrus, geraniums, and others. Researchers from more than 10 universities have carried out their work in areas of botany and taxonomy, plant physiology, and ecology; on the study of secondary plant metabolites, crop and post-harvest improvement, EO distillation and its optimization, and design of rural stills; on the study of volatile fractions from plants and flowers, obtainment of extracts with solvents and supercritical fluids (SFE-CO2), and catalytic transformation of EOs or their main components; and on the study of their diverse biological properties (antioxidant, antimicrobial, insecticidal, antiviral, and others).

2. Essential oil isolation

The primary metabolites (proteins, lipids, sugars, etc.) in plants are vital for the plant to grow, multiply, and live, while secondary metabolites are required by the plant to survive. For sure and with all the experimental details studied, the role played by secondary metabolites in plants is not completely known, because they fulfill several functions and operate through different mechanisms. Among many secondary metabolites isolated from plants, there are some very special, widely used in various branches of industry, medicine, and in many products of everyday life. This class of substances is called EOs, volatile oils, ethereal oils, or essences. Numerous substances are part of these oils; they are a complex mixture of volatile compounds with very diverse chemical nature. What most characterizes and highlights them is their smell, generally pleasant and intense, that evokes the fragrance of the plant or of the fruit or wood, from which these oils come. The essence can be remembered as the smell, for example, of a freshly cut grass or vanilla, sweet and cloying, among other aromatic tones that an EO has, formed by a complex range of volatile substances with different fragrant notes and different sensory thresholds for their perception.

Isolated from flowers (rose, orange blossom, lily, ylang-ylang), seeds (coriander, celery, carrot, anise, cardamom), leaves and stems (basil, thyme, mint, lavender, oregano), bark (cinnamon), wood (pine, sandalwood), roots (valerian, vetiver), and rhizomes (ginger, turmeric). EOs can be considered as the soul of the plants, their spirit, which characterizes, highlights, evokes, and makes them memorable in time; oils, generally, produce a pleasant sensation, especially when diluted. The EOs in the plants can be found in the different oil cells (ginger, turmeric, vanilla), in the secretory channels (pine, artemisia, anise, angelica), in the glands (citrus,

eucalyptus), or in the trichomes (many plants of Labiatae, Asteraceae, Solanaceae, Geraniaceae families). The plant material (aromatic plant), when subjected to water vapor, releases a liquid odoriferous mixture (EO) of various volatile substances; this mixture can have from 50 to more than 300 chemical substances and is composed of terpene hydrocarbons, their oxygenated derivatives, alcohols, aldehydes, and ketones, as well as ethers, esters, phenolic compounds, phenylpropanoids, and other derivatives [3].

EOs can be obtained from plant material by three main methods (**Figure 1**). (1) Steam distillation. This process is carried out with a superheated dry steam, usually generated by a boiler or steam generator, which penetrates the plant material at higher than atmospheric pressure; the steam current breaks the cells or oil channels in the plant and drags the volatile mixture, which condenses after passing through a cooling system (heat exchanger). Generally, the oils are lighter than water and with very little soluble in it; therefore, they can be separated by decantation. The exception is the clove oil, which is heavier than water and is collected under it. The steam distillation method is used to extract oils from rhizomes, roots, seeds (vetiver, valerian, ginger, anise, cardamom, etc.), and dried or fermented leaves of some plants (*e.g.*, patchouli). (2) Distillation with water-steam. In this extraction system, wet steam is used, coming from the boiling water, which passes through the plant material suspended above and supported on a mesh. Most herbaceous plants are distilled by this method. (3) Hydro-distillation is a process in which the plant material is directly immersed in water, heated to a boil. This method is used for the distillation of more delicate plant material, for example, flowers (*e.g.*, ylang-ylang, roses). The citrus peel (orange, tangerine, lime) EOs are also obtained by cold-pressing or by scraping their surfaces. The mixtures obtained by the methods mentioned above are called "essential oils"; other products, isolated by maceration in different solvents or with supercritical fluid (CO_2), are generally called "extracts" and not "oils"; among them are concrete—obtained

Steam /Water   **Essential Oil**

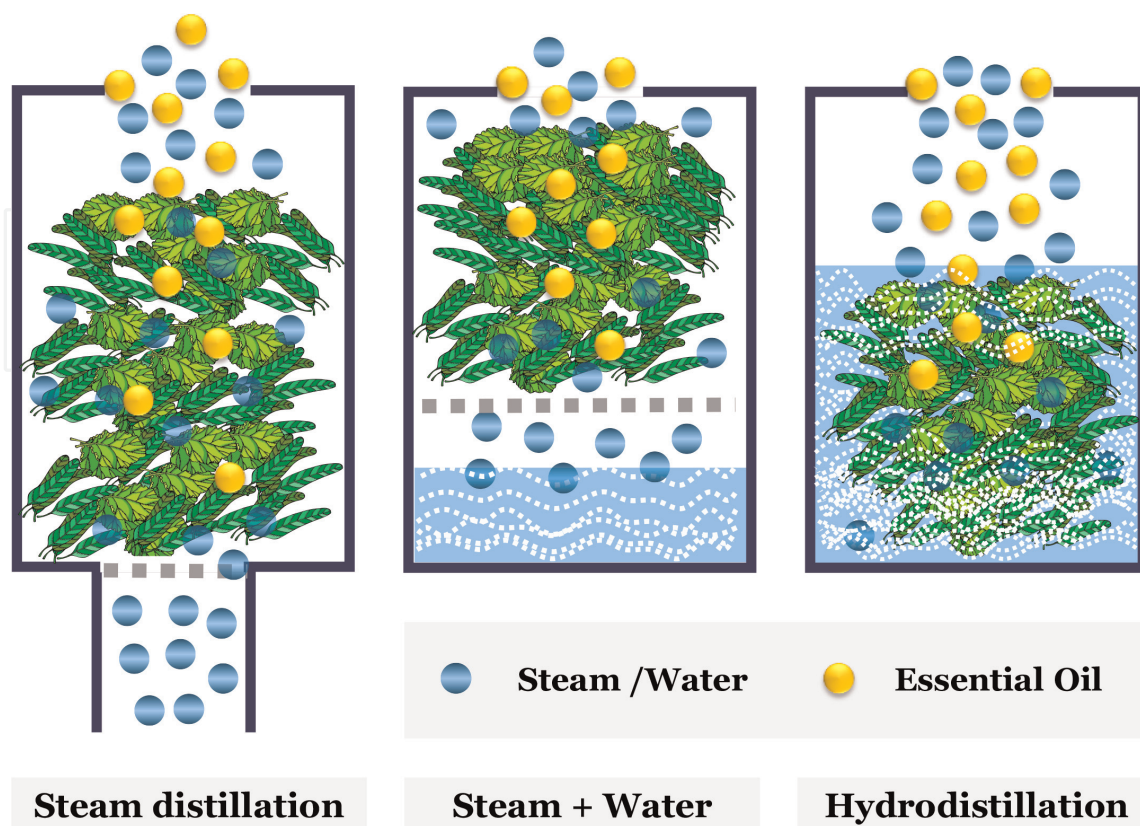**Steam distillation**   **Steam + Water**   **Hydrodistillation**

Figure 1.
Main methods of essential oil isolation.

by extraction with hydrocarbons from aromatic plants or, more frequently, flowers —and absolutes, which are separated from concrete or pomade (obtained by enfleurage) by alcohol.

The EO industrial production involves field distillation, in order to avoid the high transportation costs of large vegetal material loads from which only about 1% is going to be obtained as EO. Steam generation is one of the main components of the operation costs. Current trends point toward the use of lignocellulosic waste as biofuel for the furnace. Still capacity is determined by the crop size. The goal is to maintain the still operating for at least 300 days of the year and to schedule the harvests to avoid long storage (more than a week) of the cut vegetal material waiting for its distillation. This is mainly to prevent mold formation. Patchouli and vetiver are two exceptions to this rule, because a curing period of several days or months (vetiver) recommended to enhance oil yield and organoleptic quality.

The reality is that a large part of Colombian small growers have low purchasing power, low economic performance and productivity, and not very sophisticated technology level in rural operations and processes. Traditional agricultural production faces a complex problem that includes low prices, low profitability, and the increasingly acute lack of rural labor, because young people migrate to the cities. The EO industry is a very important rural development alternative in which the harvested vegetal material is no longer the final product, but the start of an added-value product chain. Several pilot projects, financed by the Ministry of Agriculture and Rural Development and Colciencias (Colombia's Science Funding Agency), have been carried out in the past 15 years by CENIVAM with the participation of small rural farmers associations. The common goal of these projects has been the development of the EO value chain. The economic, agronomic, and quality viability of EOs obtained in several productive units have been studied. Each unit has characteristics, as follows: 5–8 ha crop extension, 20–22 families of small growers

Figure 2.
Rural production and distillation of essential oils in Colombia (Barbosa, Santander).

involved in each project, 3 or 4 plant species cultivated per unit (palmarosa, citronella, *Lippia origanoides*, *L. alba*, rosemary, or thyme), plant nurseries, and the facilities for EO extraction in the field. Several rural stills (1 m³ retort capacity) have been designed and built by CENIVAM. A mobile autonomous version that uses a radiator as condenser received a patent [4]. The farmers are trained on good agricultural practices, post-harvest treatment, and steam distillation. All activities are accompanied by permanent technical assistance (**Figure 2**). These small rural projects constitute an opportunity for a commercializing enterprise that consolidates the various producers around quality control guidelines and provides the technical support to connect them with buyers abroad. The university provides the technical support for chemical characterization with modern instrumentation, production of technical data sheets, and quality assurance.

3. Essential oil characterization

3.1 Gas chromatographic analysis

The analytical technique routinely used for the instrumental chemical analysis of EOs is gas chromatography (GC), because the constituents of oils are volatile (monoterpenoids, esters, etc.) or semi-volatile substances (sesquiterpenoids, phenolic derivatives, etc.), whose molecular masses and boiling points do not exceed 300 a.m.u. and 300 °C, respectively. A chromatographic system comprises four fundamental blocks: (1) sample introduction system (injector), (2) separation system (column), (3) detection system for analytes eluted from the column (detector), and (4) data analysis and operation control system.

The GC can have conventional, *e.g.*, flame ionization detector (FID), or thermal conductivity detector (TCD), and spectral detectors can have an external device attached, for example, a headspace sampler, a pyrolyzer, a purge and trap (P&T) system or a thermal desorption setup, among others. Each block of the chromatographic system has its own function and its "responsibility" for the quality of the analysis and the results obtained; for example, the function of the injection system is to transfer the sample to the column quantitatively, without discrimination by molecular weight or by the volatility of the components and without their chemical alteration (decomposition, isomerization, or polymerization) (**Figure 3**). The "responsibility" of the chromatographic column in the EO analysis is high: the clear, complete (ideally) separation of all the components of the mixture must be accomplished. The separation is based on achieving different distribution constants of the components between the two phases, stationary and mobile. This is obtained by establishing the optimal operational conditions (temperature, type of mobile phase, its velocity, stationary phase polarity, carrier gas pressure, temperature program, etc.) (**Figure 4**) and by correctly choosing the chromatographic column, *i.e.*, its dimensions (length, internal diameter), chemical composition of stationary phase, its polarity and thickness, and among other factors. For the EO analysis, long columns (50 and 60 m) are used, since the oils are complex multicomponent mixtures and, above all, they have structurally very similar compounds (isomers), which require that the column has a very high resolution, which, among other factors, is achieved by increasing its length. The EOs contain compounds of very different polarities, both nonpolar (terpene hydrocarbons) and polar (alcohols, aldehydes). This implies that for their analysis, columns with different stationary phase polarities will be required.

The detection system differentiates the analyte molecules from those of the mobile phase (carrier gas), to which the detector is transparent. The response of the

A
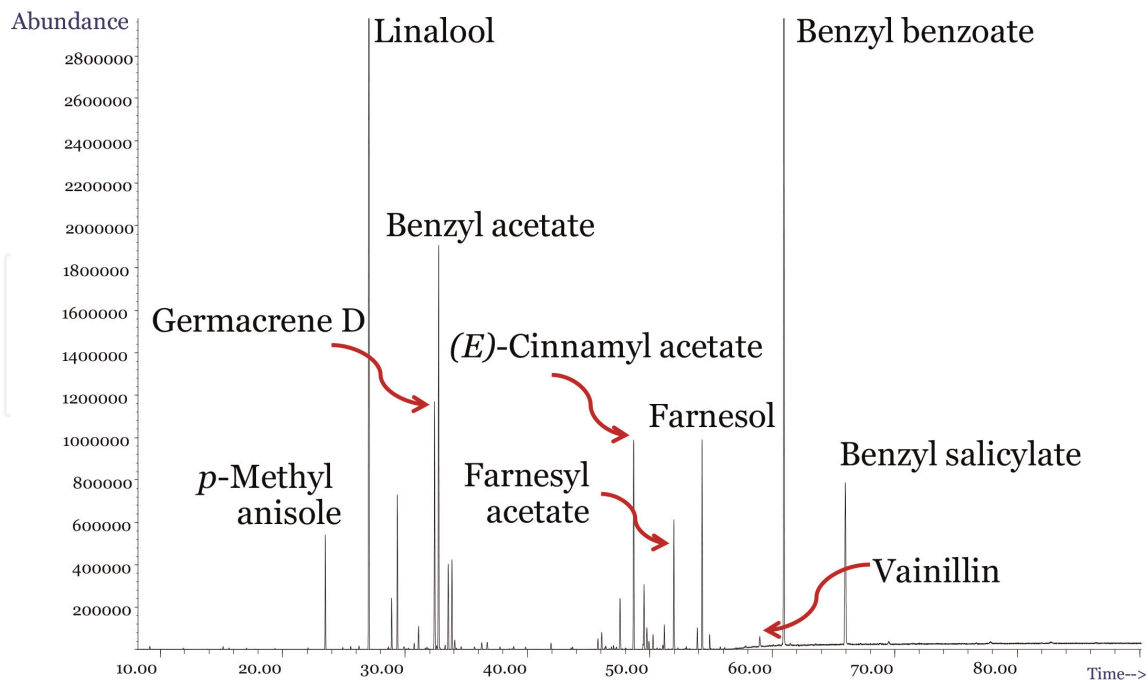
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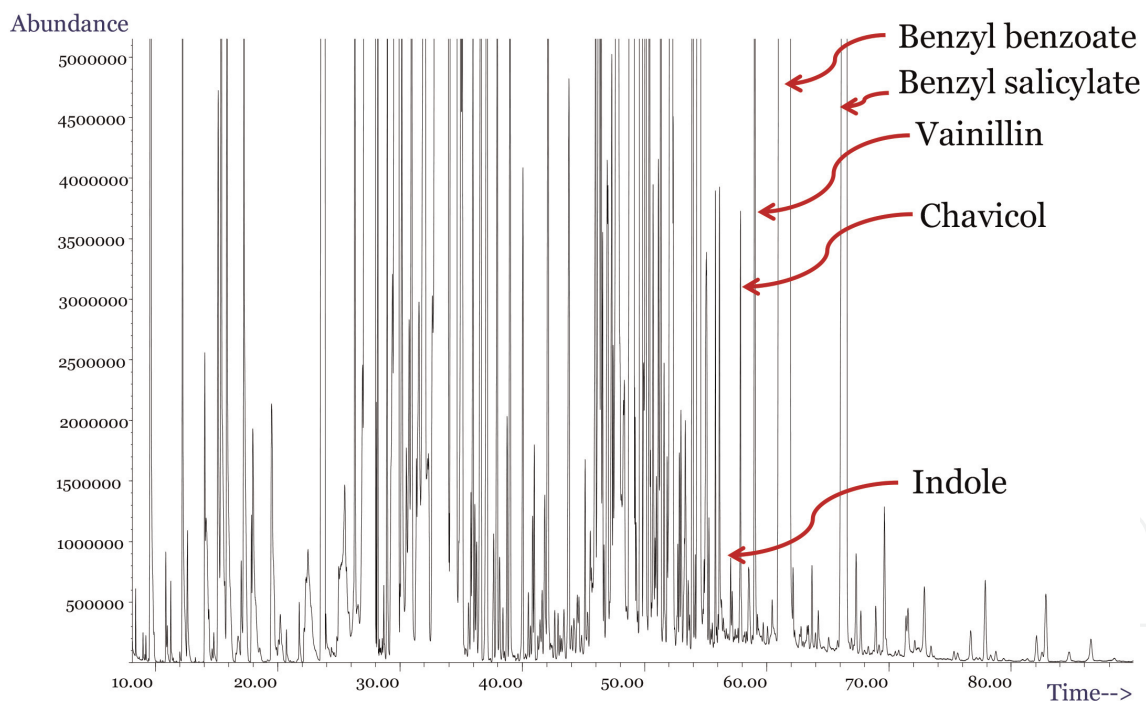
Figure 3.
*Ylang-ylang essential oil obtained by hydro-distillation from flowers. GC-MS analysis. DB-WAX column (60 m). Injection modes: **A.** Split 1, 100; **B.** Splitless. Injection volume—1 μL (250°C). Many "new" compounds appear in the chromatogram obtained in splitless mode.*

detector is based on the measurement of one of the physical properties of the system, *e.g.*, ion current, thermal conductivity, photon emission, etc. The analog signal becomes digital, graphic, *i.e.*, a chromatographic peak, which is characterized by its area (A), which is proportional to the analyte quantity or concentration (C). This permits to establish an interdependent relationship, A = f (C), and to carry out a quantitative analysis, to determine not only how many components there are in a mixture but in what proportion (quantity) they are present. Through a combination
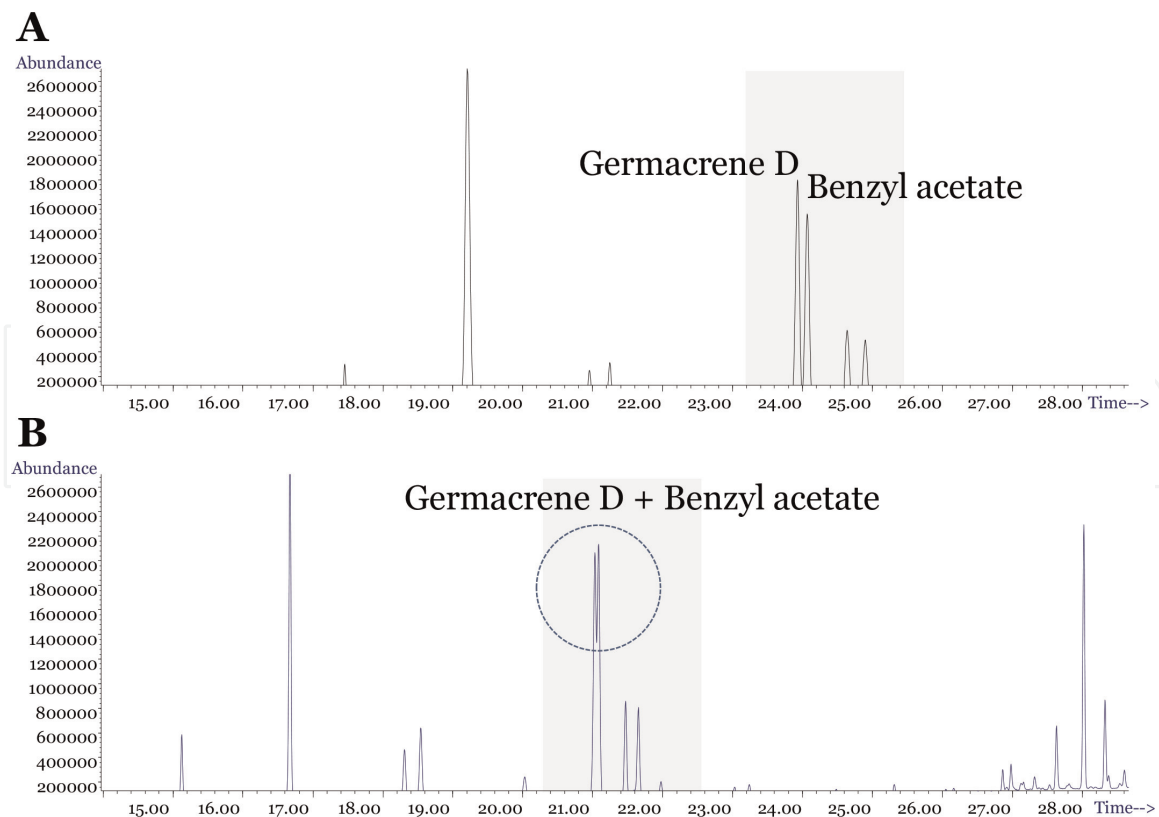
Figure 4.
Ylang-ylang essential oil obtained from flowers by hydro-distillation and analyzed by GC-MS on a polar column (DB-WAX, 60 m), using different temperature programs: A. 12 °C/min and B. 8 °C/min (chromatogram fragment). With higher temperature rate, poorer separation of germacrene D and benzyl acetate is observed.

of specialized software (data system), its accessories, interfaces, and analog-digital converters, the work of the chromatographic system and all its operational parts (hardware) is harmonized. For the EO analysis, which are very complex mixtures, two GC detectors are mainly used, namely, the flame ionization detector and the mass selective detector (MSD) or the mass spectrometry (MS) detector. The GC-FID is used to quantify the oil components.

3.2 Tentative and confirmatory identification of essential oil components

The preliminary or presumptive (tentative) identification of the EO components may be obtained once the retention indices are determined. The analysis in modern equipment uses a program for the column temperature; in these cases, linear retention indexes are calculated, which are part of many databases and bibliographic references [5, 6]. The confirmatory identification of a compound in a complex mixture analyzed by GC needs to obtain its "fingerprint," which is the mass spectrum (MS) represented by a unique combination of charged fragments (ions) generated during the breakup of the previously ionized molecule. The complementarity of the chromatographic analysis (screening) with confirmatory spectral data (mass spectra) is achieved using the combination of two techniques, GC and MS. The GC-MS coupling complements the quantitative analysis carried out by GC-FID and provides important additional information, *i.e.*, the mass spectra of all components, through which their identity can be established.

EOs contain both nonpolar (monoterpene and sesquiterpene hydrocarbons) and polar compounds (their oxygenated derivatives, aliphatic alcohols, ketones, oxides, phenolic compounds and their derivatives, phenylpropanoids, and rarely acids, among others). Their analysis is performed by GC-FID (quantitative analysis) and

by GC-MS (qualitative analysis), in two columns, with polar and nonpolar stationary phases. In columns with the nonpolar stationary phase, poly(dimethylsiloxane), PDMS, or 5% phenyl-PDMS, the elution of components happens depending on their boiling temperatures (or volatilities), that is, the retention times, t_R, increase with the decrease of the volatility and with the increase of the molecular masses and boiling points of the components (**Figure 5**). The compounds reach the end of the column in the increasing order of their boiling points. In the polar column, poly (ethylene glycol), the elution order of the components is more difficult to predict, because it is related to the intermolecular forces between the analyte and the stationary phase and depends both on the dipole moment of the molecule (the polarity) and on the possibility of hydrogen bond formation between the substance and the stationary phase.

The elution order of some compounds in columns with different stationary phases may be reversed. This often helps, together with the mass spectra and the fragmentation pattern study, to differentiate, for example, terpene alcohols from their acetates, since the latter sometimes do not exhibit molecular ions, $M^{+\bullet}$ in their mass spectra. When the chromatographic parameters (t_R, t_{RR}, or retention indices) and spectroscopic parameters, *i.e.*, mass spectra (characteristic fragmentation pattern; see **Figure 6**) of the analyte and reference substance (certified standard) coincide, a complete or confirmatory compound identification is achieved. However, when retention indexes and mass spectra are used, extracted from the specialized literature [6, 7] or from the databases (*e.g.*, spectral libraries, NIST, WILEY, Adams [7], others), and compared with the spectroscopic and chromatographic parameters of the EO component, their coincidence leads only to a recognition of the chemical structure, but not to its unambiguous, absolute identification, which requires the use of a standard compound, a pure substance with certified chemical structure. Frequently, it is necessary to isolate the compound from the mixture and purify it for further characterization through the UV, IR, MS, X-ray diffraction, NMR, elemental analysis, or high-resolution mass spectrometry (HRMS). Each one of the mentioned spectroscopic techniques contributes with some structural information, but the combined results allow to assemble the puzzle and elucidate the chemical structure unequivocally.

The biggest challenge in EO analysis is the complete separation of its components (**Figure 7**) because their frequent coelution occurs due to their very close or equal distribution constants. Some conventional strategies, *e.g.*, change of the column (polarity), temperature program, use of selective detectors, etc., can often fail or be insufficient to determine all the compounds present in the oil. Multidimensional chromatography makes it possible to separate the peaks of partially or totally co-eluted substances. For this, it uses a second column, usually orthogonal, through the "heart-cutting" operation by means of pneumatic switching valves—today with the micro-fluidic technology, between the two columns and diverting part of the eluent from the first to the second column. This method has played an important role in the development of separation techniques for complex mixtures, including EOs [8, 9]. Multidimensional chromatography requires at least two detectors and can have configurations of up to three columns in the same oven or in separate chromatographic ovens. Along with this, today one of the most modern, complete solutions for the separation of multicomponent mixtures—although not very affordable for most laboratories because of its high price—is comprehensive or total gas chromatography (Comprehensive GC × GC), whose applications and developments have grown day after day for more than two decades [10, 11].

In comprehensive gas chromatography (GC x GC), two columns are used, linked together by means of a modulator. In contrast to conventional multidimensional gas
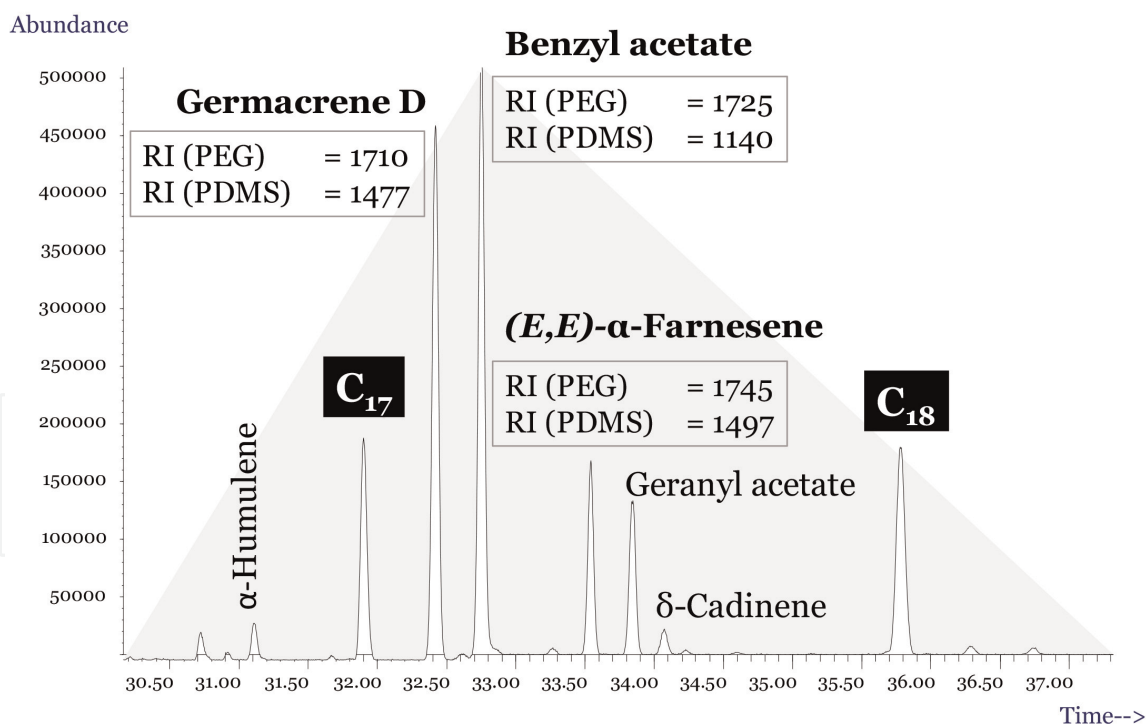
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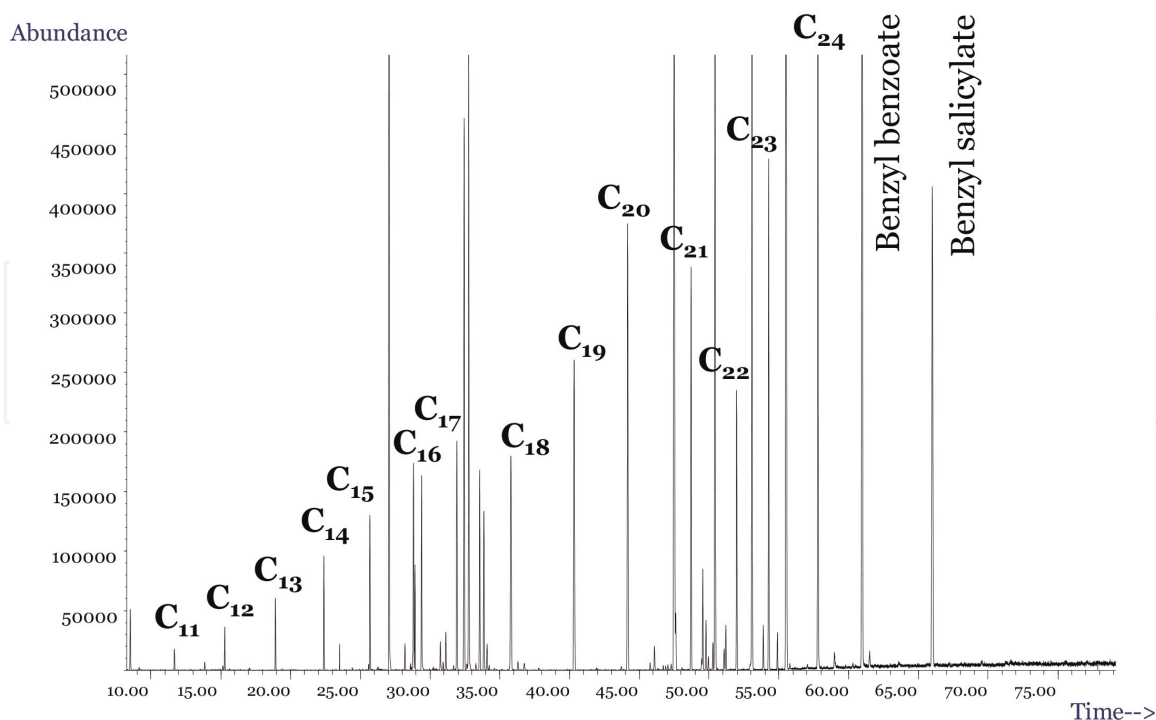
B

Figure 5.
GC-MS chromatogram of ylang-ylang essential oil isolated from flowers by hydro-distillation and analyzed by GC-MS on a polar column (DB-WAX, 60 m). A. Co-injection of the essential oil and n-paraffin mixture to calculate retention indices (RI). B. RIs of germacrene D, benzyl acetate, and (E,E)-α-farnesene measured in nonpolar [poly(dimethylsiloxane)] and polar [poly(ethylenglycol), PEG] columns.

chromatography, the GC x GC requires a single detector with high processing frequency; both columns can be in the same oven or in two separate ovens. There are different types of modulators, *e.g.*, rotary thermal modulator ("sweeper"), cryogenic "jet" system, modulators of valves, or longitudinal cryogenic modulator,

among others, which also vary in the cryogenic agent employed; more modern modulators are not cryogenic in nature. The eluent of the first column, by means of the modulator, is "split" into very small "slices," which, one after the other, enter the second column from the first column. The first column (1D) is a conventional column, with length of 25 or 30 m, and the second column (2D) is of rapid chromatography, that is, short and with a very thin internal diameter (0.1 mm or less). The stationary phases in both columns are "orthogonal," *i.e.*, if the first is nonpolar,
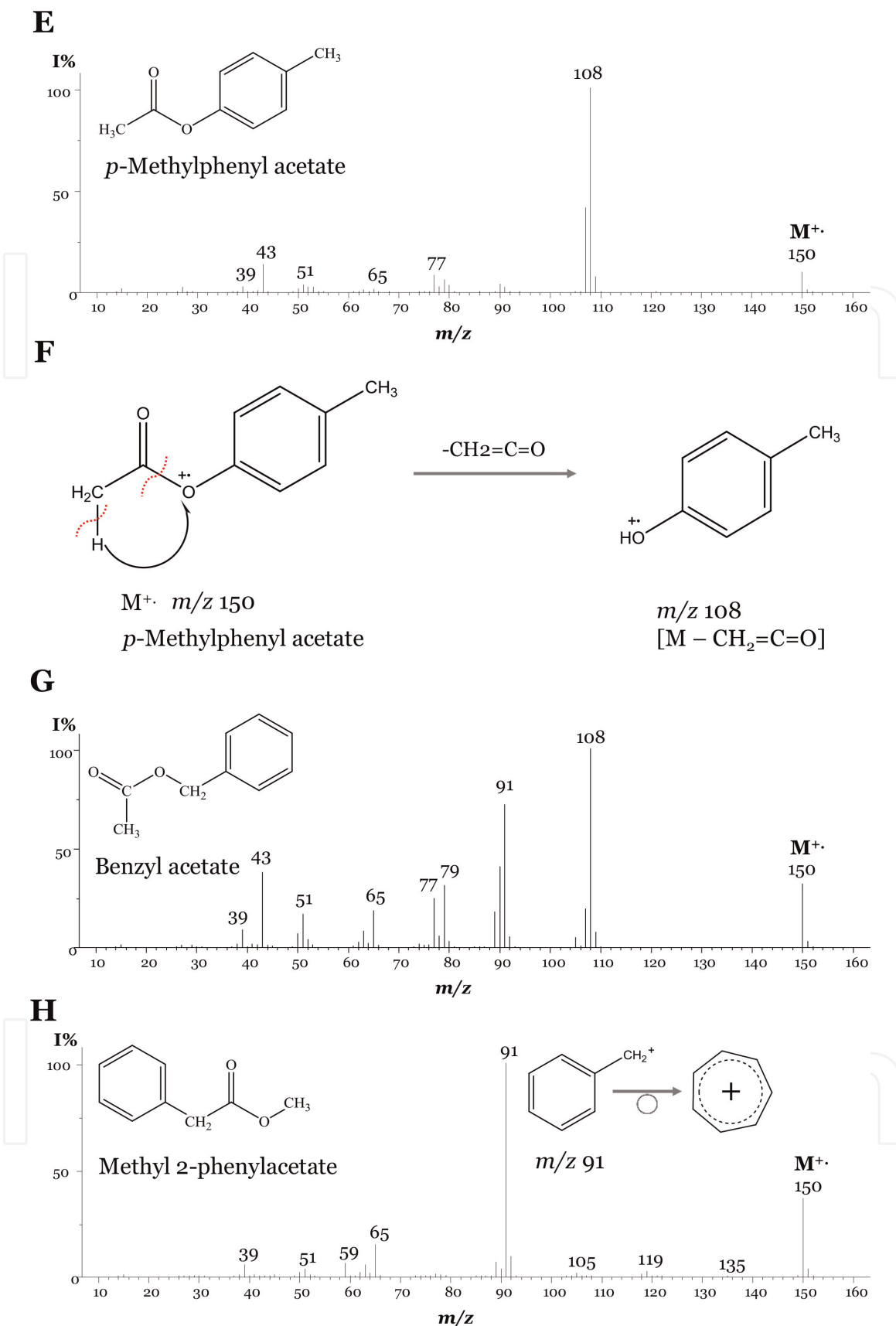
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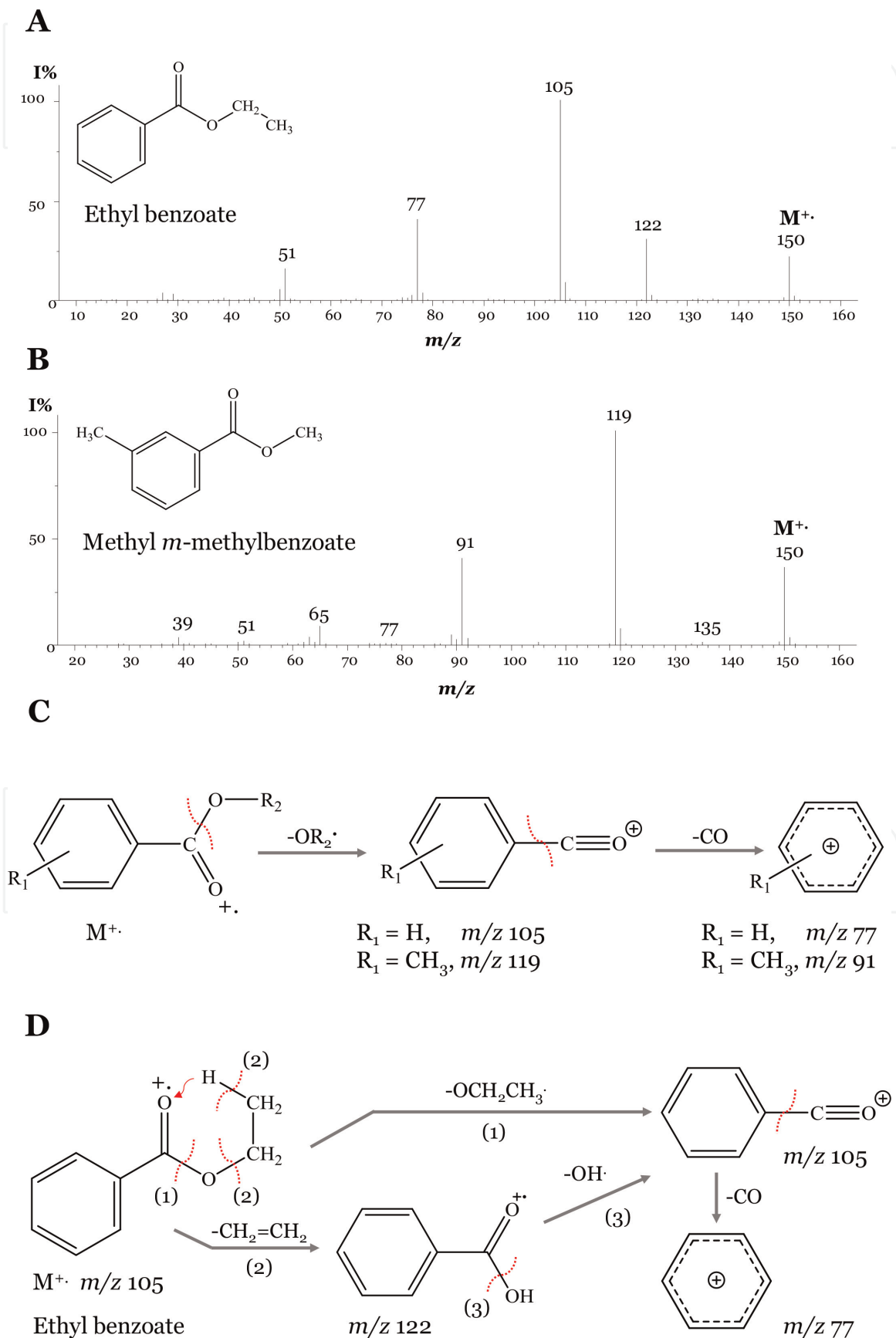
Figure 6.
Fragmentation pattern in mass spectra (electron impact, 70 eV) of some essential oil components. A. Ethyl benzoate mass spectrum. B. Methyl m-methyl benzoate mass spectrum. C. α-Rupture and typical benzoyl ion (m/z 105, 119) formation. D. McLafferty transposition of ethyl benzoate molecular ion M$^{+\bullet}$ and formation of [M–CH$_2$=CH$_2$]$^{+\bullet}$ (m/z 122) fragment. E. p-Methylphenyl acetate mass spectrum. F. Elimination of CH$_2$=C=O neutral fragment and formation of [M—42]$^{+\bullet}$, diagnostic ion for acetates. G. Benzyl acetate mass spectrum. H. Methyl 2-phenylacetate mass spectrum and the formation of tropylium ion (m/z 91) generated through benzylic excision.
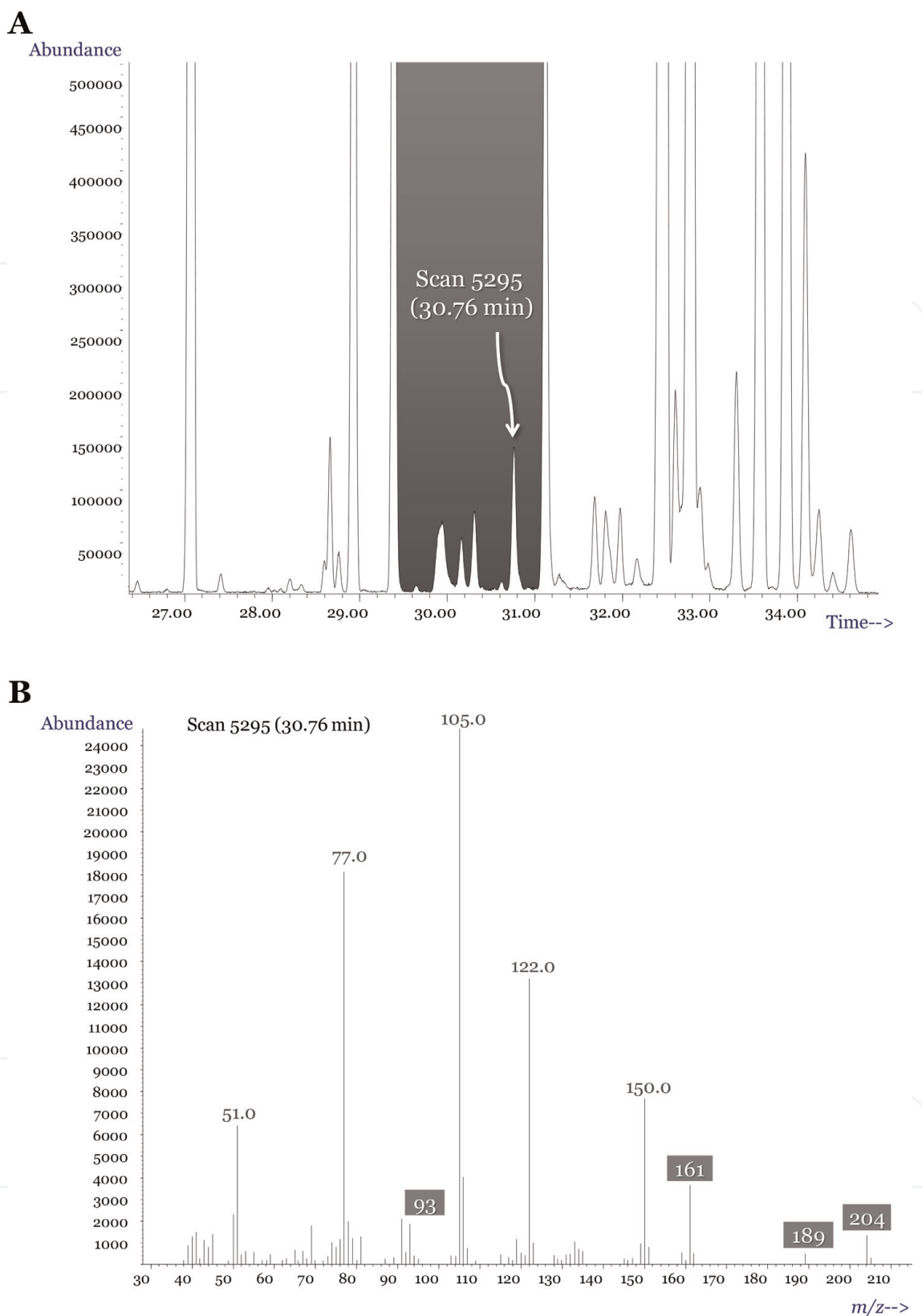
A

B

Figure 7.
*Ylang-ylang essential oil chromatogram (GC-MS) fragment. **A.** Chromatographic peak at t_R = 30.76 min is highlighted. **B.** Mass spectrum corresponded to this peak, identified by the software as ethyl benzoate (**Figure 6A**); nevertheless, the peak is "contaminated" with other compounds; the presence of typical sesquiterpene hydrocarbon ions (m/z 204, 189, 161,) is observed.*

the second column is polar, and *vice versa*. The modulation time, required for the transfer of a very small portion of eluent from the first column to the second, must be very short and similar but never longer than the elution time of the "slowest"

component in the second column. The second column, therefore, must be short and very thin and separate the components in just a few seconds. Since the second column is connected to the detector (MSD, FID, or ECD), it must have a very-high-reading and signal-processing frequency. In most cases, a time-of-flight (TOF) mass detector is used, which is the best option—though expensive—to make a quantitative analysis and identification of compounds in such complex mixtures, as are EOs (**Figure 8**).

Further technical details for EO chemical characterization and that of their components can be found elsewhere [12, 13]. In summary, EO characterization necessary for its quality control and the determination of authenticity, as part of a technical data sheet necessary for its commercialization, can be divided into four main stages or areas: (1) organoleptic properties, (2) physicochemical determinations, (3) qualitative and quantitative analysis of the components present in the oil (chemical composition), and, finally, (4) some other determinations, *e.g.*, pesticide residues, traces of heavy metals, etc.

3.3 Chemical compositions of essential oils obtained from tropical plants grown in Colombia

CENIVAM has studied Colombian plants widely used in popular medicine or in culinary, for example, anise [14], oregano [15], rue [16, 17], and other species introduced from Asia, such as lemongrass, citronella, ginger, citrics [18–20], vetiver, and ylang-ylang [21–23], as well as several native species, among others, *Copaifera officinalis* [24], *Spilanthes americana* [25], *Lepechinia schiedeana* [26], *Lippia alba* [27], *Xylopia americana* [28], *Hyptis umbrosa* [29], *Callistemon speciosus* (sims) DC. [30], *Swinglea glutinosa* [31], *Satureja viminea* [32], and *Lippia origanoides* [33], with emphasis on the comparative study of extraction methods [34–40]. **Table 1** summarizes the composition of several *Lippia* EOs, according to compound families. The knowledge of the chemical composition has been the basis for the interpretation of the results of bioactivity assays such as genotoxicity
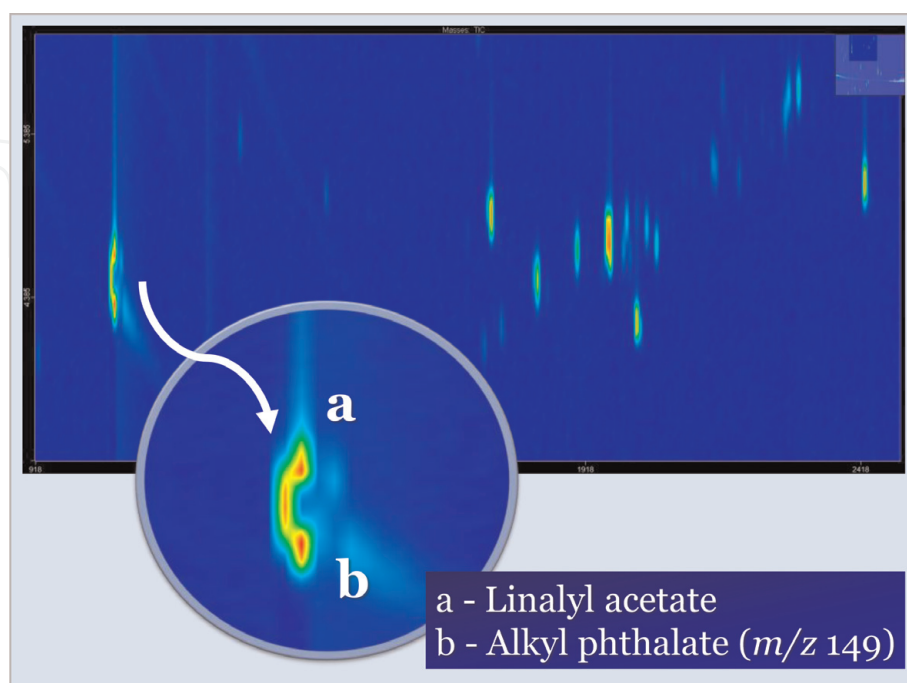

Figure 8.
Fragment of GCxGC-HRMS-TOF chromatogram of ylang-ylang essential oil contaminated with plasticizer (phthalate) traces. m/z 149 is a base peak in alkyl phthalates' mass spectra.

Compound family	Relative amount, %											
	L. alba, chemotypes				*L. origanoides*, chemotypes			*L. citriodora*	*L. micromera*	*L. americana*	*L. graveolens*	*L. dulcis*
	Carvone	Citral	Citral + Carvone	Myrcenone	Thymol	Carvacrol	Phellandrene					
Monoterpene hydrocarbons	31.5	4.1	24.6	12.6	14.7	31.0	45.7	15.4	44.9	19.1	12.2	0.3
Oxygenated monoterpenes	61.4	62.4	52.7	71.6	2.1	2.0	7.4	49.7	2.8	1.6	2.0	5.6
Oxygenated compounds (phenylpropanoids)	—	6.9	2.7	0.0	63.3	57.7	2.0	4.4	47.7	0.2	78.1	7..9
Sesquiterpene hydrocarbons	4.9	11.9	16.2	6.6	5.3	3.9	29.1	11.0	3.2	52.3	5.3	54.0
Oxygenated sesquiterpenes	0.3	5.1	1.1	—	0.4	—	5.2	12.8	0.5	10.7	0.2	19.1

Table 1.
Relative amounts of compound families in essential oils of various Lippia *species grown in Colombia.*

[41–43], antiviral [44] and antifungal [45–52] activities, insect repellence [53–59], antioxidant capacity [60–65], cytotoxic [30, 66–68], antituberculosis [69], and anti-protozoarial activities [70, 71]. A few examples are highlighted in the following section.

4. Some biological activities of essential oils obtained in Colombia

EOs have been used in phytotherapy and folk medicine for their good odor and antibacterial, antifungal or insecticidal activities. Phenols, alcohols and aldehydes are EO components capable of crossing the cell wall, and in doing so, they alter its permeability and may cause leakage of macromolecules, loss of ions, structure disruption, and, eventually, cell death. This cytotoxicity enables EO applications against human pathogens or parasites and for the preservation of vegetal and marine products. Due to their large number of constituents, EOs affect several targets simultaneously, and this may be the reason for the lack of microorganism resistance development or adaptation. Besides cytotoxicity, the antioxidant properties of EOs are generally invoked as an indication of their potential benefits for human health. This is related to the notion that many diseases are due to high oxidative stress generated by diet, environmental contaminants, or work habits. However, the prooxidant properties of some EO components can play a protective role by promoting the removal of damaged cells. The mitochondria produce reactive oxygen species which can oxidize phenolic compounds (EO components) and give rise to reactive phenoxyl radicals which accelerate the general cell damage [72].

Genus *Lippia* (Verbenaceae family) has been the focus of attention of Colombian researchers (**Figure 9**). *L. alba* and *L. origanoides* EOs have appeared as the prominent representatives of this genus, after the evaluation of various *Lippia* genus EOs for antioxidant [33], antiviral [44], antimicrobial [69], antiprotozoal [71], and antigenotoxic [41, 42] activities.

Lippia origanoides Kunth (mountain oregano) is a good example of the aromatic plant biodiversity found in CENIVAM studies. It is an aromatic shrub found in the wild in northern South America and Central America. At least four different chemotypes have been distinguished according to differences in EO composition [34, 73]. Further research showed notorious differences in the compositions of the various chemotype extracts. Several uses of *L. origanoides* infusions in popular medicine have been related to antimicrobial and analgesic activities due to phenylpropanoids and flavonoids found among its secondary metabolites. The detection of thymol and carvacrol as main EO constituents and pinocembrin, naringenin, quercetin, and luteolin in mg/g amounts in extracts of various *L. origanoides* chemotypes supports the recognition of this species as a promising source of bioactive substances. *L. origanoides* is the second most studied species of the *Lippia* genus, preceded by *L. alba*. The useful bioactive properties found for *L. origanoides* oil have aroused interest in commercial applications such as food additive, preservative, or pest control agent, among others. *L. origanoides* oil is an important ingredient of various current chicken food commercial products. The most widely known sources of thymol and carvacrol are thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*), both of Eurasian origin. Thymol and carvacrol contents in thyme EO are in the range 37–55% and 0.5–5.5%, respectively (ISO 19817:2017). Oregano EO contains around 22% thymol and 18% carvacrol. Thymol and carvacrol are major components in three L. *origanoides* chemotypes. Several projects conducted in CENIVAM have related the variations in EO composition with the steam distillation conditions, with the phenological stage, the agricultural conditions, and the post-harvest treatment.
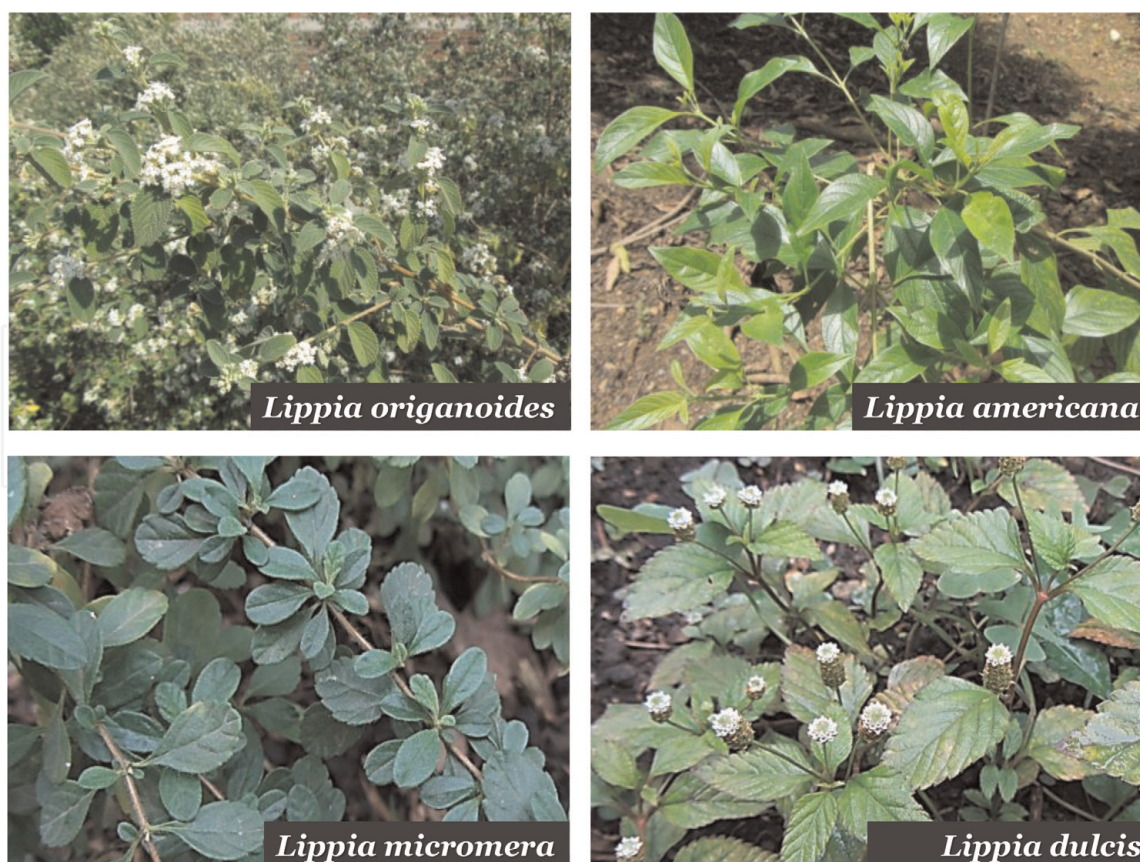
Figure 9.
Some tropical aromatic plants of Lippia *genus (Verbenaceae family): L. origanoides, L. americana, L. micromera, and L. dulcis.*

More than 30 botanical outings were organized by CENIVAM to various Colombian regions in the Andes, the Eastern Plains, the Caribbean, and the Pacific coasts. Over 1100 accessions of medicinal and aromatic plants were collected and taxonomically identified, under the due permit of access to the genetic resource. Hydro-distillation and steam distillation of these samples produced close to 1000 different EOs that were subjected to chemical characterization using GC techniques provided with FID and MS systems. Tests for antioxidant, antimicrobial, antiviral, antiparasitic, immunomodulatory, photoprotective, and antigenotoxic activities of the EOs revealed that more than 45% of these oils were highly active in one or two assays. The following sections highlight just a few of the interesting findings obtained in the search for natural ingredients with biological properties that may enable the development of products for the pharmaceutical, cosmetics, hygiene, or food industries.

4.1 Cytotoxicity

Following the recognition of cytotoxicity and antioxidant capacity as main determinants of EO potential pharmaceutical applications, Olivero and co-workers [64] used the brine shrimp assay and the measurement of thiobarbituric acid induced in rat liver microsomes by a Fenton reagent to evaluate 13 EOs from Colombian plants for cytotoxicity and antioxidant capacity, respectively. Mean effective concentrations (EC_{50}) below 100 μg/mL were registered for the *Ocotea* sp., *Tagetes lucida*, and *L. alba* (citral chemotype) EOs. Moderate values (130–174 μg/mL) were obtained for the *Elettaria cardamomum* and *L. alba* (carvone chemotype, Tolima) EOs. No antioxidant activity ($EC_{50} > 1$ mg/mL) was found for the *Minthostachys mollis*, *L. alba* (carvone chemotype, Cundinamarca), and *Piper sancti-*

felisis EOs. LC_{50} cytotoxicity values between 4.36–64.3 and 1.2–20.8 μg/mL, for 24 and 48 h exposure, respectively, were obtained. Most tested EOs can be considered cytotoxic (LC_{50} < 10 μg/mL), but bioactivity was highly dependent on EO chemotype, extraction mode, and plant growing location.

L. alba EOs of different origins in Colombia showed cytotoxicity in the *Artemia franciscana* assay at concentrations in the range from 7 to 21 μg/mL. The differences were attributed to compositional variations caused by the geographical habitat and environmental factors (temperature, light intensity) [74].

A study of cytotoxic activity of EOs from the Verbenaceae and Asteraceae families included 36 species of various origins. These oils were tested on Jurkat, HeLa, HepG2, and Vero cell lines [75]. None of the tested EOs was cytotoxic, except that from *Ambrosia arborescens*, which showed IC_{50} of 16 ± 3.4 μg/mL and was not active against the tested tumor cell lines. All the Verbenaceae family EOs examined produced dose-dependent inhibition on the growth of HeLa cells with determination coefficient R^2 > 0.7. Four *L. alba* citral chemotype EOs and one oil of the *L. alba*, carvone chemotype, were active against HeLa cells. This activity was attributed to the presence of a target on HeLa that is not present on HepG2 and Jurkat cells.

4.2 Antioxidant activity

A study of 12 EOs of 7 *Lippia* species growing in Colombia employed GC-FID and GC-MS methods for their chemical characterization and the ORAC and ABTS assays for their antioxidant activity evaluation [76]. The ORAC and ABTS methods explore radical scavenging mechanisms in which the fundamental step is either the proton transfer (ORAC) or the electron transfer (ABTS) [77]. The EOs with high phenylpropanoid content showed higher antioxidant capacity in both assays. The ORAC antioxidant activity of these oils was five or more times superior to those of butyl hydroxytoluene (BHT) and α-tocopherol, which are antioxidants used commonly in commercial products. This superiority was maintained, although at a smaller proportion, in the ABTS test. The ORAC and ABTS values measured individually for carvacrol and thymol were close to the values obtained for the EOs that contain them as main components. For the remaining oils, not rich in phenylpropanoids, there was no clear relationship between the oil's antioxidant activity and that of its main constituents (**Table 2**). The *L. americana* EO and the phellandrene-rich *L. origanoides* chemotype EO have mono- and sesquiterpene hydrocarbons as main components. They showed poor antioxidant activity under the ABTS assay conditions but did have higher antioxidant activity than BHT in the ORAC assay. This is consistent with the evaluation of individual unsaturated nonaromatic hydrocarbon terpenes (*trans*-ß-caryophyllene, α-phellandrene, ɣ-terpinene), which were capable of scavenging radicals by proton transfer (ORAC conditions) but were completely inactive under the ABTS assay conditions (electron transfer). All the 12 EOs examined, those of *L. alba* (carvone), *L. alba* (citral), *L. alba* (carvone+citral), *L. alba* (myrcenone), *L. origanoides* (carvacrol), *L. origanoides* (thymol), *L. origanoides* (phellandrene), *L. citriodora*, *L. micromera*, *L. americana*, *L. graveolens*, and *L. dulcis* exhibited higher antioxidant capacity than BHT and α-tocopherol in the ORAC assay, and this makes them very good candidates to become ingredients of final products in substitution of synthetic antioxidants.

4.3 Antiviral activity

EOs from plants of the Labiatae and Verbenaceae families are considered very useful in folk medicine, as antibacterials, antivirals, antifungals, antioxidants, and

Essential oil (chemotype) or standard compound	Main compounds	Essay ($\pm$ s, n = 3)		
		Yield, % w/w	ORAC (μmol Trolox®/g compound)	ABTS (μmol Trolox®/g compound)
L. graveolens	6-Methyl-5-hepten-2-one (4.9%), α-terpinene (3.0%), thymol (64 6%), carvacrol (12 2%), *trans*-β-caryophyllene (2.9%)	1.2	3990 $\pm$ 58	5410 $\pm$ 48
L. citriodora	Limonene (10.7%), 1,8-cineole (5.0%), neral (15.6%), geranial (18.9%), spathulenol (4.7%)	0.1	3630 $\pm$ 40	41 $\pm$ 2
L. origanoides (carvacrol)	*p*-Cymene (12%), carvacrol (46.2%), γ-terpinene (9.6%)	4.4	3400 $\pm$ 120	5200 $\pm$ 109
L. origanoides (thymol)	γ-Terpinene (5.0%), thymol (54.5%), thymyl acetate (4.8%)	3.1	2840 $\pm$ 72	5090 $\pm$ 42
L. origanoides (phellandrene)	*p*-Cymene (11.2%), limonene (7.2%), *trans*-β-caryophyllene (11.3%), α-phellandrene (9.9%)	1.5	1820 $\pm$ 82	310 $\pm$ 2
L. micromera	*p*-Cymene (13.1%), thymol (29.1%), thymyl methyl ether (14.9%)	1.0	2050 $\pm$ 78	2750 $\pm$ 80
L. alba (citral)	Geranial (27%), neral (21%), geraniol (6.0%)	0.9	2000 $\pm$ 77	24.8 $\pm$ 0.4
L. alba (carvona)	Limonene (30.2%), carvone (50.3%)	0.8	1340 $\pm$ 54	126.4 $\pm$ 0.7
L. americana	Sabinene (7.4%), *trans*-β-caryophyllene (12.2%), germacrene D (16.3%)	0.5	1200 $\pm$ 27	239 $\pm$ 4
Carvacrol			3410 $\pm$ 50	4609 $\pm$ 6
Thymol			3000 $\pm$ 103	5700 $\pm$ 125
Trans-β-caryophyllene			2800 $\pm$ 109	N.D.
γ-Terpinene			1766 $\pm$ 8	N.D.
α-Phellandrene			1040 $\pm$ 18	136 $\pm$ 3
α-Tocopherol			550 $\pm$ 13	2429 $\pm$ 7
BHT			457 $\pm$ 9	4760 $\pm$ 23
1,8-Cineole			299 $\pm$ 5	N.D.
p-Cymene			219 $\pm$ 2	N.D.

Table 2.
Main constituents and antioxidant capacity of Lippia essential oils.

insecticides. The antiviral activity of 40 EOs of the Labiatae and Verbenaceae families and some monoterpenes were evaluated on human herpes virus types 1 and 2 using the end point titration technique [78]. Samples that showed reduction factor of viral titer in comparison to control without treatment (*Rf*) at least against one viral type, at concentrations lower than or equal to 100 μg/mL, were considered active. *Hyptis mutabilis* oil showed a high activity against both viruses (HHV-1 and HHV-2), with *Rf* values of 10^3 and 10^2, respectively, at a concentration of 50 μg/mL. *Lepechinia vulcanicola* and *Mintostachys mollis* EOs showed the same reduction factor of viral titer against both viral types at a concentration of 100 μg/mL. *Lepechinia salviifolia* was moderately active against both viruses at the same concentration

(100 μg/mL). *Ocimum campechianum* EO showed relevant (1×10^3) and mild (1×10^1) activity against HHV-1 and HHV-2 at concentrations of 100 and 50 μg/mL, respectively. *Lepechinia salviifolia* and *Rosmarinus officinalis* oils showed moderate anti-herpetic activity against HHV-1 and HHV-2, respectively.

The *in vitro* inhibitory effect of *L. alba*, *L. origanoides*, *Origanum vulgare*, and *Artemisia vulgaris* EOs on yellow fever virus was investigated by exposing African green monkey kidney (Vero) cells to EO prior to virus exposure [79]. None of the EOs studied was cytotoxic on these cells. The minimum concentration of the EO that inhibited virus titer by more than 50% (MIC) was determined by virus yield reduction assay. Preincubation of virus with selected EO for 24 h at 4°C before adsorption on Vero cell inhibited the subsequent extracellular virus titer. Vero cells were exposed to EO 24 h at 37 °C before the adsorption of untreated virus. The presence of EO in the culture medium enhanced the antiviral effect: *L. origanoides* oil at 11.1 μg/mL produced a 100% reduction of virus yield, and the same result was observed with *L. alba*, *O. vulgare*, and *A. vulgaris* oils at 100 μg/mL.

4.4 Antigenotoxicity

Since plants are exposed daily to the sun radiation, they have evolved mechanisms that protect them from the effects of overexposure, such as damages to the DNA. When DNA suffers a damage, the cell responds with a set of actions that was discovered in 1975 by Miroslav Radman [80], who assigned the name of SOS response. The Pasteur Institute developed a colorimetric assay to detect carcinogens, based on this response in which the exposition to UV causes the DNA damage whose extent is associated with the intensity of light absorbance by a chromophore [81]. This SOS chromotest is highly sensitive to UV. A modified version has been used by Fuentes and collaborators [82] to identify plants of Colombian flora that may be a source of genoprotective compounds. Their application of the SOS chromotest in a survey of 50 extracts obtained with supercritical CO_2 from aromatic plants grown in Colombia permitted to identify those that significantly reduced UV-induced genotoxicity depending on their concentration, as follows: *Baccharis nitida*, *Solanum crotonifolium*, *Hyptis suaveolens*, *Persea caerulea*, and *L. origanoides*. Volatile secondary metabolites have been the subject of antigenotoxicity tests. *L. alba*, *L. micromera* and *L. origanoides* EOs were found antigenotoxic, and the evaluation of their main constituents showed that carvacrol, thymol, citral, *p*-cymene, and geraniol inhibited the UV-induced genotoxicity in the SOS chromotest [83].

4.5 Repellence

Synthetic insecticides are the most frequent pest control method in crop production and storage. However, their application has negative effects on environmental resources, elimination of beneficial insects, and toxicity for susceptible species and humans, who represent the last link in the food chain. EOs have attracted attention in recent years as potential pest control agents due to their insecticidal, repellent, and/or antifeedant properties. Stored products of insect pest control are important in managing post-harvest grains, food products and processed goods. *Tribolium castaneum* (Herbst) is one of the most common insect pests worldwide of flourmills, grocery shops and warehouses. Jaramillo *et al.* [84] examined the repellent effect of Colombian *Croton malambo* (Karst) EO against *T. castaneum* using the area preference method. A filter paper was divided in halves. On one half, equal volumes of different concentrations of EO dissolved in acetone (0.00002, 0.0002, 0.002, 0.02, and 0.2 μL/cm²) and the other with acetone only as

control. These halves were joined, and a fixed number of insects were released on the center. Observations on the number of insects present on both the treated and untreated halves were recorded after 2 and after 4 h. The highest repellent activity was observed at an EO concentration of 0.2 μL/cm^2. Repellence values of 86% ± 5 (2 h) and 92% ± 3 (4 h) were observed, which were higher than those obtained for a commercial repellent at the same concentration and exposure times (78% ± 5 and 76% ± 9, respectively).

Weevils that consume flour (*Tribolium castaneum*), peanuts and wheat bread (*Ulomoides dermestoides*) merit attention alongside other insects of major concern in crop production and storage of cereals and other products. Alcala and co-workers [85] used the area preference method to show that EOs of *Elettaria cardamomum*, *Salvia officinalis* and *L. origanoides* (carvacrol chemotype) had repellent action against both pest insects, while the repellency in the controls was null. This repellency increased when the EO concentrations were higher. None of the EOs presented attractant action for either of the exposure times. A 100% repellency was obtained at the highest concentration tested (1.6% v/v), except for *S. officinalis* against *U. dermestoides* at 2 h of treatment that had a 97% of repellency. Mean repellent concentration (RC50) values showed that *E. cardamomum*, *S. officinalis*, and *L. origanoides* had better repellent properties against *U. dermestoides* than a commercial preparation that contained 15% of ethyl butylacetylaminopropionate. The carvacrol-rich chemotype of *L. origanoides* was the most potent, with RC50 values of 0.220 and 0.207% (v/v), for *T. castaneum* and *U. dermestoides*, respectively.

Several tropical diseases for which there is no vaccine yet (yellow fever, Zika fever, chikungunya, dengue) are transmitted by *Aedes aegypti*. The strategies to prevent these illnesses involve the use of insecticide or repellent agents. Since several pesticides have deleterious environmental effects and affect humans, there is a strong interest in finding EOs and plant extracts that can be effective in controlling *Aedes aegypti*. The guideline that good larvicide candidates are substances with LC$_{50}$ < 100 mg/L [86] shows the importance of the finding that the EOs from *L. origanoides* (LC$_{50}$ = 54 mg/L) and *Swinglea glutinosa* (LC$_{50}$ = 66 mg/L) had an improved performance when used as a mixture (LC$_{50}$ = 38 mg/L). Other EO binary mixtures showed similarly interesting activity (*Turnera diffusa* and *S. glutinosa*, LC$_{50}$ = 64 mg/L; *L. alba* and *S. glutinosa*, LC$_{50}$ = 49 mg/L) [87].

5. Conclusions

Colombia's geographic and botanical conditions favor the development of its EOs agroindustry to convert this country into an important provider to the ever-growing EO world market. The initial offer will consist of EOs from aromatic plants of European and Asian origins, which are well-known and commonly traded in the international market. However, the results from the very small survey of Colombia's biodiversity indicate that there are many promising alternatives for future market expansion. The evaluation of biological activities of EOs obtained from plants growing in Colombia points toward many opportunities to develop a wide range of products that employ them as active ingredients.

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Conflict of interest

The authors declare that they do not have conflict of interest.

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