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Prospecting for non-timber forest products by chemical analysis of four species of Lauraceae from the Amazon region of Colombia

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Abstract

Lauraceae is a family of woody plants of economic importance mainly for their commercial exploitation as timber, as well as spices/food. Nonetheless, overexploitation is causing a decline in both the population and the associated ecosystems due to the lack of sustainability strategies and knowledge of alternative ways of utilization. The focus of this research was to determine if the secondary metabolites found/identified in the volatile fractions/ethyl acetate extracts of *Aniba panurensis*, *Nectandra cuspidata*, *Ocotea cymbarum* and *O. myriantha* from the Amazon region of Colombia (Departamento de Caquetá) would be promising/interesting for industry, so that uses/exploitation other than timber could be recommended. In this work, the chemical compositions by GC–FID/MS of the volatile fractions/total extracts (by HS–SPME/SDE/maceration) of the trunk wood of these trees were determined. The results were: (i) the volatile fractions/extracts of *A. panurensis* were composed of 88–94% benzenoid-type aromatic esters (benzyl salicylate and benzoate); (ii) *N. cuspidata* contained 95% sesquiterpenes (α-copaene and α-cubebene/germacrene D) by HS–SPME, 89% oxygenated and hydrocarbonated sesquiterpenes (τ-cadinol and δ-cadinene) by SDE, and 87% sesquiterpenes and aporphine alkaloids (α-copaene/germacrene D/δ-cadinene/α-cubebene and dicentrine/dehydrodicentrine) by solvent extraction; (iii) *O. cymbarum* contained mainly 63% sesquiterpenes and monoterpene ethers (α-copaene/*trans*-calamenene and eucalyptol) by HS–SPME, 63–85% of monoterpene alcohols (α-terpineol/borneol)/hydrocarbons (α-/β-pinenes)/ethers (eucalyptol) and phenylpropanoid ethers (methyleugenol) by SDE/solvent extraction; and (iv) for *O. myriantha*, the constituents per family were 91% sesquiterpenes (bicyclogermacrene/germacrene D)—HS–SPME, 72% sesquiterpene alcohols and sesquiterpenes/monoterpenes (spathulenol and bicyclogermacrene/δ-3-carene)—SDE, and 69% benzenoid-type aromatic esters and sesquiterpene hydrocarbons/alcohols (benzyl salicylate and bicyclogermacrene/α-cadinol)—solvent extraction. In conclusion, the main constituents identified in the woods (volatile fractions/extracts) of the species could be isolated and sustainably used/exploitated due to their bioproperties, as well as for their fragrant properties, some of which could be harnessed by different sectors/types of industries.

Keywords NWFP/NTFP, *Aniba panurensis*, *Nectandra cuspidata*, *Ocotea cymbarum*, *Ocotea myriantha*, Volatile fractions/ethyl acetate extracts, HS–SPME/SDE/EAE/GC–MS

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Introduction

The term "non-wood forest products" (NWFP) refers to those biological products (tangible goods) obtained from forests, other wooded lands and trees outside forest that are not timber or fuelwood; such products must be for human use/benefit. Derived products could include whole plants (herbs, shrubs/trees; medicinal or not) or their parts (fruits, leaves, bark, trunks, roots, seeds) for applications in medicine (drugs), food (edible products—fruits/seeds/roots/rhizomes)/spices/dyes, pharmaceutical/cosmetic/perfumery (essential oils/gums/resins/extracts/fractions), as well as sources of natural ingredients/raw materials, among others. NTFP harvesting/processing would offer great employment/economic opportunities for the poor rural populations surrounding the forest areas containing the biological resource but would also contribute to tropical forest conservation processes [1–3].

On the other hand, Lauraceae is a large family of perennial woody plants (ca. 55 genera and 3000–3500 species) widely distributed and mostly from tropical/sub-tropical latitudes/areas, such as Southeast Asia, Madagascar and Central/South America; some species are of great economic importance due to their commercial exploitation, e.g., *Aniba rosaedora*, *Cinnamomum camphora*, *C. cassia*, *C. zeylanicum*, *Laurus nobilis*, *Ocotea odorifera*, *Persea americana*, *P. caerulea*, and *Sassafras albidum*. The species numbers for the genera of interest are 48 *Aniba* spp., 120 *Nectandra* spp., and 400 *Ocotea* spp.; they are mostly timber trees/shrubs with glands containing fragrant oils [4–11]; however, many others are used as spices/fragrances for their pleasant aromas and medicinal properties (analgesic, anti-bacterial/fungal, anti-inflammatory, febrifuge, antinociceptive, anti-tumor/neoplastic, cytotoxic, antituberculous, antitussive, anti-dysenteric, antiparasitic/antimalarial, antirheumatic, cardiovascular effects, against gastrointestinal/urogenital disorders, snakebites, etc.). The main chemotaxonomic markers of Lauraceae species are lignans/neolignans; in addition, they contain other secondary metabolites as alkaloids, flavonoids, diterpenoids, steroids, miscellaneous, and volatile compounds [terpenoids/benzenoids/phenylpropa(e)noids—which constitute essential oils (EO)] [12–15].

An important fact is that Colombia has a wide diversity of species (ca. 255 spp.) of the Lauraceae family, represented by the genera *Ocotea* (75 spp.) and *Nectandra* (28 spp.), the most diverse and widely distributed in the country according to the Herbario Nacional de Colombia [16]. Meanwhile, the genus *Aniba* has only 20 spp. in the entire national territory. In the southern region of the country (Departamento de Caquetá) where tropical rainforest predominates, the Yarí-Caguán Reserve (municipalities of San Vicente del Caguán/Cartagena del Chairá) has an abundance of species of the Lauraceae family, which are exploited for commercialization, because they are timber, e.g., *Aniba panurensis*, *Nectandra cuspidata*, *Ocotea cymbarum* [syn. *Mespilodaphne cymbarum* (Kunth) Trofimov], and *O. myriantha*. Regardless of this use, these four species have ethnobotanical uses and fragrances that could suggest other applications besides timber harvesting [17, 18].

For instance, *A. panurensis* (syn. *A. gonggrijpii* Kosterm., *Aydendron panurense* Meisn.) is native to South America and is distributed in the northern region (e.g., Brazil, Colombia, Surinam, etc.); it is commonly named amarillo (yellow)/laurel oloroso, miratava/jiaocu (Amazon region of Colombia), camphor, canelón (Bolivia/Venezuela), sapito (Bolivia), cujacco ñomemba (Ecuador), palo de rosa (Peru), rozenhout (Surinam) and louro-amarelo (Brazil) [4, 17, 19, 20]. Different parts of the tree (up to 21 m, with red/purple fruits) have a characteristic/pleasant, penetrating odor; and according to the scientific literature consulted on the Brazilian plant, it contained essential oil (leaves) and styrylpyrone-type compounds, neolignans, benzoic acid esters, flavonoids, and indolizinium alkaloid (isolated from branches/wood (trunk)/barks). In addition, the extracts (EtOH)/fractions (hexane/CH_2Cl_2)/constituents obtained/isolated (kawapyron and indolizinium/indol alkaloids) from the plant were effective against bacteria (*Staphylococcus simulans*, *S. aureus*, and MRSA), parasites (*Trypanosoma cruzi* and *Leishmania amazonensis*) and fungi (*Candida albicans*); they also showed antioxidant capacities ($DPPH^{\cdot}$/$ABTS^{+\cdot}$ assays), cytotoxicity (MRC5 line) and inhibitions on the acetylcholinesterase enzyme (AChE) [21–27].

Furthermore, *N. cuspidata* (syn. *Aydendron laurel* Nees, *N. membranacea* (Sw.) Griseb., *Ocotea pichurium* Kunth.) is a shrub/tree (up to 21 m with a pungent fragrant odor on the leaves/branches/bark; edible drupoid fruits) native to America and is distributed from Mexico to Paraguay (including the Amazon countries). Some vernacular names are laurel(illo)/amarillo guacharaco/finto, laurel sabanero (negro), moena, lengua de venado (Colombia), aguacatillo (Mexico), laurel (Honduras), canelo (Nicaragua), laurel(ito) amarillo (rastrojero) (Venezuela), kerati/pisi (shirua) (Guiana/Surinam), moena(illa)/roble amarilla/blanca (Peru), yobini (Bolivia) and louro (branco/preto/tamanco)/canelão-seboso (Brazil). The stem-bark is used in ethnomedicine (Bolivia) as a treatment for stomachache and the fruit coat is applied as a dye; while in Brazil, the decoctions of seed and branch are astringent/febrifuge/tonic and gastric sedative, respectively. In Peru, the plant is use as a condiment and food, but also, as an antidiarrheal, antifebrifuge and

antidysenteric [17, 19, 20, 28–31]. The fruits are edible (used as food) and EOs and individual constituents such as sesquiterpenoids (costic, 3-isocostic and ilicic acids), alkaloids (glaziovine, apoglaziovine), diterpenoids, steroid (sitosterol), megastigmane (7-megastigmen-3β,6β,9ξ-triol) and polyprenoids (ficaprenol-12, ficaprenal-12) have been isolated from the different parts of the plant (leaves/branches/fruits). The EtOH extract of tree stem bark showed antimalarial activity and EOs containing sesquiterpenoids or monoterpenes had activities as antibacterial/cytotoxic and toxicity (brine shrimp). In addition, the acetone extract of the bark was antibacterial, antifungal and cytotoxic [32–38].

For its part, *Mespilodaphne cymbarum* (syn. *Alseodaphne cymbarum* (Kunth.) Kostel., *Licaria cymbarum* (Kunth.) Pittier, *O. cymbarum* Kunth.) is a tree (up to 23 m with fragrant odor in its parts; fleshy fruits) native and distributed in the northern region of South America (e.g., Colombia, Ecuador, French Guiana, Venezuela, Brazil), and its common names are aguarrás/s(z)asafrás/laurel aguacatillo/caparrapí/incibe/imbauba/cascarillo/Amacey (Colombia), sadi (Venezuela), sassafras orenoque/tirinkamwi (French Guiana), pau-sassafrás/canela-sassafás-preta/Brazilian sassafras oil (Brazil). The plant is used as a spice (bark and flower cups) and a source of EO (wood oil and it is medicinal) and fuel; likewise, the stem has diuretic, emmenagogue and tonic properties, and it contained bitter constituents. In Guiana/Suriname/French Guiana and Colombia, respectively, the plant (extracts) is used against poisoned arrows and to heal sores/wounds, treat respiratory/skin disorders, against malaria, as a repellent, purgative (indigenous) and antirheumatic [9, 17, 19, 39–42]. In addition, some allylphenols (apiol/dillapiol), neolignans (benzofuranes, burchellin, dehydrodieugenols), flavonoids (catechin derivatives) and sesquiterpenoids (oplopanone), sterols (stigmasterol/sitosterol) and fragrant liquid ("huile de sassafras") have been isolated from the wood/bark. An interesting molecule is burchellin (neolignane isolated from wood), which was active against *Aedes aegypti* larvae and *T. cruzi* trypomastigotes, and non-toxic on macrophages (BALB/c). It is worth noting that the wood EO from Brazilian tree was used as a substitute for sassafras oil due to its high safrole content [43–49].

Finally, *O. myriantha* (syn. *Mespilodaphne myriantha* Meisn., *Oreodaphne myriantha* Meisn.) is an evergreen tree/shrub (up to 22 m with ellipsoide/globose fleshy fruits) distributed in the Amazon countries of America. Its main vernacular names are garza muena (Peru), laurel espina (hediondo)/palo amarillo (picure) (Venezuela); in Colombia, it is known as amarillo balsudo/laurel baboso/buruchió/buruchigai-moho (Amazonas/Caquetá). Like most Lauraceae, its parts emit a pleasant penetrating odor [17, 19, 50]. In the scientific literature consulted, no report was found on the chemical constituents or biological activities on this plant and its parts.

Therefore, in this work, the chemical compositions of the volatile fractions (by HS–SPME/SDE) and ethyl acetate extracts (by simple maceration) of trunk woods of the *Aniba panurensis*, *Nectandra cuspidata*, *Ocotea cymbarum* and *O. myriantha* from the Amazon region of the departamento de Caquetá (Colombia) were determined by GC–FID/MSD, to establish if their secondary metabolites would be promising/interesting for the industry, so that other applications/uses/exploitations than timber would be recommended.

Text mining

The text mining analysis related to the bibliometric exploration on the topic of interest of this manuscript was based on two search equations (Supplementary Materials) which were run on the Scopus database (Elsevier, BV, 2023); the search found 1262 indexed entries. The graphs of the co-occurrence analysis generated by the bibliometric analysis for each search equation through the Scopus database are displayed in Fig. 1. As can be seen therein, many specific words correlated with the keywords/search equations were found in the database; nonetheless, the words *non-timber forest products*/*forestry*/*forest management*/*livelihood*/*conservation*, in that order, had the greatest occurrence for each search equation. In contrast, the scientific dynamics from 2010 to 2023 indicated that 2022/2020/2021/2014/2019 were the years with the highest science activity with 142/141/140/125/111 records, respectively. Considering the areas of knowledge, Agricultural/Biological Sciences, Environmental Science and Social Sciences were the most important for the keyword *non-timber forest products*; whereas for the names of the four species were Agricultural/Biological Sciences, Pharmacology/Toxicology/Pharmaceutics and Biochemistry/Genetics/Molecular Biology. Finally, the top five of countries according to the number of records (196–76) based on the equation were E.E.U.U., Brazil, India, Indonesia and United Kingdom. The top five Latin American countries were Brazil, Mexico, Colombia, Peru and Argentina with 162-10 records.

When a search equation (Supplementary Materials) was used, which included the terms *non-timber forest products* and the *four names of the species of interest*, no records were found. This result is relevant because it revealed the "*gap*" of information on this topic, creating an opportunity for the generation of new knowledge. In other words, based on the information that would be gathered on the chemical compositions of the four species under study and their potential to be used as natural ingredients/raw material for the pharmaceutical/

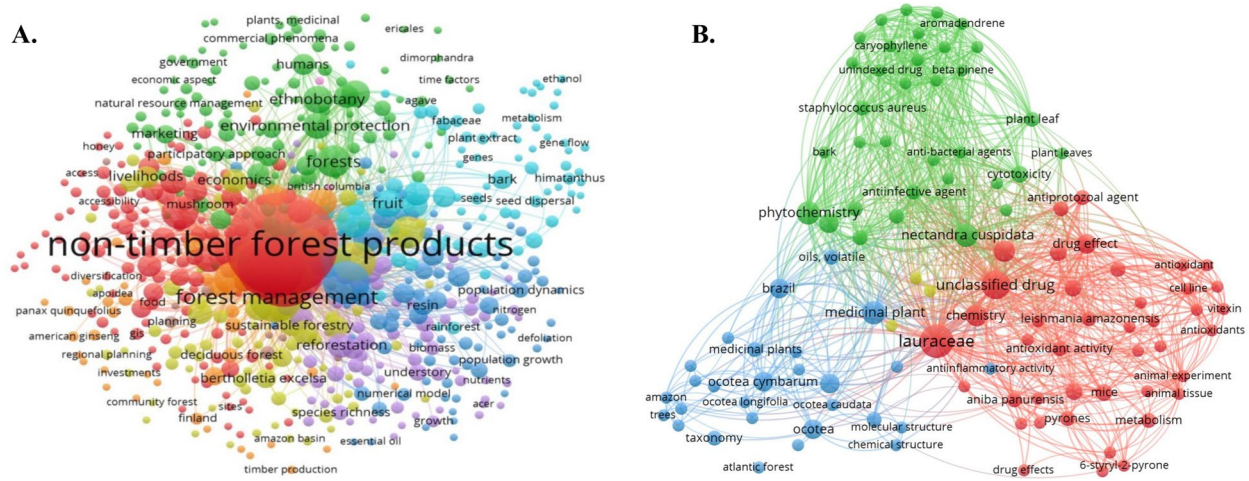


Fig. 1 Co-ocurrence diagrams according to the search equations used for the scientometric analysis. **A** non-timber forest products; **B** four species under study

perfumery/cosmetic industries or medicine, the species could be prioritized for sustainable exploitation as *non-wood forest products*.

Methods

Plant material

Samples (trunk woods consisting of sapwood/heartwood) from *Aniba panurensis*, *Nectandra cuspidata*, *Ocotea cymbarum* and *O. myriantha* were collected in the Unidad de Ordenación Forestal Yarí/Caguán (UOF), Amazon Forest Reservation, Cartagena del Chairá (Caquetá—Colombia) in August–September 2015. The UOF is a protected forest reserve (ca. 840,000 ha), containing non-stable (ca. 26%) and stable (ca. 49%) forest zones. On the other hand, the taxonomic identification of the species was carried out by Francisco Rojas-Triana at the Herbario Forestal UDBC—Univ. Distrital Francisco José de Caldas, and the collection of plants was made under Resolution No. 738 of July 8, 2014, conferred by the Agencia Nacional de Licencias Ambientales (ANLA).

In addition, trunk woods were sampled as reported by Bárcenas Pazos [51] with the main objective of establishing the physical–mechanical properties of the timbers; for this, 3–4 individuals (plants) of each species were randomly selected (distanced ca. 5–10 km, in a stable forest zone), from which three sections were isolated (lower, middle and upper parts, in triplicate) and rectangular blocks (62.5 cm^3) were taken (in triplicate) for each of them. For the determination of the physical–mechanical properties, a set of representative samples of the wood of each species was considered; as well as another set of samples was used for the respective chemical analysis. From the latter group, the wood samples (blocks of each section/tree, dehydrated/stored (on shelves in paper bags, not stacked) at room temperature (humidity controlled) until extraction process) were chipped (very thin and short slices), mixed/homogenized and subjected to three extraction methods (headspace solid-phase microextraction, simultaneous distillation–extraction and simple maceration) to obtain the secondary metabolites.

Obtaining the volatile fractions/total extracts

Headspace–solid-phase micro-extraction

Vapor phase constituents of the trunk wood were trapped using a SPME device (Supelco), with a polydimethylsiloxane (PDMS)-coated fiber (100 µm), sampling the headspace. The plant samples (1 g) were thermally preconditioned (50 °C) for 10 min, and then, the fiber was exposed to the headspace of each sample (separately) at 50 °C during 30 min (non-equilibrium conditions according to Muñoz [52] and Field et al. [53]). After sampling was completed, the SPME fiber was desorbed (5 min, 250 °C) with the analytes inside the GC–MS inlet port [54]. All samples were prepared/analyzed in duplicate.

Simultaneous distillation–extraction

In addition, the volatile fractions of the trunk wood (10 g) of *A. panurensis*, *N. cuspidata*, *O. cymbarum,* and *O. myriantha* were isolated by the Likens & Nickerson microscale apparatus, modified by Godefroot et al. [55], using CH_2Cl_2 (2 mL) as the extraction solvent for 2 h. The extracts were dehydrated with anhydrous sodium sulfate, and 1 µL of each extract (individually) was analyzed by GC–MS. All samples were prepared/analyzed in duplicate.

Maceration process

Total extracts of the four wood samples were obtained by maceration using ethyl acetate as the solvent. The plant material (30 g) was in contact with the solvent (100 mL) under stirring for 7 days at 25 °C. The extracts were concentrated to 1 mL, dehydrated with anhydrous sodium sulfate, and analyzed by GC–MS [54]. All samples were prepared/analyzed in duplicate.

Chemical analysis by GC–FID/MSD

Each volatile fraction/total extract was analyzed using a Trace 1310 gas chromatograph with a flame ionization detector (FID, 250 °C) along with an ISQ Series mass spectrometer (Thermo Fisher Scientific), with a split/splitless inlet (split ratio, 10:1), liquid autosampler (AI/AS 1310 Series, Thermo Fisher Scientific), or manual injection (SPME). A Rxi®-1 ms column (crossbond PDMS, 30 m × 0.25 mm ID × 0.5 µm df, Restek) was useful for the separation by individual constituents. The GC oven temperature setting was adjusted as described by Muñoz-Acevedo et al. [54]. Chromatographic and spectroscopic data were processed and analyzed through Thermo Xcalibur™ (Version 2.2 SP1.48, Thermo Fisher Scientific) and Automated Mass Spectral Deconvolution and Identification System software (AMDIS®, Build 130.53, Version 2.70). Linear temperature-programmed retention indices were calculated from a homologous series of C_7–C_{35} aliphatic hydrocarbons and analyzed under the same conditions as the samples by GC–FID/MSD. Then, the constituents were identified by comparing their mass spectra with those of the available databases/libraries (NIST11, NIST Retention Index, and Wiley9) along with the certified reference standards of some terpenes, and the linear retention indices reported and consulted in the existing literature [56–60]. As an important point, some constituents that could not be unequivocally identified, information about their molecular ions ($M^{+\cdot}$) and base peaks (BP—100% intensity) are reported in Table 1.

Cluster analysis of data

To carry out *Cluster* analysis with the data, Statistica software (version 10, data analysis software system, StatSoft, Inc.) was used to establish the best correlation between chemical compositions, methods of analysis and the samples of plant material used.

Results and discussion

Taxonomic identification

The four Lauraceae species were identified as *Aniba panurensis* (Meisn.) Mez (UDBC36928), *Nectandra* cf. *cuspidata* Nees & Mart. (Sw.) Griseb. (UDBC36978), *Mespilodaphne* cf. *cymbarum* (Kunth) Trofimov = *Ocotea* cf. *cymbarum* Kunth (UDBC36975) and *Ocotea* cf. *myriantha* (Meisn.) Mez (UDBC36979), which were species used as timber with large volumes of harvesting and mobilization in the sampling area (Departamento del Caquetá) between August–September 2015.

Uses for timber/non-timber forest products

The trunk woods of the four species from the Amazon Region, which are light-to-medium-heavy (0.613–0.666 g/cm^3) woods, have been used to make rafts, furniture and the core of lathed boards. In addition, the three species called amarillos/laurel (yellows/bay), *A. panurensis*, *N. cuspidata* and *O. cymbarum*, are exploited for flooring, finishing, carpentry and construction, according to Solórzano et al. [18] and León [61]. On the other hand, the results of the mechanical properties (low-to-medium strength values), reported by Solórzano et al., determined the promising uses of these woods, i.e., for the manufacture of handicrafts, packaging, stowage, exterior/interior finishes, stairs, post/beams, roofing, musical instruments, toys, pencils, etc.

Even so, the promising uses of timber mentioned above, based on uncontrolled/excessive anthropogenic extractive activity on these large trees, could put at risk the populations of these four species, the forest cover of this region, as well as the associated ecosystems. For these reasons, it is necessary to find other alternative uses that allow sustainability but also provide them with high/greater value (e.g., sources of new/known chemical substances that could be useful in medicine, food, flavorings/perfumes/cosmetics, and pharmaceutical/agrochemical industries).

Chemical compositions of volatile fractions and extracts

An important aspect to consider in this work was the possibility that the volatile profile could be significantly changed/lost from the plant material over time. Nonetheless, the plant material used was dry wood (sapwood/heartwood) composed mainly of lignocelullose and vegetable cells, which would influence the biosynthesis/preservation/retention/release/function of some secondary metabolites (termed extractives and varying between 2% and 10%) over time and ecological environment; subsequently, they can be extracted and used. These metabolites include terpenoids (volatile and fragrant components), benzenoids (ester derivatives) and alkaloids in nature [62, 63]. Thus, the structural complexity (biopolymers) of the sapwood/heartwood does not allow the complete and fast release of secondary metabolites (volatile, semivolatile, and non-volatile); only those constituents (volatile) found at the surface level of the wood (or in the bark) could be partially lost. For the exhaustive extraction of all of them from wood, it is necessary to increase the surface area of the sample (transforming

Table 1 Chemical composition of secondary metabolites (> 1.0%) isolated/analyzed by HS–SPME/SDE/SM/GC–MS from wood trunks of four Lauraceae species

No	Constituents	$R_{I\text{-}Cal}$	$R_{I\text{-}Lit}$	[a]Relative amounts, %											
				A. panurensis			*N. cuspidata*			*O. cymbarum*			*O. myriantha*		
				SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM
1	Benzaldehyde	930	931	Trace	5.5 ± 0.1	–	–	–	–	–	–	–	–	–	–
2	α-Pinene[c]	933	933	–	Trace	–	–	–	–	4.4 ± 0.1	13 ± 1	9.9 ± 0.1	–	1.1 ± 0.1	–
3	Camphene	942	943	–	–	–	–	–	–	Trace	1.2 ± 0.2	–	–	–	–
4	β-Pinene[c]	969	973	–	–	–	–	–	–	1.5 ± 0.1	5.0 ± 0.2	2.8 ± 0.2	–	–	–
5	Benzyl alcohol	1000	1005	–	1.6 ± 0.2	–	–	–	–	–	–	–	–	–	–
6	δ-3-Carene[c]	1005	1007	–	–	–	–	–	–	–	–	–	Trace	9.5 ± 0.1	–
7	*p*-Cymene	1008	1011	–	Trace	–	–	–	–	0.8 ± 0.1	1.6 ± 0.2	1.4 ± 0.1	–	–	–
8	1,8-Cineole[c]	1015	1020	–	Trace	–	–	–	–	11 ± 1	15 ± 1	8.6 ± 0.1	Trace	–	–
9	Limonene[c]	1017	1020	–	Trace	–	–	–	–	Trace	1.6 ± 0.1	–	–	–	–
10	Terpinolene	1075	1079	–	–	–	–	–	–	Trace	Trace	–	–	4.2 ± 0.1	–
11	Fenchol	1096	1100	–	–	–	–	–	–	Trace	2.3 ± 0.1	–	–	–	–
12	Borneol	1141	1148	–	–	–	–	–	–	1.2 ± 0.1	5.5 ± 0.2	–	–	–	–
13	Terpinen-4-ol[c]	1169	1164	–	–	–	–	–	–	Trace	4.3 ± 0.1	2.9 ± 0.1	–	–	–
14	α-Terpineol[c]	1178	1175	–	Trace	–	–	–	–	2.7 ± 0.2	25 ± 2	30 ± 1	–	–	–
15	α-Terpineol acetate	1337	1333	–	–	–	–	–	–	3.1 ± 0.1	Trace	Trace	–	–	–
16	Unidentified sesquiterpene ($M^{+\cdot}$ 204, BP 95)	1337	–	–	–	–	–	–	–	1.0 ± 0.1	–	–	–	–	–
17	δ-Elemene	1343	1338	–	–	–	2.8 ± 0.1	–	1.4 ± 0.1	–	–	–	2.3 ± 0.1	–	1.9 ± 0.1
18	α-Cubebene	1345	1350	Trace	–	–	10 ± 1	4.8 ± 0.1	12 ± 1	–	–	–	6.1 ± 0.2	1.3 ± 0.1	3.0 ± 0.1
19	Cyclosativene	1356	1360	–	–	–	1.4 ± 0.1	–	0.9 ± 0.1	2.4 ± 0.1	–	–	1.2 ± 0.1	–	–
20	Methyleugenol[c]	1366	1378	–	–	–	–	–	–	4.3 ± 0.1	11 ± 1	8.5 ± 0.1	–	–	–
21	α-Copaene	1374	1384	2.1 ± 0.2	–	–	18 ± 1	0.9 ± 0.1	17 ± 1	17 ± 2	–	3.3 ± 0.1	5.0 ± 0.2	1.3 ± 0.1	2.8 ± 0.1
22	β-Elemene	1380	1387	–	–	–	3.1 ± 0.1	–	–	1.1 ± 0.1	–	–	1.8 ± 0.1	–	–
23	β-Cubebene	1394	1395	–	–	–	1.3 ± 0.1	4.0 ± 0.1	3.3 ± 0.1	–	–	–	Trace	–	1.4 ± 0.1
24	Thujpsadiene[b]	1393	1405	–	–	–	–	–	–	1.9 ± 0.2	–	–	–	–	–
25	Aristolene	1417	1423	–	–	–	–	–	–	0.8 ± 0.1	–	–	2.4 ± 0.1	–	–
26	β-Caryophyllene[c]	1424	1428	–	–	–	3.7 ± 0.2	–	1.0 ± 0.1	–	–	–	2.5 ± 0.1	–	1.1 ± 0.1
27	β-Copaene	1426	1430	–	–	–	–	–	–	–	–	–	1.3 ± 0.1	–	–
28	6,9-Guaiadiene	1430	1435	–	–	–	0.8 ± 0.1	–	–	–	–	–	3.6 ± 0.2	–	2.0 ± 0.1
29	9-*epi*-(E)-Caryophyllene	1432	1429	–	–	–	1.9 ± 0.2	–	–	1.0 ± 0.1	–	–	Trace	–	–
30	γ-Elemene	1433	1432	–	–	–	1.3 ± 0.1	–	Trace	–	–	–	0.8 ± 0.1	–	0.8 ± 0.1
31	Aromadendrene	1439	1445	–	–	–	–	–	–	–	–	–	2.5 ± 0.1	–	–
32	*trans*-Muurola-3,5-diene	1450	1454	–	–	–	2.5 ± 0.2	Trace	0.8 ± 0.1	–	–	–	–	–	–
33	α-Humulene[c]	1454	1455	–	–	–	4.2 ± 0.1	Trace	1.6 ± 0.2	–	–	–	2.2 ± 0.2	–	–

Table 1 (continued)

No	Constituents	$R_{I\text{-}Cal}$	$R_{I\text{-}Lit}$	[a]Relative amounts, %											
				A. panurensis			*N. cuspidata*			*O. cymbarum*			*O. myriantha*		
				SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM
34	Unidentified sesquiterpene ($M^{+\cdot}$ 204, BP 95)	1459	–	–	–	–	–	–	–	–	–	–	–	–	2.0 ± 0.1
35	*trans*-Cadina-1(6),4-diene	1460	1470	–	–	–	1.1 ± 0.1	2.2 ± 0.2	–	–	–	–	–	–	–
36	γ-Muurolene	1461	1471	–	–	–	6.3 ± 0.2	Trace	2.2 ± 0.1	2.0 ± 0.1	–	–	1.6 ± 0.1	–	–
37	Germacrene D[c]	1464	1473	0.9 ± 0.1	–	–	9.5 ± 0.1	3.8 ± 0.2	15 ± 1	–	–	–	11 ± 2	4.1 ± 0.1	4.0 ± 0.2
38	β-Selinene	1470	1482	–	–	–	1.2 ± 0.1	–	Trace	Trace	–	–	5.6 ± 0.2	2.5 ± 0.1	2.3 ± 0.1
39	*trans*-Muurola-4(14),5-diene	1473	1476	–	–	–	1.9 ± 0.1	1.4 ± 0.1	–	–	–	–	–	–	–
40	Valencene	1486	1483	–	–	–	–	–	–	–	–	–	1.1 ± 0.1	–	–
41	*epi*-Cubebol	1490	1492	–	–	–	–	3.4 ± 0.1	1.9 ± 0.1	–	–	–	–	–	–
42	γ-Amorphene	1490	1495	–	–	–	1.7 ± 0.1	–	–	–	–	–	–	–	–
43	α-Selinene	1492	1497	–	–	–	1.7 ± 0.1	–	–	Trace	–	–	–	–	–
44	Bicyclogermacrene	1496	1500	–	–	–	–	–	1.1 ± 0.1	–	–	–	22 ± 1	11 ± 1	13 ± 1
45	α-Muurolene	1496	1495	–	–	–	2.9 ± 0.1	Trace	1.1 ± 0.2	1.3 ± 0.1	–	–	2.7 ± 0.2	–	–
46	γ-Cadinene	1499	1507	Trace	–	–	4.4 ± 0.1	3.8 ± 0.2	–	–	–	–	1.5 ± 0.1	–	–
47	*trans*-β-Guaiene	1499	1503	–	–	–	–	–	–	–	–	–	1.2 ± 0.1	–	–
48	β-Himachalene	1500	1501	–	–	–	–	–	–	–	–	–	1.2 ± 0.1	–	–
49	Cubebol	1510	1512	–	–	–	–	–	3.9 ± 0.1	–	–	–	–	–	–
50	δ-Amorphene	1512	1513	–	–	–	1.1 ± 0.2	–	–	–	–	–	–	–	–
51	*trans*-Calamenene	1513	1513	–	–	–	1.1 ± 0.1	Trace	Trace	7.4 ± 0.1	Trace	0.9 ± 0.1	1.9 ± 0.1	Trace	–
52	Unidentified sesquiterpenoid ($M^{+\cdot}$ 220, BP 93)	1515	–	–	–	–	–	–	–	1.1 ± 0.1	–	–	–	–	–
53	δ-Cadinene	1515	1517	1.8 ± 0.1	–	–	8.9 ± 0.1	21 ± 2	12 ± 1	5.5 ± 0.2	Trace	1.7 ± 0.1	7.8 ± 0.2	4.4 ± 0.1	4.2 ± 0.1
54	Elemicin	1522	1531	–	–	–	–	–	–	Trace	1.4 ± 0.1	–	–	–	–
55	*trans*-Cadina-1(2),4-diene	1525	1523	–	–	–	1.1 ± 0.2	1.6 ± 0.1	1.3 ± 0.1	Trace	–	–	Trace	–	–
56	α-Calacorene	1531	1531	–	–	–	Trace	Trace	–	6.4 ± 0.2	Trace	0.9 ± 0.1	Trace	–	–
57	Elemol	1537	1535	–	–	–	–	–	–	–	–	–	1.2 ± 0.1	6.2 ± 0.2	2.9 ± 0.1
58	Furopelargone A	1538	1540	–	–	–	–	–	–	2.4 ± 0.1	Trace	–	–	–	–
59	Spathulenol	1545	1552	–	–	–	–	Trace	–	–	Trace	–	1.8 ± 0.2	12 ± 1	6.5 ± 0.1
60	Germacrene D-4-ol	1547	1559	–	–	–	–	1.0 ± 0.1	–	–	Trace	–	–	–	–
61	*trans*-Nerolidol	1548	1550	–	–	–	–	–	–	3.3 ± 0.1	2.3 ± 0.1	4.5 ± 0.2	–	–	–
62	Germacrene B	1550	1557	–	–	–	1.9 ± 0.1	Trace	Trace	–	–	–	1.2 ± 0.1	–	1.2 ± 0.1
63	Unidentified sesquiterpene ($M^{+\cdot}$ 204, BP 95)	1576	–	–	–	–	–	–	–	–	–	–	–	–	3.2 ± 0.1
64	Globulol	1580	1588	–	–	–	–	–	–	0.8 ± 0.1	–	–	–	5.7 ± 0.2	–

Table 1 (continued)

No	Constituents	$R_{I\text{-}Cal}$	$R_{I\text{-}Lit}$	[a]Relative amounts, %											
				A. panurensis			*N. cuspidata*			*O. cymbarum*			*O. myriantha*		
				SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM	SPME	SDE	SM
65	Viridiflorol	1584	1590	–	–	–	Trace	Trace	–	–	–	–	–	2.4 ± 0.1	–
66	Guaiol	1589	1602	–	–	–	–	–	–	–	–	–	–	3.2 ± 0.1	–
67	Rosifoliol	1598	1600	–	–	–	–	–	–	–	–	–	–	2.4 ± 0.1	–
68	1,10-di-*epi*-Cubenol	1604	1611	–	–	–	Trace	1.1 ± 0.2	Trace	–	–	–	–	–	–
69	1-*epi*-Cubenol	1616	1623	–	–	–	Trace	7.9 ± 0.1	0.8 ± 0.1	1.6 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	–	6.0 ± 0.2	6.3 ± 0.2
70	*allo*-Aromadendrene epoxide	1622	1622	–	–	–	–	–	–	–	–	–	–	2.7 ± 0.1	–
71	τ-Cadinol	1625	1626	–	–	–	1.0 ± 0.1	26 ± 1	3.3 ± 0.2	–	–	Trace	–	–	–
72	τ-Muurolol	1625	1631	–	–	–	–	1.1 ± 0.1	–	–	–	–	–	–	–
73	γ-Eudesmol	1630	1632	–	–	–	–	–	–	–	–	–	–	3.9 ± 0.2	–
74	Cubenol	1631	1633	–	–	–	Trace	–	Trace	–	–	Trace	Trace	2.1 ± 0.1	–
75	δ-Cadinol	1633	1646	–	–	–	–	7.0 ± 0.2	–	–	–	–	–	2.3 ± 0.1	–
76	Unidentified sesquiterpenoid ($M^{+\cdot}$ 222, BP 81)	1636	–	–	–	–	–	–	–	–	–	–	–	2.7 ± 0.1	–
77	α-Cadinol[c]	1639	1641	–	–	–	–	3.8 ± 0.1	Trace	Trace	–	Trace	–	3.9 ± 0.2	9.0 ± 0.2
78	Cadalene	1652	1654	–	–	–	–	–	–	3.8 ± 0.2	1.0 ± 0.1	3.4 ± 0.2	–	–	–
79	10-nor-Calamenen-10-one	1698	1706	–	–	–	–	–	–	0.9 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	–	–	–
80	Benzyl benzoate[c]	1718	1722	14 ± 1	25 ± 1	11 ± 1	–	–	–	–	–	–	–	Trace	2.3 ± 0.1
81	Oplopanone	1734	1740	–	–	–	–	–	–	–	–	1.0 ± 0.1	–	–	–
82	Unidentified sesquiterpenoid ($M^{+\cdot}$ 234, BP 133)	1762	–	–	–	–	–	–	–	–	–	8.2 ± 0.2	–	–	–
83	Unidentified sesquiterpenoid ($M^{+\cdot}$ 232, BP 91)	1791	–	–	–	–	–	–	–	–	–	1.4 ± 0.1	–	–	–
84	Benzyl salicylate[c]	1839	1836	79 ± 2	67 ± 1	68 ± 1	–	–	Trace	–	Trace	–	0.8 ± 0.1	6.0 ± 0.1	29 ± 1
85	Verimol K	2006	2050	Trace	Trace	7.7 ± 0.2	–	–	–	–	–	–	–	–	–
86	Piperonylbenzofuran neolignan [$M^{+\cdot}$ 300.11 (BP)][b]	2479	–	–	–	0.7 ± 0.1	–	–	–	–	–	–	–	–	–
87	Allyl-piperonylbenzofuran neolignan [$M^{+\cdot}$ 340.13 (BP)][b]	2615	–	–	–	8.8 ± 0.2	–	–	–	–	–	–	–	–	–
88	NI	2629	–	–	–	1.8 ± 0.1	–	–	–	–	–	–	–	–	–
89	Dicentrine	2937	–	–	–	–	–	–	5.8 ± 0.1	–	–	–	–	–	–
90	Dehydrodicentrine	3307	–	–	–	–	–	–	7.2 ± 0.2	–	–	–	–	–	–

[a] Area normalization based on GC–FID analysis; $R_{I\text{-}Cal}$: Retention index calculated; $R_{I\text{-}Lit}$: Retention index of literature; SPME: Headspace–solid-phase micro-extraction; SDE: simultaneous distillation–extraction; SM: Simple maceration (ethyl acetate extraction); [b]Tentatively identified; [c]Compared to the respective certified reference standard; $M^{+\cdot}$: Molecular ion; BP: Base peak (100% intensity); NI: not identified

it into very small particles, chips or pellets). This type of matrix ensures that secondary metabolites could be isolated/obtained later (days, weeks, and months) by different extraction methods. Some well-known examples from the scientific literature on the use of woods in commercial applications/uses (inner/outer bark, chips, shavings/flakes, sawdust or small pieces of wood) for the isolation of bio-ingredients or raw material of fragrant products for the perfumery and condiments/spices industries are cinnamon (*C. zeylanicum*), sandalwood (*Santalum album*), cedarwood (*Juniperus virginiana*), guaiac-wood (*Bulnesia sarmienti*), laurel (*L. nobilis*), balsam of Peru (*Myroxylon pereira*), camphor (*C. camphora*), ishpingo (*Ocotea quixos*), sassafras (*O. cymbarum*) and rosewood/bois de rose (*Aniba rosaeodora*, *A. duckei*) [5, 64–66].

Furthermore, it is very important to clarify why ethyl acetate was chosen as the extraction solvent, as well as the non-equilibrium conditions used for the isolation of volatile fractions by HS–SPME. Thus, ethyl acetate was chosen as the solvent because of its intermediate polarity, low boiling point (77 °C, easy removal by distillation), ability to isolate both non-polar (preferably)/polar (with less preference) and low to relatively high-molecular weight molecules, low cost, low log P (log P 0.71—hydrophobicity/hydrophilicity), low water solubility (8.7%w/w), and low toxicity (non-toxic to the environment) [67, 68]. For HS–SPME, non-equilibrium conditions [69] were chosen and carried out due to the complexity of volatile mixture (very common circumstance for flavor and fragrances fields, > 20 constituents) with which the partition equilibrium varies constituent-to-constituent (different chemical nature) and therefore, there are different equilibria [70], and the occurrence of the competitive and displacement processes of terpenes during absorption when the temperature and time are higher than 50 °C and 30 min, respectively [52, 53].

In this way, the identified chemical constituents (88–100%) in the volatile fractions and ethyl acetate extracts of the trunk woods from the four Lauraceae species are listed in Table 1, according to the elution order of the total ion chromatograms (Figure S1a–c—Supplementary Material). In accordance with the table, both volatile fractions (VF) and ethyl acetate extract (EAE—%yield: 7.0%) of trunk woods from *A. panurensis* were mainly constituted by benzenoid-type aromatic esters [benzyl salicylate (67 ± 1–79 ± 2%) and benzyl benzoate (11 ± 1–25 ± 1%)] with relative amounts of 94% (HS–SPME), 92% (SDE) and 88% (EAE). In addition, EAE and SDE extracts, and HS–SPME contained neolignan derivatives of piperonylbenzofuran (10%), aromatic aldehydes/alcohols and tricyclic/bicyclic sesquiterpene hydrocarbons, respectively. The differentiable constituents of *A. panurensis* were (i) α-copaene and δ-cadinene by HS–SPME, (ii) benzaldehyde and benzyl alcohol by SDE, and (iii) verimol k (benzyl 2,6-dihydroxybenzoate) by EAE.

Comparison of the results on chemical compositions with the science literature consulted showed some similarities and/or differences. For example, the leaf EO from Brazilian *A. panurensis* consisted of sesquiterpene hydrocarbons represented by β-caryophyllene (33.5 ± 0.3%), germacrene D (25.4 ± 0.4%) and α-copaene (7.5 ± 0.1%) as described by Alcântara et al. [71]. In addition, some kawapyrone/neolignans were isolated from the EtOH extract of branches (hexane/CH_2Cl_2 fractions) [21–23, 27]; as well as an indolizinium alkaloid, styrylpyrones and four esters of benzoic acid (with cinnamyl alcohol), 10 neolignans (benzofuran/tetrahydrofuran/bicyclooctane guianin/bicyclooctane canellin types), five styrylpyrones and six flavonoids from wood and/or bark [4, 25, 26, 72–74].

In the case of the chemical constituents (volatile/non-volatile) of the wood of Colombian *A. panurensis*, they were different from those identified/recognized in the Brazilian species according to the reports mentioned above. However, Gottlieb and Kubitzi [75] reviewed/studied the chemomarkers found in *Aniba* spp. from the Amazon area; the metabolites were styryl(aryl)-pyrones and benzyl (derivatives) benzoates, without neolignans. However, other authors [26, 76–79] reported that trunk wood of *Aniba* sp., *A. burchellii* and *A. terminalis*, besides containing esters derived from benzoic acid, had several piperonylbenzofuran neolignans.

Thus, *A. panurensis* from Colombian Amazon region contained benzyl benzoate/salicylate [fragrant solid/liquid mixture isolated from EAE (picture of extract in Figure S1c—(A))—mass spectra in Figure S2], in agreement with [75]; but in addition, the wood sample presumably contained two neolignan derivatives of piperonylbenzofuran [$M^{+\cdot}$ 300.11 ($C_{17}H_{16}O_5$) and $M^{+\cdot}$ 340.13 ($C_{20}H_{20}O_5$)—Figure S3]. These neolignan derivatives were isolated and their structures have not yet been fully elucidated; however, according to the ions at *m/z* 77, *m/z* 91, *m/z* 103, *m/z* 105, *m/z* 115 and *m/z* 135 recorded in their mass spectra, these ions would originate from the fragmentation of the piperonyl group (Scheme 1, in Figure S3) of the structures. Moreover, due to the difference in *m/z* 40 mass units between the spectra of the $C_{17}H_{16}O_5$ and $C_{20}H_{20}O_5$ neolignans, it was indicative of the absence and presence of the allyl group in the two structures, respectively; this presumptive allyl-piperonyl lignan showed structural similarity (fragmentation pattern) to an allyl-piperonyl benzofuran [$(M+1)^{+\cdot}$ 341.13 (25%), $M^{+\cdot}$ 340.13 (100%)] reported by Gottlieb et al. [80]. Furthermore, other *Aniba* species also contained the same type of benzenoid esters, e.g., *A. affinis* [benzyl

benzoate (29%)], *A. firmula* [benzyl benzoate (64%), benzyl salycilate (36%)], *A. gardneri* [benzyl benzoate (44–78%)], *A. guianensis* [benzyl benzoate (45–59%), benzyl salicylate (6–17%)], *A. permollis* [benzyl benzoate (+), benzyl salicylate (+)] [72, 81].

Another interesting report was carried by Courtois et al. [82], who studied (by HS–SPME/GC–MS) the volatile organic compounds released by tropical trees (including Lauraceae) from French Guiana; these authors stated that the bark/leaves of *A. panurensis* mainly emitted α-/β-pinenes, *p*-cymene, limonene, β-caryophyllene/α-humulene, α-copaene/δ-cadinene and germacrene D, as volatile organic compounds [this chemical composition was also different from that of this work, but some monoterpenes coincided although in very low relative amounts (< 0.3%)]. This type of compounds (terpenes) would have a defensive function against fungi/bacteria/insects [83].

It is important to mention (considering the greater exploitation/application/use) that some extract/fraction/kawapyrone of the Brazilian tree were effective against bacteria [*S. simulans* (MIC 8–125 μg/mL), *S. aureus* (MIC 8–125 μg/mL) and MRSA (MIC 8–250 μg/mL)] and parasites [*T. cruzi* (epimastigote IC_{50} 20 ± 2 μg/mL, EC_{50} 79 μg/mL; trypomastigote IC_{50} 11 ± 2–21 ± 2 μg/mL) and *L. amazonensis* (promastigote EC_{50} 67 μg/mL)]; as well, EtOH extracts of leaves/twigs showed antioxidant capacities [$DPPH^{\cdot}$ assay, EC_{50} 14.4 ± 0.1–28 ± 2 μg/mL] and AChE inhibition (IC_{50} 1.3 mg/mL); and, the indolizinium alkaloid was an antifungal agent [against *C. albicans* (MIC 0.25 μg/mL), *Cryptococcus neoformans* (MIC 0.25 μg/mL), *Enterococcus faecium* (MIC 0.25 μg/mL)].

Regarding the chemical composition of the second Lauraceae species (*N. cuspidata*) under study, the classification of the most abundant volatile components by chemical families was: (i) HS–SPME: bicyclic (42%)/tricyclic (30%)/monocyclic (23%) sesquiterpene hydrocarbons; (ii) SDE: bicyclic sesquiterpene alcohols (47%) and bicyclic (32%)/tricyclic (10%) sesquiterpene hydrocarbons; and, (iii) EAE (%yield: 2.2%): tricyclic (32%)/bicyclic (20%)/monocyclic (20%) sesquiterpene hydrocarbons and aporphine alkaloids (13%). The constituents highlighted by family of compounds mentioned were: α-copaene (18 ± 1%)/α-cubebene (10 ± 1%)/germacrene D (9.5 ± 0.1%)—HS–SPME; τ-cadinol (26 ± 1%) and δ-cadinene (21 ± 2%)—SDE; α-copaene (17 ± 1%)/germacrene D (15 ± 1%)/δ-cadinene (12 ± 1%)/α-cubebene (12 ± 1%) and dicentrine/dehydrodicentrine—EAE. Once the wood sample was processed at basic pH, a fraction enriched with aporphinic alkaloids was obtained, among which dicentrine (95%) was identified, followed by dehydrodicentrine (4%).

When the compounds identified in the volatile fractions of Colombian *N. cuspidata* were compared with those found in the available scientific literature, they differed. Thus, the EOs (obtained by HD–GC/MS) of the leaves/branches/fruits (ripe and green) from Brazilian "laurelillo" were constituted by (i) γ-elemene (19%), (E)-caryophyllene (14%), bicyclogermacrene (13%) and δ-elemene (11%)—leaves; (ii) unidentified sesquiterpenoid (24%, $M^{+\cdot}$ 234)—branches; and (iii) δ-elemene (10%/7%), (E)-caryophyllene (7%), bicyclogermacrene (7%/6%), γ-elemene (6%), and (E)-nerolidol (6%/7%)—fruit oils (green/ripe), as reported by Margalho et al. [34]. While da Silva et al. [33] identified (E)-caryophyllene (27%) and bicyclogermacrene (16%) as main components in leaf EO.

Furthermore, Farias et al. [84] enunciated that bicyclogermacrene (28–50%) and viridiflorol (13–19%) were the most abundant compounds found in the leaf and branch EOs. Furthermore, Nascimento et al. [85] determined in (i) fresh leaf EO—bicyclogermacrene/(E)-caryophyllene (20% for each) and γ-elemene/germacrene B (12% for each); (ii) dried leaf EO—(E)-caryophyllene (23%), bicyclogermacrene (19%), γ-elemene (11%) and germacrene B (10%); (iii) fresh fruit EO—germacrene D (22%) and (E)-caryophyllene (12%); and (iv) dried fruit EO—(E)-nerolidol (8%), γ-elemene/caryophyllene oxide/(E)-caryophyllene (7% for each). Finally, Wu et al. [38] identified α-pinene (22%), δ-cadinene (14%), β-pinene (13%) and α-copaene (12%) as the main constituents of the leaf EO from Costa Rica. Despite these compositional differences, most species considered in this discussion consisted mainly of sesquiterpene hydrocarbons (> 50%).

If the composition of the trunk extract is considered, again some differences were found with the scientific reports reviewed. That is, Batista et al. [32] isolated nine compounds from the EtOH extract of the leaves; the metabolites were 7-megastigmen-3β,6β,9ξ-triol, glaziovine/apoglaziovine (proaporphine alkaloids), costic acid (sesquiterpenoid), α-tocopherol, β-sitosterol and three polyprenols (ficaprenol-12, ficaprenal-12, phytol), which were not identified in the EAE of the wood of this work. In addition to sesquiterpenoids, EAE contained dicentrine and dehydrodicentrine (aporphine alkaloids), which are reported for the first time in this species; whilst glaziovine/apoglaziovine were not found in the extract. The mass spectra (Figure S4) of dicentrine [$M^{+\cdot}$ 339.10 (64%), *m/z* 338.11 (100%)] and dehydrodicentrine [$M^{+\cdot}$ 337.09 (100%), *m/z* 322.07 (85%)] were compared with those available in scientific reports [86, 87] and both the intensities/distributions of the ions and fragmentation patterns coincided completely.

Taking into consideration the biological activities of the species, the leaf EO of the Brazilian tree demonstrated

antibacterial and antitumor properties, i.e., it was effective against *Escherichia coli* (MIC 20 µg/mL), *Bacillus cereus* (MIC 312 µg/mL) and *S. aureus* (MIC 625 µg/mL), and on the MCF-7 cell line (IC_{50} 117 ± 12 µg/mL) [33]. Besides, the hydroalcohol extract (at 250 mg/kg) of *N. cuspidata* stem bark showed in vivo antimalarial effects against *Plasmodium vinckei* (percent growth inhibition: 83%) and *P. berghei* (percent growth inhibition: 61%) [35]. Antileishmanial (*L. amazonensis*) and/or antioxidant effects of the extract (EtOH)/fraction (ethyl acetate—EAF, MeOH:H_2O—MF)/isolated compounds (epicathechin, isovitexin and vitexin) were also verified. Significant IC_{50} values as antiparasitic were for (i) EAF [amastigote: 4.4 ± 0.6 µg/mL (SI > 30), promastigote: 33.3 ± 0.8 (SI > 226)] and (ii) MF [amastigote: 6 ± 1 µg/mL (SI 87), promastigote: 19 ± 1 (SI > 27)]. Likewise, EAF also had the highest antioxidant capacity, which was comparable to that of BHT and quercetin (IC_{50}—$DPPH^{\cdot}$ 6.5 ± 0.1 µg/mL, $ABTS^{\cdot+}$ 4.8 ± 0.1 mmol ET/g; FRAP 2.37 ± 0.03 mmol ET/g; ORAC 34 ± 2 mmol ET/g; TP 387.1 ± 0.2 mg GAE/g) [88]. Furthermore, Ferreira et al. [89] established that EAF and some polyphenols isolated from "louro preto" leaves (EtOH extract) could be able to protect fibroblastic cells (L-929) and mice skin of the UVB-induced inflammation and oxidative stress. Moreover, Werka et al. [37] reported that the leaf EO was toxic (LC_{50} 4 µg/mL) against *Artemia salina*, and *S. aureus* (MIC 156 µg/mL). Finally, Arnobio et al. [90] demonstrated that an extract of *N. cuspidata* was not capable of damaging the human chromosome, as well as, it had no genotoxic potential.

Otherwise, tricyclic (21%)/bicyclic monoaromatic (14%)/bicyclic (11%) sesquiterpene hydrocarbons, and bicyclic monoterpene ethers (11%) were in the majority by HS–SPME; while monocyclic monoterpene alcohols (30–34%), bicyclic monoterpene hydrocarbons (13–19%) and ethers (9–15%), and phenylpropanoid ethers (9–12%) were by SDE and EAE (%yield: 3.6%), based on the categorization by component families for *O. cymbarum*. In addition, a group defined as unknown sesquiterpenoids (12%) was related to EAE. It is noteworthy that the term "sesquiterpenoid" refers to an oxygenated sesquiterpene (condensed formula $C_{15}H_{24}O/C_{15}H_{26}O$) which could have a mass spectrum with molecular ion above *m/z* 204 and between *m/z* 218–238 and characteristic ions varying based on its molecular mass, while "sesquiterpene" refers to a sesquiterpene hydrocarbon (consisting of carbon and hydrogen only—$C_{15}H_{24}$) with a mass spectrum with molecular ion of *m/z* 204 and characteristic ions such as *m/z* 79, 81, 91, 93, 105, 107, 119, 121, 133, 147, 161, 175, 189.

Finally, the main individual constituents were: α-copaene (17 ± 2%) and eucalyptol (11 ± 1%)—HS–SPME; α-terpineol (25 ± 2–30 ± 1%), eucalyptol (8.6 ± 0.1–15 ± 1%), α-pinene (9.9 ± 0.1–13 ± 1%) and methyleugenol (8.5 ± 0.1–11 ± 1%)—EAE and SDE. Meanwhile, an unidentified sesquiterpenoid ($M^{+\cdot}$ 234) was the fifth most abundant constituent (8.2 ± 0.2%) in EAE. Some distinguishable components for *O. cymbarum* were terpinen-4-ol/cadalene, α-terpineol acetate, *p*-cymene/1-*epi*-cubenol, fenchol and furopelargone A (a monoaromatic bicyclic sesquiterpene ketone) by HS–SPME/SDE/EAE. Figure 2 displays the chemical structures of the main constituents found in each volatile fraction/ethyl acetate extract.

When discussing the chemical constituents of *O. cymbarum* trunk, these differed according to the comparison of reports in the consulted literature [47]. Thus, Güenther [91] mentioned that the main constituent of the *O. cymbarum* EO was safrole (90–93%) [92], which was considered a chemical biomarker of the species. While de Diaz et al. [93] stated that wood EO (from the Amazon) was mainly enriched with α-phellandrene and *p*-cymene, and to a lesser extent with β-pinene and eugenol. In addition, Zoghbi et al. [49] identified α-selinene (26%), δ-cadinene (19%) and terpinen-4-ol (9%) in bark EO. In contrast, Delgado Ávila et al. [94] found that cascarillo (tree trunk sap) and sassafras (tree trunk/bark) EOs from the Colombian Orinoquia region consisted of α-pinene (47%)/camphor (21%) and α-terpineol (35%)/α-pinene (18%), respectively. These volatile compositions were different from those of the tree in this study, i.e., α-terpineol (2.7 ± 0.2–30 ± 1%), α-copaene (3.3 ± 0.1–17 ± 2%), 1,8 cineole (8.6 ± 0.1–15 ± 1%), α-pinene (4.4 ± 0.1–13 ± 1%) and methyleugenol (4.3 ± 0.1–11 ± 1%); although, the Colombian Orinoquia EO also consisted of α-terpineol.

Alternatively, from wood/stem bark/leaves (ethanol/hexane extracts) were isolated: (i) piperonylic acid and 1-nitro-2-phenylethane [95, 96], (ii) dehydrodieugenol, its monomethyl ether, and dehydrodieugenol-B [95], (iii) apiol, dillapiol, 4-hydroxy-2,3,5-trimethoxyallylbenzene, apiolglycol and lyoniresinol [43], (iv) burchellin (neolignan) [44], and (v) biseugenol [97]. Despite that, these components were not identified in the ethyl acetate extract related to this study. Among the promising bioproperties associated with *O. cymbarum* are the following, burchellin isolated from the tree wood/stem bark was antilarval against *A. aegypti* [46] and antiparasitic on *T. cruzi* [44]. Likewise, the same neolignan and its stereoisomers had antiviral effects against coxsackie virus B3 [98]. Besides, 1-nitro-2-phenylethane showed a hypotensive response through active vascular relaxation in rats [99], as well as an antinociceptive effect in mice [100]. Finally, biseugenol isolated from leaves (hexane extract) attenuated inflammation and angiogenesis [99].

Finally, bicyclic (54%)/monocyclic (19%)/tricyclic (18%) sesquiterpene hydrocarbons (by HS–SPME), bicyclic

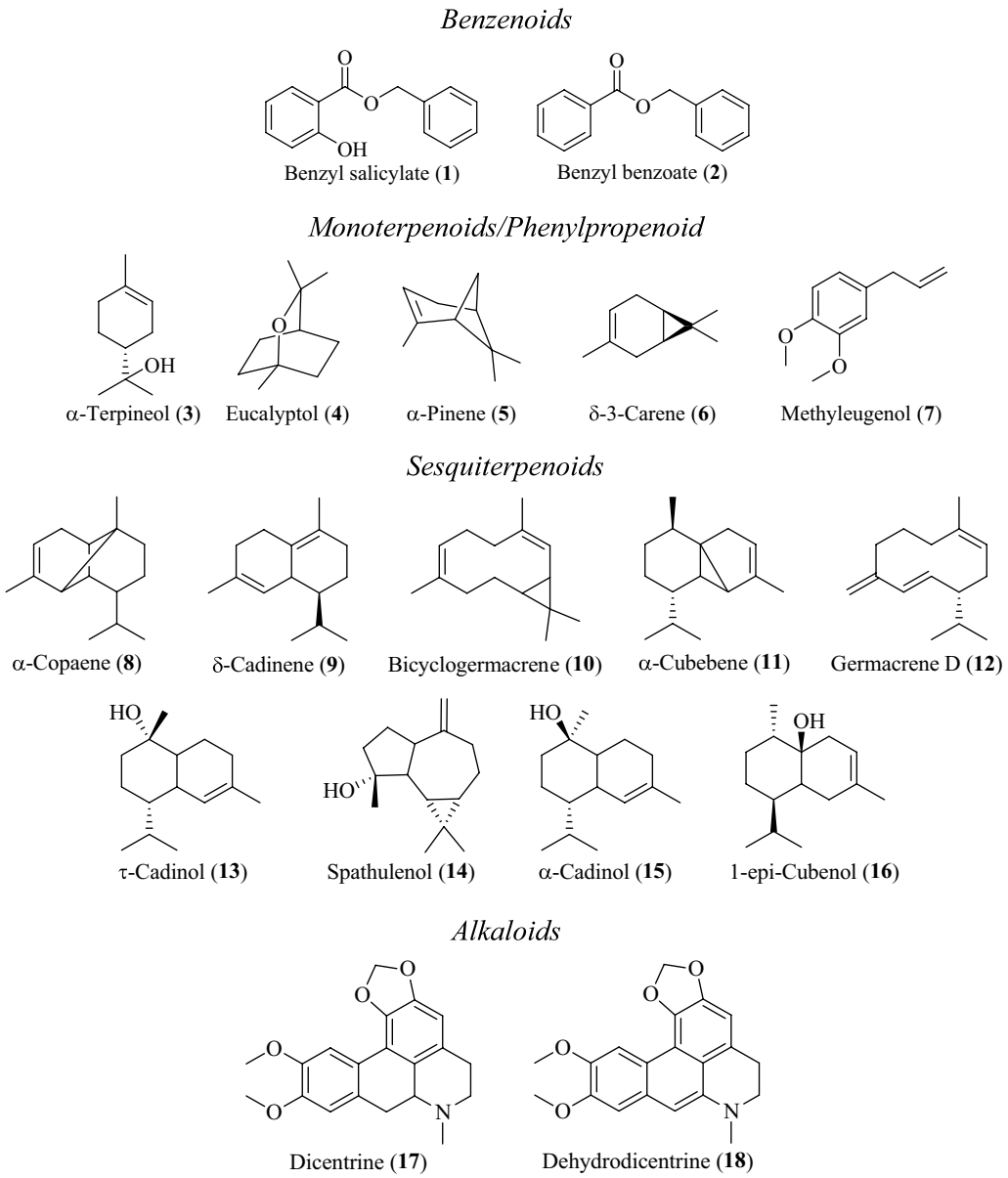


Fig. 2 Main types and chemical estructures of the compounds identified in the volatile fractions and extracts from four Lauraceae species

(24%)/tricyclic (20%) sesquiterpene alcohols, and bicyclic sesquiterpene (17%) and monoterpene (11%) hydrocarbons (by SDE), along with benzenoid-type aromatic esters (31%), bicyclic sesquiterpene hydrocarbons (23%) and alcohols (15%) (by EAE—%yield: 2.9%) were in the majority as compound families of *O. myriantha*. For SDE and EAE, some sesquiterpenoids (3–5%) were not identified but they were included in the table. The most representative compounds were: HS–SPME—bicyclogermacrene (22 ± 1%) and germacrene D (11 ± 2%); SDE—spathulenol (12 ± 1%), bicyclogermacrene (11 ± 1%) and δ-3-carene (9.5 ± 0.1%); and, EAE—benzyl salicylate (29 ± 1%), bicyclogermacrene (13 ± 1%) and α-cadinol (9.0 ± 0.2%).

The final discussion on the constituents identified in the *O. myriantha* wood was not possible, because the search in the science literature on the chemical components of this plant did not yield any results (no reports) related to them. Therefore, this discussion will be based on the constituents found in other plants belonging to *Ocotea* spp. (other than *O. cymbarum*). For example, the leaf EO of *O. porosa* contained bicyclogermacrene (25%) as main constituent [101]; while in the EOs of leaves from *O. indecora*, *O. nutans*, *O. macrophylla*, *O. macropoda*, *O.*

caudata, *O. gardneri* and *O. cernua* were identified bicyclogermacrene (11–56%) and/or germacrene D (5–58%) [33, 102–108]; as well as, for the leaf EOs of *O. limae* and *O. silvestris*/*O. discolor*/*O. leucoxylon* were reported spathulenol (13%)/bicyclogermacrene (11%) and germacrene D (54–73%)/bicyclogermacrene (9–30%), respectively [47, 105, 107–109]. As a final note, the profiles of the volatile/semi-volatile constituents of *O. myriantha* were in agreement with the aforementioned reports on the main class of constituents, which were of the sesquiterpenoid type.

Whether the biological properties of *Ocotea* spp. mentioned above are considered, *O. porosa* EO was an anticancer (MCF-7 and B16F10 lines) and insecticidal (*Cimex lectularius*) agent by topical application [101]; while *O. limae* EO had an acaricidal action on *Tetranychus urticae* [109]. To close, EOs of *O. indecora* and *O. silvestris* showed antifungal activity against *Candida parapsilosis* [107, 108].

Cluster analysis applied to the data set allowed drawing a hierarchical tree (Fig. 3) with two principal groupings according to the similarities/differences between the chemical compositions of the four Lauraceae species. The figure showed that the chemical profiles for any extraction method used for *A. panurensis* trunk were similar, with some differences for EAE (Group I); in this case, the chemically predominant family was the benzenoid-type aromatic esters (88–94%). While the remaining compositions of the other species (*N. cuspidata*, *O. cymbarum* and *O. myriantha*) were closer to each other, which were gathered in Group II (where sesquiterpenoids predominated in their great majority). This result is very interesting/important because it agrees with what was reported by Rohwer [50], who stated that the genera *Nectandra* and *Ocotea* are closer and their names are interchangeable (as synonyms) in biological descriptions due to their similar morphological/botanical characteristics (they have four locules in the anthers), as well as certain chemomarkers.

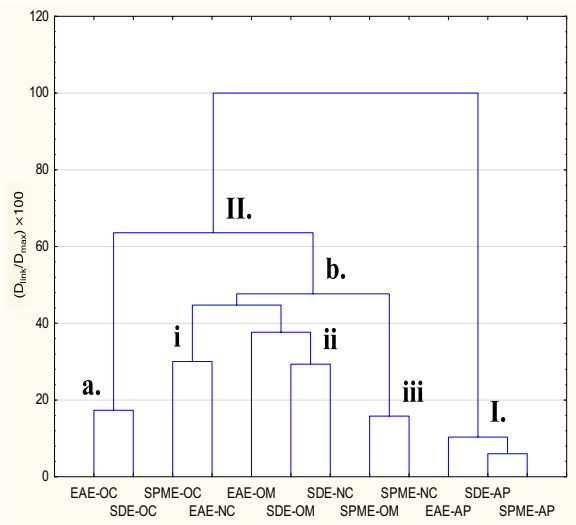


Fig. 3 Hierarchical tree acquired by Cluster analysis showing four groupings based on similarities/differences of the secondary metabolites identified in the trunks of *A. panurensis* (AP), *N. cuspidata* (NC), *O. cymbarum* (OC) and *O. myriantha* (OM)

Two subgroups also emerged from Group II: Sub-G "a" linked to SDE and EAE of *O. cymbarum* which contained mainly monoterpene alcohols (34–38%), hydrocarbons (13–19%) and ethers (9–15%); Sub-G "b" related to HS–SPME/SDE/EAE of *N. cuspidata*/*O. myriantha* along with HS–SPME of *O. cymbarum* which were mostly represented by sesquiterpene hydrocarbons (24–98%) and alcohols (4–52%). From this subgroup, a third hierarchy with three mini-groups (i/ii/iii) was derived; mini-group i was interrelated with *N. cuspidata* EAE and *O. cymbarum* SPME containing primarily sesquiterpene hydrocarbons (58–73%). While mini-group ii joined the SDE of *O. myriantha* and *N. cuspidata*, characterized mainly by sesquiterpene alcohols (28–45%) and hydrocarbons (24–47%). Furthermore, mini-group iii was related to the HS–SPME of *O. myriantha* and *N. cuspidata*, in which the sesquiterpene hydrocarbons (95–98%) represented their volatile fractions. Although the chemical compositions determined for *N. cuspidata* belonged to Sub-G "b", these were the most dissimilar, appearing in the three different mini-groups; the constituent types responsible for this differentiation were sesquiterpene hydrocarbons (98%), sesquiterpene alcohols (45%), and sesquiterpene hydrocarbons (73%)/alkaloids (13%) for HS–SPME, SDE and EAE, respectively.

Finally, as one of the most relevant aspects of this manuscript are the prospective uses of the main chemical constituents identified in the volatile fractions/extracts of the four Lauraceae species from the Colombian Amazon region, these were determined/compiled according to the reviewed science literature. Thus, some constituents have been mainly used as fragrance and flavoring ingredients (for perfumery, cosmetic and oral care products), food flavoring (for beverages), fixatives/solvents (for perfumery or chemical industry). Detailed information on these uses is included in Table S1, which contains the names of the 18 major components identified in the samples analyzed along with the type of compound, biological activities, toxicity, maximum permissible levels, industrial applications and interest for the industry. It is very important to highlight the potential that these individual constituents could have for uses/exploitation other than the industries mentioned above, but also, that the biological properties demonstrated by an individual compound

could be altered when interacting with other constituents in a mixture (e.g., extracts) producing synergistic or antagonistic effects.

As demonstrated, ***benzyl salicylate*** (**1**) was a good antifungal (MIC < 40–160 μg/mL for six dermatophytes) and insecticidal (non-/arthropods) substance, as well as anti-inflammatory and phytotoxic [110–112]. Just as benzyl salicylate is widely used in the cosmetic/perfumery industries as an active ingredient/raw material, so is ***benzyl benzoate*** (**2**). In addition, this molecule has been applied topically as a treatment for scabies and lice; it presented antifungal, antimite, pediculicidal (dogs), repellent (ticks/chiggers/mosquitoes), oestregonic and phytotoxic (herbicidal) activities, and as an arthropod/non-arthropod pest control. This constituent was also a semiochemical against *Euglossa* spp./*Euplusia* spp./*Megalopta* spp. (attractant), *Nematus prasinus* (pheromone), *Calindoea trifascialis* (allomone) and *Anomala octiescostata* (kairomone) [110, 111, 113–115].

In the case of ***α-terpineol*** (**3**), besides being one of the most important monoterpene alcohols for the flavor industry, it has presented antiviral, antiinflamatory and anticancer effects on the Herpes simplex virus type 1 (IC_{50} 22 ± 15 μg/mL), mice and A549/MCF-7 cell lines (%inhibition: 46 ± 2–95 ± 2% at 100 μg/mL; IC_{50} 33 ± 5 μg/mL), respectively. As well, its antimicrobial proficiency was proved against Gram+/Gram−/yeast strains [mainly *S. aureus*, *E. coli* (MIC 0.78 μL/mL), *Klebsiella pneumoniae*, *C. albicans* (MIC 60 μg/mL for all of them)] and as a chemical messenger against *Euclytia flava* (attractant) and *Podisus maculiventris* (pheromone) [114, 116–121].

Another important ingredient used as a flavoring/fragrance in large quantities is ***eucalyptol*** (**4**), which has demonstrated to be repellent (mosquitoes), larvicidal (*A. aegypti*), anti-inflammatory [suppressing arachidonic acid metabolism/cytokine production; 80–100% inhibition release of cytokines at ≥ 4 μM)], expectorant/mucolytic, antinociceptive and antiseptic, as well as penetration enhancer for topical delivery on the skin. In addition, eucalyptol is an allomone, attractant, pheromone and kairomone against *Stenus* spp./*Oxyops* sp., *Eufriesea* spp./*Euglossa* spp./*Eulaema* spp./*Euplusia* spp./*Adelphocoris* spp., *Frankliniella* sp., *Apis* sp./*Bombus* sp./*Coralina* sp. [114, 121–127].

Some interesting bioproperties of ***τ-cadinol*** (**13**) were antiparasitic [on *T. cruzi*—IC_{50} 18 μM (trypomastigotes) and 15 μM (amastigotes)], anticancer [MCF-7 (ED_{50} 2 μM), A-549 (ED_{50} 5 μM) and HT 29 (ED_{50} 8 μM) cell lines], toxicity (*A. salina* LC_{50} 7 μg/mL), acaricidal (*D. pteronyssinus*), antibacterial (*S. aureus*/MRSA) and antifungal [on 12 strains, e.g., *T. mentagrophytes* (MIC 2.3 μg/mL)]. In addition, it is a semiochemical on *Acanthella cavernosa*/*Heliconius erato* (pheromone), *Periplaneta americana* (attractant) and *Subulitermes baileyi* (allomone) [114, 128–136].

In contrast, few but very interesting biological potentials were described for ***bicyclogermacrene*** (**10**), e.g., cytotoxic/antitumor/cancer effects on HFF/B16F10-Nex2/HCT/HL60 cell lines (murine melanoma/colon carcinoma/leukemia) (IC_{50} 1.5 ± 0.1–4.4 ± 0.5 μg/mL) and as a semiochemical agent against *Lemnalia africana*/*Sinularia mayi*/*Parerythropodium fulvum* (pheromone) and *Thyrinteina arnobia* (attractant) [114, 137]. Likewise, ***δ-cadinene*** (**9**) (one of the most abundant sesquiterpenes in plants) is a semiochemical on *Sinularia mayi*/*Papilio glaucus*/*Byasa alcinous*/*Plebeia droryana*/*Apis mellifera*/*Nannotrigona testaceicornis* (pheromone), *Reticulitermes okinawanus*/*Semanotus japonicus* (allomone) and *Tritoma bipustulata*/*Dacne bipustulata*/*Gonipterus platensis* (attractant). As well, this cadinane-type sesquiterpene had a good cytotoxic effect against BT-20 (3.9 μg/mL), HeLa (3.7 μg/mL) and OVCAR-3 cell lines. Finally, this cadinene showed insecticidal action against larvae of *Anopheles stephensi*/*A. aegypti*/*Culex quinquefasciatus* (LC_{50} 8–10 μg/mL) (malaria, dengue and filariasis vectors) [114, 138, 139].

As for ***α-copaene*** (**8**), it had an antineoplastic effect on MCF-7 and non-cytotoxic action against N2a-NB cell lines, but moderate cytotoxicity on human lymphocytes. Furthermore, α-copaene is a semiochemical against *Hypothenemus* spp./*Pityoborus* sp./*Pseudopityophthorus* spp./*Ambrosiodmus* spp./*Xyleborus* spp./*Xylosandrus* sp./*Ceratitis* sp./*Plebeia* sp. (attractant) and *Euceraphis* sp./*Apis* sp./*Meliponula* sp./*Plebeia* sp./*Heliconius* spp./*Papilio* sp./*Byasa* sp. (pheromone) [114, 140–142].

Considering ***germacrene D*** (**12**), which is another of the most common constituents in plant EOs, it has demonstrated to be effective against various types of cancer, e.g., HL60/B16F10-Nex2/HCT/HFF cell lines (IC_{50} 4.4 ± 0.2–10.5 ± 0.7 μg/mL); as well as antibacterial actions against strains of *B. subtilis*, *S. aureus*, *Proteus vulgaris*, *Shigella dyssenteriae*, *Salmonella typhi* and *K. pneumoniae* (MIC 30–90 μg/mL). While the antiradical capacities on $DPPH^{\cdot}$ and $OH^{\cdot}$ radicals presented IC_{50} values of 80 μg/mL and 91 μg/mL, respectively. Last but not least, this sesquiterpene activated the receptor neuron of *Heliothis virescens*, acting as a semiochemical [139, 143, 144].

Finally, ***dicentrine*** (**17**), an aporphine-type alkaloid, was considered. Thus, dicentrine is a powerful therapeutic agent due to its relevant bioproperties, e.g., anticancer/tumoral (on 21 cell lines with IC_{50} values between 0.4 ± 0.2–27 ± 2 μM with the most susceptible in decreasing order being HCE-6, TE II, Molt-4, CE 81 T, CESS, HL-60, K562, MS-G2, CE 48 T and COLO 201; and on other 13 cell lines with IC_{50} values between 0.6 and

6 µg/mL), toxic (LC_{50} 8 µg/mL on *A. salina*), antiparasitic (on chloroquine-sensitive/resistant *Pl. falciparum*, *T. cruzi* trypomastigote, *T. brucei brucei*, *L. infantum* amastigote), larvicidal (on *A. aegypti*), AChE inhibitor (IC_{50} 7 µM), antihypertensive/hypolipidaemic, potent/selective α1-adrenoceptor antagonist in vascular smooth muscle/human hyperplastic prostate, vasorelaxant, antiplatelet thromboxane B2 formation and antinociceptive [144–159].

Conclusions

It was reported that, (i) for the first time, the chemical composition of the volatile fractions and ethyl acetate extract of *O. myriantha* trunk; (ii) the high content of benzenoid-type aromatic esters in the wood of Colombian *A. panurensis*; (iii) the unusual presence of the aporphine alkaloids dicentrine and dehydrodicentrine in the *N. cuspidata* trunk. In addition, (iv) chemical similarity was found/evidenced between the wood of the trunks of *Nectandra* sp. and *Ocotea* spp. under study. Finally, (v) the most abundant chemical constituents identified as secondary metabolites (particular/uncommon) in the four species, which would have promising bioactivities and uses in the industry (based on the scientific literature reviewed), would allow recommending other non-timber use/exploitation for these trees, e.g., fragrance ingredients/raw materials, semiochemicals, insecticides, anticancer/antitumoral/antiparasitic/antimicrobial/antiviral/anti-AChE/antinociceptive/vasorelaxing substances, etc., with which some sustainability strategies could be created along with productive projects (including closing the cycle—orange or circular economy).

Abbreviations

$ABTS^{+\cdot}$	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical-cation
AChE	Acetylcholinesterase enzyme
BP	Base peak
$DPPH^{\cdot}$	2,2-Diphenyl-1-picrylhydrazyl radical
EAE	Ethyl acetate extract(ion)
EO(s)	Essential oil(s)
EtOH	Ethanol
GAE	Gallic acid equivalent
GC–FID	Gas chromatography–flame ionization detector
GC–MS	Gas chromatography–mass spectrometry
HD	Hydrodistillation
HS–SPME	Headspace–solid-phase microextraction
IC_{50}	50% Inhibitory concentration
LC_{50}	50% Lethal concentration
$M^{+\cdot}$	Molecular ion
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
NWFP/NTFP	Non-wood (timber) forest products
PDMS	Polydimethylsiloxane
SDE	Simultaneous-distillation extraction
VF	Volatile fractions

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s10086-024-02148-y.

Supplementary Material 1.

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Author contributions

Conceptualization, writing—review and editing, HS–SPME analysis, GC–FID/MS analysis, multivariate analysis, A.M.-A.; sample collection (trunks), writing—review and editing, multivariate analysis, R.L.-C.; sample preparation, obtaining SDE and EA extracts, M.C.G.; sample preparation, obtaining SDE and EA extracts, isolation of alkaloids/benzenoid esters, O.J.C.; bibliometric analysis, writing—review, M.C.-D.; writing—review and editing, M.C. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The main raw data for this manuscript are included in the supplementary materials. However, if any reviewer, editorial member or reader requires additional information, please contact the corresponding author to send the information directly.

Declarations

Competing interests

The authors declare that the information in this manuscript does not have any potential competing interests.

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