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Phytochemical analyses of eight plants from two provinces of Ecuador by GC-MS

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Abstract
Vegetal extracts are mixes of secondary metabolites that exhibit a broad spectrum of pharmacology effects. The aim of this work was to identify the chemical constituents in ethanol extracts of eight plants from Ecuador and to relate it with biological activities reported for those compounds. Extracts were obtained from leaves of *Coriandrum sativum*, *Cynara scolymus*, *Artemisia absinthium*, *Cnidoscolus chayamansa*, *Melissa officinalis*, *Moringa oleifera*, *Bougainvillea spectabilis*, and *Lippia citrodora* by maceration with ethanol. Characterization of chemical composition was carried out by the technique of gas chromatography-mass spectrometry. Study showed that Ecuadorian plants are source of essential fatty acids and derivatives, benzenoids, and terpenoids such as phytosteroles and pentacyclic triterpenes. According to literature, almost all of constituents identified in the eight Ecuadorian plants have shown biological activity, such as antimicrobial and antitumor activities. This confirms that Ecuadorian plants could be important resources of bioactive compounds for treatment of illnesses such as cancer.

Keywords: medicinal plants, bioactive constituents, phytosterols, vitamin E, phytol, fatty acids

1. Introduction
Even nowadays, many American communities use herbal medicine as the first response to illness. Several studies have demonstrated that Ecuador has a megadiversity of plants, which are used as wood, ornamental, food and especially traditional medicine [1-6]. Ethnobotanical field studies aimed at a contribution to natural products research and/or the conservation of cultural heritage [7-8]. In fact, medicinal plants are intensely studied in Andes region in which Ecuadorian can include these plants in their quotidian and substitute the conventional medicines [9]. In this study, the chemical compositions of eight Ecuadorian medicinal plants belong to different botanical families were evaluated by gas chromatography/mass spectrometry as a contribution of the chemotaxonomic knowledge for these species in the region.

2. Materials and Methods
2.1 Sampling
Sample of plants were collected in natural ecosystems from Machala and Santa Rosa (province of El Oro, relative humidity: 65-85 %, average temperature 26 °C), and Cuenca (province of El Azuay, relative humidity: 82 %, average temperature 21 °C). Samples were identified by botanical Jesús Inca as *Coriandrum sativum* L. [1753] (Apiaceae), *Cynara scolymus* L. [1753] (Asteraceae), *Artemisia absinthium* L. [1753] (Asteraceae), *Cnidoscolus chayamansa* McVaugh [1944] (Euphorbiaceae), *Melissa officinalis* L. [1753] (Lamiaceae), *Moringa oleifera* Lam. [1783] (Moringaceae), *Bougainvillea spectabilis* Willd. [1799] (Nyctaginaceae), and *Lippia citrodora* (Paláu) Kunth [1818] (Verbenaceae), which were processed without previous storage. The species were selected according to their ethno-botanical uses and bioactivity reported.

2.2 Extracts
Leaf of each plant were separated from their other botanical parts, powdered and extracted with ethanol (Puriss. p.a., Fluka Chemie, Seelze, Germany). In each case, solvent was evaporated in a rotary evaporator Heidolph (~11 mbar, 40 °C), for obtaining crude extracts.

2.3 Chromatographic analyses
Solutions of 20-30 µL of each extracts dissolved in 1 mL of chloroform (99,0-99,4 % GC, Sigma-Aldrich, St. Louis, MO, USA) were injected in an Thermo Scientific Trace 1300 chromatograph, with source of electronic impact ionization (70 eV) and an injector type Split

(10:1) at 280 °C with auto-sampling. The apparatus was equipped with a TG-5MS (Thermo Scientific) column of 30m×0.25mm DI×0.25mm of thickness). Initial temperature in oven was 40 °C (during five minutes), with a ramp of 5 °C/min until 310 °C. Helium was used as eluent with a flux of 1 ml/min. A Triple Quadrupole TSQ 8000 Mass Spectrometer was coupled to chromatograph, which was performed at EI of 70 eV, transference line at 280 °C and ion source at 220 °C; scan was carried out from 50 to 500 m/z. Analyses were performed by triplicate. Identification was based on the comparison of obtained mass spectra with spectra stored in the libraries NIST-2011 and WILEY (9 ed.) databases, selecting the compounds with a confidence match upper to 90 %.

3. Results

Chromatograms of the eight Ecuadorian plant extracts are shown on Fig. 1 to 8, in which there are about three-ten peaks with high intensity. Constituents identified in these plants are summarized in Table 1. Results indicated the presence of several types of secondary metabolites; especially those belong to the terpenoids family, including steroids and pentacyclic triterpenes. Other compounds were fatty acid and their ester derivatives, benzenoids, and some volatile hydrocarbons.

In the chromatogram of *C. sativum* leaves-extract (Fig. 1), the four main signals are shown at retention time (RT) of 38.65 min, 40.77 min, 41.46 min, and 42.05 min. The compounds associated with these values were palmitic acid, visnagin, phytol and linolenic acid, respectively. Furthermore, two important phytosterols, stigmasterol and β-sitosterol, appeared at 58.68 min and 59.38 min, respectively. While peak at 62.14 min was related to α-tocopherol.

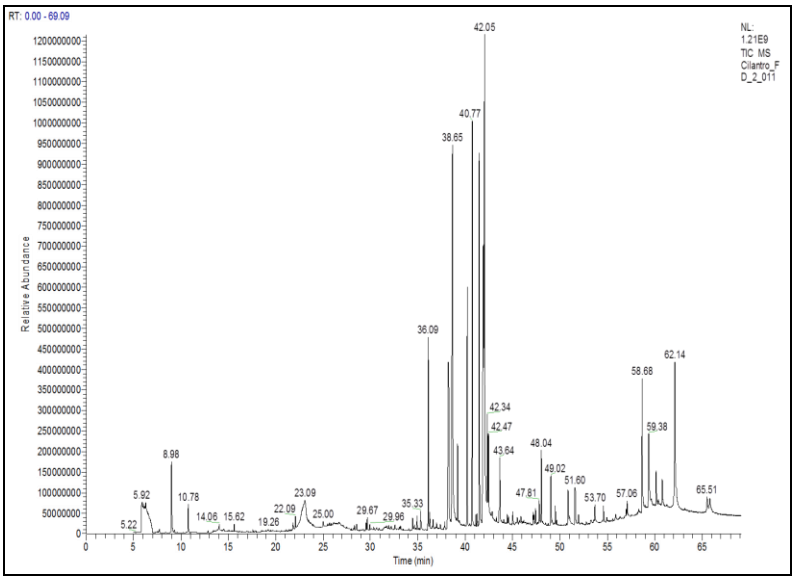

Fig 1: Chromatogram of ethanolic leaves-extract of *Coriandrum sativum* L.

The main compounds on chromatogram of *C. scolymus* leaves-extract (Fig. 2) were 4-hydroxy-4-methyl-2-pentanone, palmitic acid, phytol, linolenic acid, squalene and elasterol, which appeared at RT of 8.92 min, 38.64 min, 41.48 min, 42.02 min, 52.81 min, and 59.36 min, respectively. Fig. 3 shows the chromatogram of *A. absinthium* leaves-extract, on which three important signals appear at RT 41.46 min, 59.40 min, and 60.59 min. These values were associated with the compounds phytol, γ-sitosterol and lupeol, respectively.

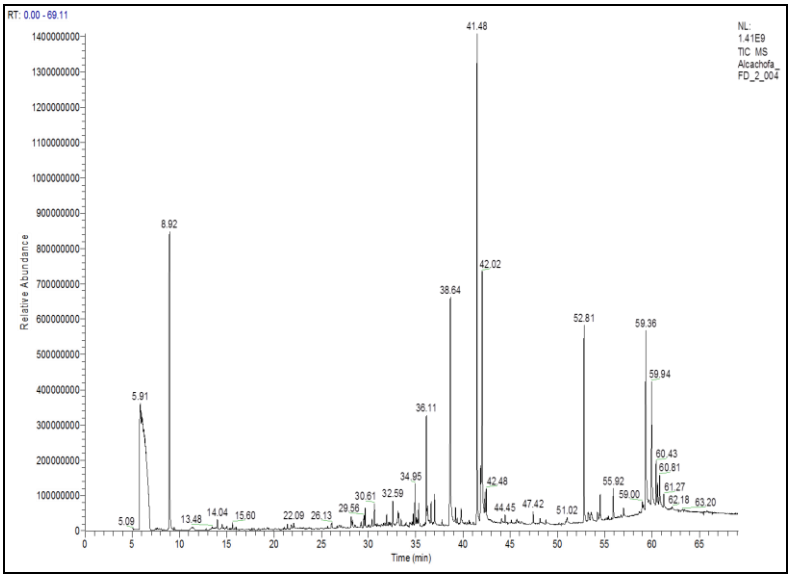

Fig 2: Chromatogram of ethanolic leaves-extract of *Cynara scolymus* L.

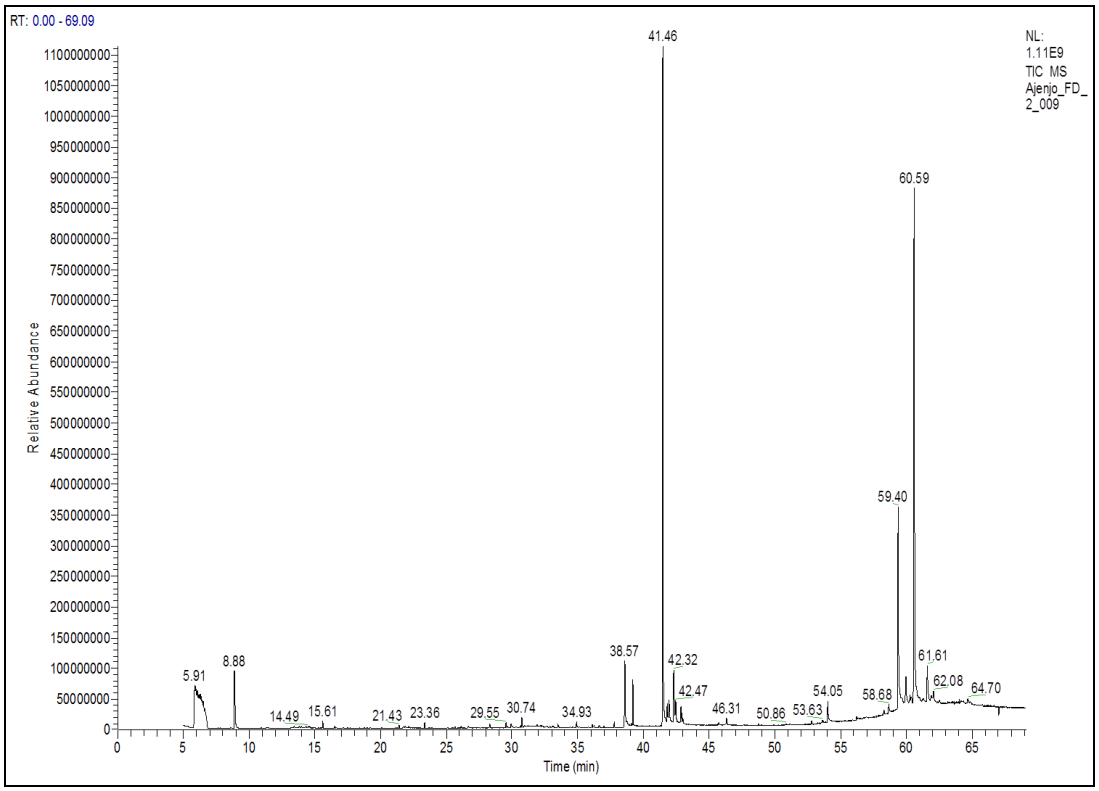

Fig 3: Chromatogram of ethanolic leaves-extract of *Artemisia absinthium* L.

Chromatogram of *C. chayamansa* leaves-extract (Fig. 4) also shows three main signals, which appear at RT of 41.47 min, 59.41 min, and 62.02 min. Similarly, the compounds associated with these signals were phytol, β-sitosterol and lupeol acetate, respectively. The main constituents identified in *M. officinalis* leaves-extract were 2,2-diethoxypropane and γ-sitosterol, which appeared in the chromatogram of Fig. 5 at RT of 6.17 min, and 59.41 min, respectively. Other minor compounds identified were 4-hydroxy-4-methyl-2-pentanone (8.93 min), phytol (41.46 min), and α-tocopherol (56.96 min).

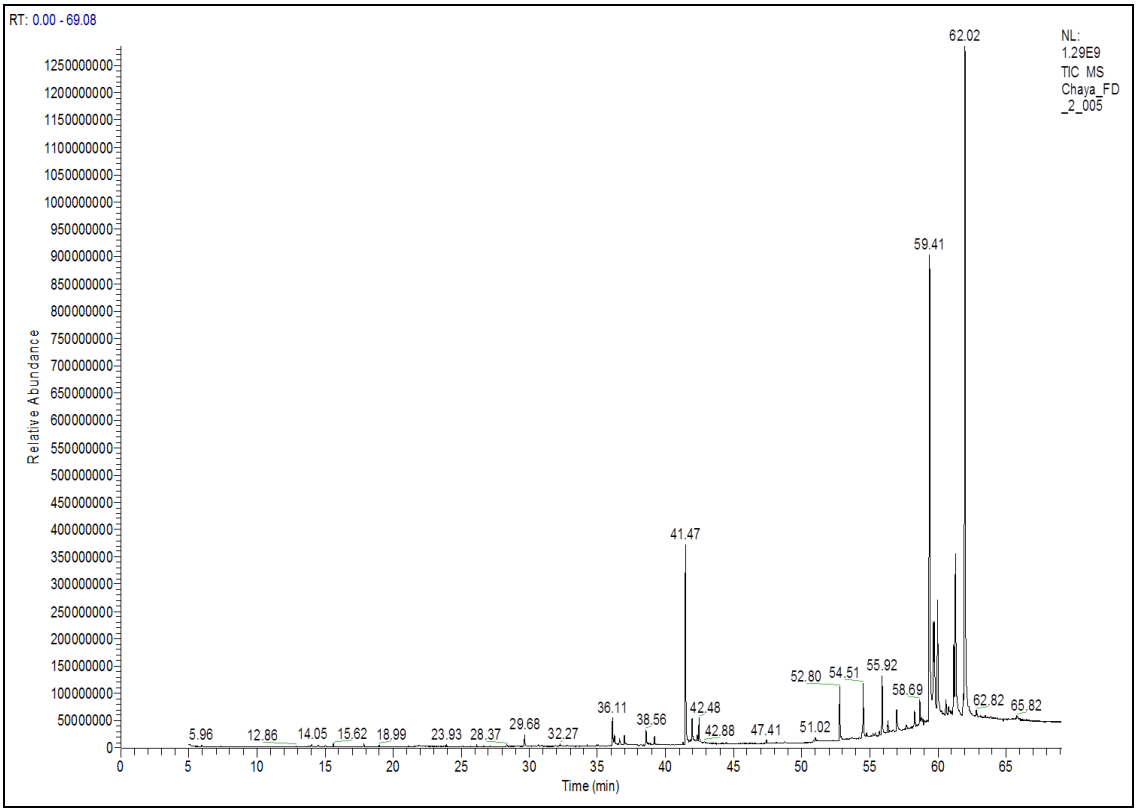

Fig 4: Chromatogram of ethanolic leaves-extract of *Cnidoscolus chayamansa* Mc Vaugh.

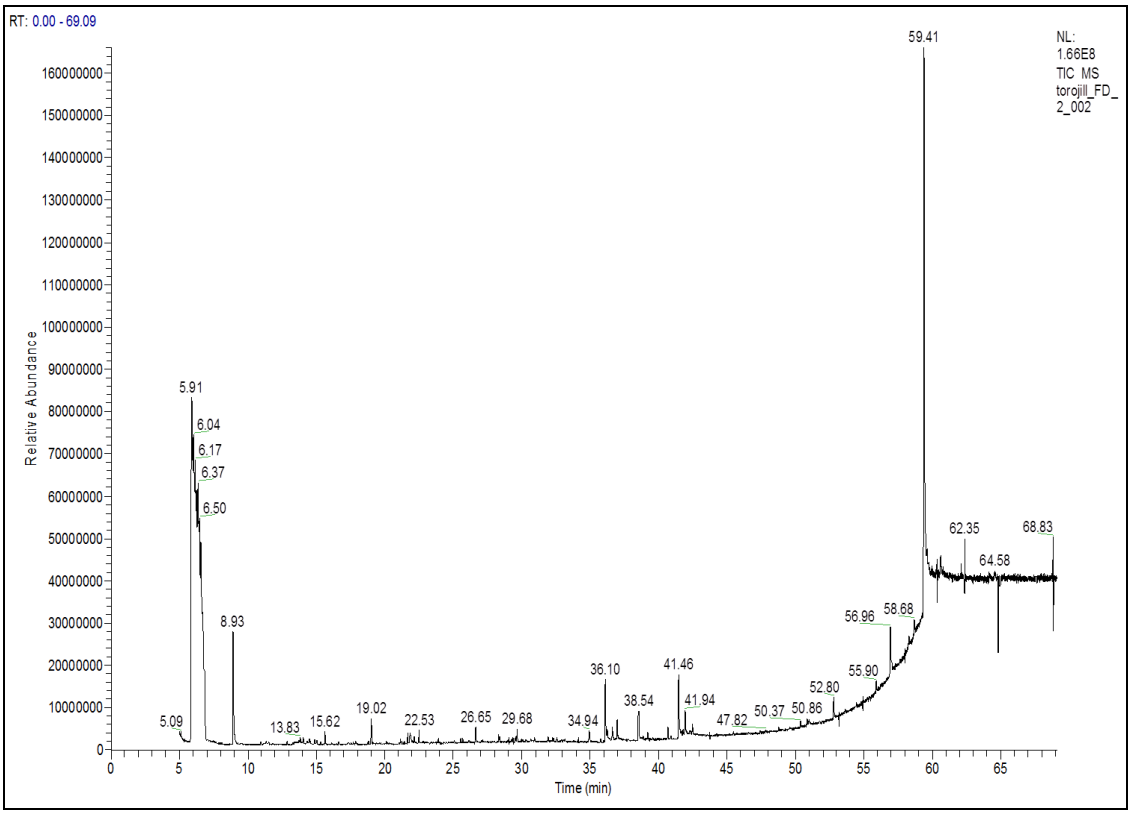

Fig 5: Chromatogram of ethanolic leaves-extract of *Melissa officinalis* L.

Chromatogram of *M. oleifera* leaves-extract (Fig. 6) shows five main constituents, which appear at RT of 38.62 min, 41.46 min, 42.03 min, 56.97 min, and 59.63 min. These signals were associated with the compounds palmitic acid, phytol, linolenic acid, α-tocopherol, and fucosterol, respectively. In the chromatogram of *B. spectabilis* leaves-extract (Fig. 7), seven main compounds were identified as 4-hydroxy-4-methyl-2-pentanone, 3-O-methyl-D-glucose, palmitic acid, ethyl palmitate, petroselinic acid, ethyl oleate, and stigmasterol, which occur at RT of 8.90 min, 33.45 min, 38.58 min, 39.19 min, 41.92 min, 42.42 min, and 59.37 min, respectively.

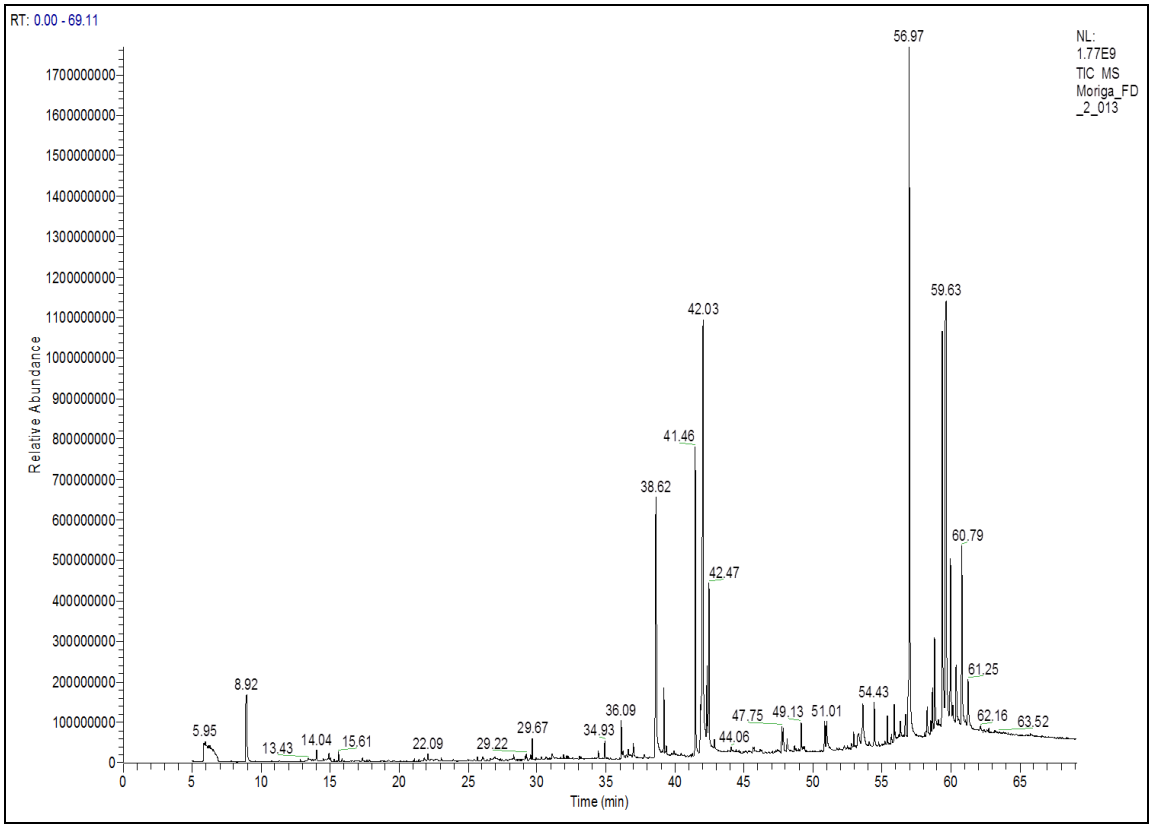

Fig 6: Chromatogram of ethanolic leaves-extract of *Moringa oleifera* Lam.

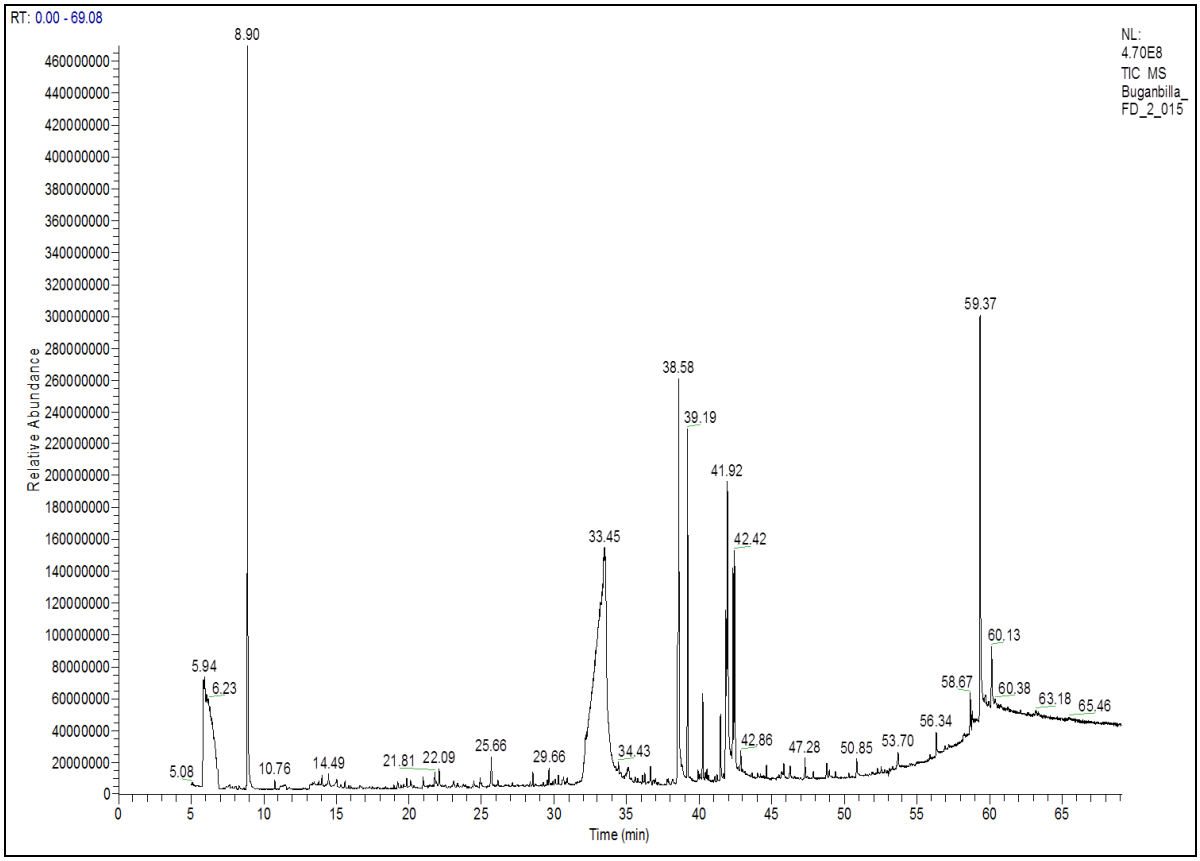

Fig 7: Chromatogram of ethanolic leaves-extract of *Bougainvillea spectabilis* Willd.

Geraniol and fernenol methyl ether were the main constituents identified in the chromatogram of *L. citrodora* leaves-extract (Fig. 8) at RT of 22.09 min, and 59.04 min, respectively. Other compounds found in this specie were the terpenoids β-citronellal (19.02 min), neral (21.67 min), geranial (22.52 min), caryophyllene (26.64 min), and phytol (36.09 min).

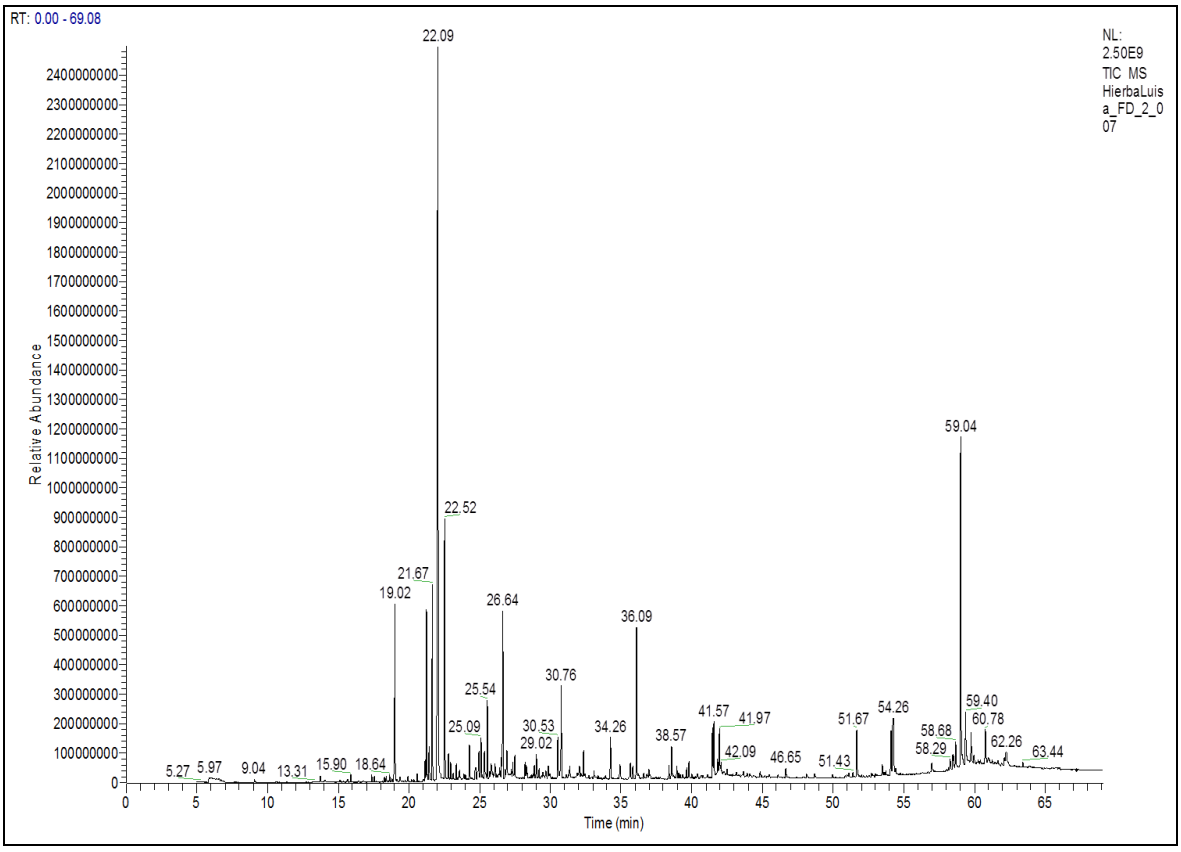

Fig 8: Chromatogram of ethanolic leaves-extract of *Lippia citrodora* (Paláu) Kunth.

As shows in Table 1, *L. citrodora* and *C. chayamansa* exhibited terpenoid compositions, especially monoterpenes and triterpenoids, respectively, while the other plants had a broad variety of constituents in their respective composition.

Table 1: Secondary metabolites identified in eight plants from Ecuador by GC-MS.

Compound	*C. sativum*	*C. scolymus*	*A. absinthium*	*C. chayamansa*	*M. officinalis*	*M. oleifera*	*B. spectabilis*	*L. citrodora*
Monoterpenoids								
Neral (β-citral)					•			•
Geranial (α-citral)					•			•
Geraniol								•
Geranyl acetate								•
β-citronellal					•			•
β-citronellol								•
Sesquiterpenoids								
8-bromo-neoisolongifolene								•
Caryophyllene								•
Caryophyllene oxide								•
α-cadinol								•
Diterpenoids								
Phytol	•	•	•	•	•	•		
Phytol acetate	•	•			•			•
Triterpenoids								
Squalene		•		•				
α-Tocopherol	•				•	•		
γ-Tocopherol		•		•				
δ-Tocopherol				•				
Pentacyclic triterpenoids								
β-amyrin				•		•		
Lupeol			•					
Lupeol acetate				•				
Farnenol methyl ether								•
Steroids								
Elasterol		•						
Fucosterol						•		
Stigmasterol	•	•					•	
β-Sitosterol	•			•				
γ-Sitosterol (clionasterol)			•		•	•		
24-methylenecycloartanol						•		
(3β)-cycloartenol acetate		•						
Fatty acid and derivatives								
Palmitic acid (C16:0)	•	•	•		•	•	•	
Ethyl palmitate (C16:0)	•						•	
Petroselinic acid (C18:1, *n*-12)							•	
Ethyl oleate (C18:1, *n*-9)							•	
Ethyl linoleate (C18:2, *n*-6)			•					
Isopropyl linoleate (C18:2, *n*-6)	•							
Ethyl linolelaidate (C18:2, *n*-6, *E*)	•							
Linolenic acid (C18:3, *n*-3)	•	•				•		
Ethyl linolenate (C18:3, *n*-3)						•		
Carbohydrates								
3-*O*-methyl-D-glucose							•	
Aromatic derivatives								
Phenylacetaldehyde			•					
Diethyl benzalmalonate	•							
Visnagin	•							
Other								
2,2-diethoxypropane	•	•	•		•		•	
4,4-dimethyl-1,3-dioxane	•							
4-hydroxy-4-methyl-2-pentanone	•	•	•		•		•	

4. Discussion

Three volatile hydrocarbons were found among constituents with low RT (that is, lower than 15 min). Two of them were 2,2-diethoxypropane (acetone, diethyl acetal, 6.03±0.16 min) and 4-hydroxy-4-methyl-2-pentanone (diacetone alcohol, 8.92±0.04 min), which were detected in five of the eight plant extracts (Table 1), being relatively relevant to *C. scolymus* (Fig. 2), *M. officinalis* (Fig. 5), and *B. spectabilis* (Fig. 7). The first compound has also been identified by CG-MS in the ethanol extract of *Ampelocissus latifolia* (Roxb.) tuberous root [10], the antifungical *n*-butanol fraction of methanolic leaf extract of *Kochia indica* Wight [11], and the antibacterial material of *Ulva lactuca* ethanolic extract [12]. The second has been reported as constituent of essential oils obtained from several species, such as dried flowering aerial parts of Iranian *Phlomis bruguieri* Desf. [13], *Foeniculum vulgare* Mill seeds from Rawalpindi, Pakistan [14], *Solanum quitoense* Lam. fruits (naranjilla) cultivated in Costa Rica [15], and Nepalese *Acorus*

calamus L. rhizomes [16], several of which exhibited antimicrobial activities. Furthemore, this compound has been identified as one of the major constituents of the cattle anal odour, which showed repellence to brown ear tick *Rhipicephalus appendiculatus* [17]. The third compound was 4,4-dimethyl-1,3-dioxane (10.78±0,01 min), which was detected only in the extract of *C. sativum* (Fig. 1). It has reported that this volatile compound is directly related to the freshness and quality indices during whiting (*Merlangius merlangus*) conservation [18, 19].

At RT between 15 and 35 min were found: phenylacetaldehyde benzenoid (15.61±0.1 min) in the *A. absinthium* extract (Fig. 3); some monoterpenes and sesquiterpenes, especially in *L. citrodora* extract (Fig. 8), where geraniol (22.09±0.1 min) was the most relevant constituent; and 3-*O*-methyl-D-glucose (33.45±0.01 min) carbohydrate in *B. spectabilis* extract (Fig. 7). Phenylacetaldehyde has been recognized as one of the compound contributor to fresh tomato aroma [20], and as one of aroma components of semi-fermented teas made in Korea [21], honey [22], and coffee [23, 24]. Furthermore, this has shown attractant and repellent functions against several insects [25, 26]. Geraniol is a common acyclic monoterpene of the essential oils obtained from several aromatic plants, which is widely used as a fragrance chemical in both cosmetic and household products [27]. It has exhibited various biochemical and pharmacological properties, especially against a broad range of cancers, including breast, lung, colon, prostate, pancreatic, skin, liver, kidney and oral cancers, by abolishing various properties of tumor cells that allow them to adapt and survive under selective pressure [28]. However, it has been reported that geraniol does not present genotoxic and/or clastogenic/aneugenic effects in human cells with and without liver enzyme metabolization, and they advised caution in the use of this substance by humans, since a significant reduction in viability of HepG2 and a marked decrease in cell viability on normal PBMC were verified[29]. But, in contrast, it has also indicated that geraniol treatment increases anti-oxidative defenses in mice liver, with no signs of liver toxicity, affecting only, slightly, CYP enzyme activities, reason for which it can be considered safe even at high doses and for long periods of time [30]. Additionally, geraniol has exhibited the most potent of twelve terpenes in inhibiting intracellular bacillary dysentery-causing *Shigella sonnei* at a concentration of 42 µM, and found that doses of geraniol between 350 and 62.5 mg kg^{-1} improved *Galleria mellonella* larval survival compared to *Shigella*-infected untreated larvae [31].

3-*O*-methyl-D-glucose is a non-metabolized carbohydrate that is used as a marker to assess glucose transport by evaluating its uptake within various cells and organ systems, and recently it is suggested as a CEST-contrast agent for tumor detection [32, 33]. A glycoside with a terminal 3-*O*-methylglucose, named cucumarioside D, was found in the sea cucumber *Eupentacta fraudatrix*, which exhibited moderate cytotoxic and hemolytic activity [34]. In this sense, this carbohydrate could be associated with complex glycosides in *B. spectabilis*.

Only two diterpenes were found in the compositions of some Ecuadorian plants, which appeared at RT between 35 and 45 min together with several fatty acids (saturated, monounsaturated and polyunsaturated) and their derivatives, and an halogenated sesquiterpene. Among diterpenes, phytol acetate (36.10±0.02 min) was common for four species (*C. sativum*, *C. scolymus*, *M. officinalis*, and *L. citrodora*) (Table 1), while phytol (41.47±0.02 min) was common for six of them (Table 1), being particularly important in the *C. scolymus* and *A. absinthium* compositions (Fig. 2 and 3).

Phytol isolated from *Leptadenia pyrotechnica* has been suggested as a potential surface disinfectant, because it showed antimicrobial activity against *Escherichia coli*, *Candida albicans* and *Aspergillus niger*, it was not toxic, and it was stable on surfaces such as stone, MDF, and steel, until 36 hours [35]. Phytol extracted from *Abutilon indicum* exhibited cytotoxicity on *Schizosaccharomyces pombe* cells at 0.37 µM concentration, by affecting the growth and viability [36]. This compound has also shown anti-inflammatory activity [37], and potent antiproliferative activity against human lung adenocarcinoma cell line A549 [38]. While, phytol acetate has been identified by GC-MS as main constituent or trace in bioactive extracts of several plants, such as *Pistia stratiotes* [39], *Eichhornia crassipes* [40], and *Arum palaestinum* Boiss [41], among others.

With respect to fatty acids, palmitic acid (hexadecanoic acid, C16:0, RT: 38.60±0.04 min) was identified in six of the eight species, but it seems being one of the main constituent in four of them (*C. sativum*, *B. spectabilis*, *M. oleifera*, and *C. scolymus*). Antimicrobial and anti-inflammatory activities, among others, have been attributed to fatty acids and their derivatives. Hexadecanoic acid isolated from hydroid *Aglaophenia cupressina* Lamoureoux at concentration of 30 ppm was bactericidal against *Salmonella typhi* by damaging its cell membrane structure [42]. While, *n*-hexadecanoic acid isolated from *Kigelia pinnata* leaves exhibited significant IC50 with value of 0.8 µg mL^{-1} against HCT-116 cells, due to its interaction with DNA topoisomerase-I [43]. Moreover, it was reported that *n*-hexadecanoic acid might function as an anti-inflammatory agent, because it had shown significant inhibitory activity in the enzyme kinetics study of PLA2, entropy driven strong binding to the enzyme shown by ITC analysis, high active site binding affinity shown by forming binary complex crystals with PLA2 in their 1:1 molar solution, and its binding at the active site of the enzyme in the X-ray structure [44].

Ethyl palmitate (hexadecanoic acid ethyl ester, RT: 39.19±0.01 min) was identified in *C. sativum* and *B. spectabilis* extracts, which has shown its effectiveness in combating inflammation in several experimental models [45]. Many fatty acid ethyl esters are used as secure skin-conditioning agent-emolient of several cosmetic formulations, beside fragrance ingredient, binder, and hair conditioning agent, among others [46]. Several fatty acid ethyl esters are the main constituents in *B. correcta* female rectal glands at different ages, which could be involved in intraspecific communication, as pheromone compounds [47]. It suggests that the biosynthesis of these constituents by Ecuadorian plant could be associated with possible ecological interactions.

Petroselinic acid (C18:1, *n*-12, RT: 41.92±0.01 min) and ethyl oleate (C18:1 Et, *n*-9, RT: 42.42±0.01 min) were monounsaturated derivatives identified only in the *B. spectabilis* extract. While, alpha-linolenic acid (ALA, C18:3, *n*-3, RT: 42.03±0.02 min) was common in *C. sativum*, *C. scolymus*, and *M. oleifera* extracts. Intake of ALA has been recommended for its beneficial effects in human health [48], especially as neuroprotective agent [49]. Higher ALA levels could be associated with lower odds of new T1 and T2 gadolinium enhancing lesions in a cohort of multiple sclerosis patients [50]. Linolenic acid derivatives identified were ethyl linoleate (in *A. absinthium* extract, RT: 42.32±0.01 min) and isopropyl linoleate (in *C. sativum* extract, RT: 65.51±0.01 min. Ethyl linoleate is a noncytotoxic anti-melanogenesis chemical, through Akt/GSK3β/β-catenin signal pathway,

which suggests its possible use as skin-whitening agent [51]. While isopropyl linoleate isolated from *Clerodendrum phlomidis* leaf showed good antioxidant activity in DPPH assay with an IC50 value of 61.13 μg mL^{-1} [52].

8-bromo-neoisolongifolene was the halogenated sesquiterpene found in the composition of *L. citrodora* at RT 41.57±0.01 min (Fig. 8). This compound has been reported in *Polygonum minus* Huds essential oil [53], and *Euphorbia paralias* leaf and seed extracts [54].

At RT higher than 45 min appeared the aromatic compound visnagin (48.04±0.01 min) in *C. sativum*, and triterpenoids, including squalene (52.81±0.02 min) found in *C. chayamansa* and *C. scolymus*, tocopherol derivatives (55.92 min – 62.14 min), pentacyclic triperpenes (59.04 min – 62.02 min), and steroids (58.68 min – 61.25 min). Visnagin is a furanochromone isolated mainly from *Ammi visnaga* L., and its anticancer activity against several human cell lines has been reported [55, 56]. Additionaly, visnagin was found to prevent cerulein induced acute pancreatitis and associated multi organ dysfunction syndrome via its antioxidant and anti-inflammatory properties, where the effects are partly mediated by modulation of Nrf2/NFκB pathway [57]. Furthermore, it has shown herbicidal activity causing membrane destabilization, photosynthetic efficiency reduction, inhibition of cell division, and cell death [58].

Squalene is an intermediate or precursor in phytosterols, pentacyclic triterpenes, cholesterol and various hormones biosynthesis, which has broad applications in food industry and cosmetics and in prevention and treatment of human diseases, such as it reduces skin damage by UV radiation, LDL levels, and cholesterol in the blood, prevents the suffering of cardiovascular diseases, and has antitumor and anticancer effects against ovarian, breast, lung, and colon cancer [59, 60]. This compound has been extensively used as an excipient in pharmaceutical formulations for disease management and therapy, due to its significant dietary benefits, biocompatibility, inertness, and other advantageous properties [61].

Three isoforms of vitamin E, such as α-tocopherol, δ-tocopherol, and γ-tocopherol, were found distributed in five of the eight species (Table 1), being α-tocopherol a relatively important constituent in the *M. oleifera* extract (Fig. 6). Vitamin E is a generic term for a group of fat-soluble antioxidant nutrients consisting of four tocopherols and four tocotrienols [62, 63, 64]. α-, β-, γ-, and δ-tocopherols contain a chromanol ring system and a phytyl chain containing 16 carbons, differences among them depend upon the number and position of methyl groups on the chromanol ring [62], but only one α-tocopherol satisfies the criteria of being a vitamin and its deficiency leads to ataxia in humans [64, 65]. Other forms of vitamin E such as γ-tocopherol, δ-tocopherol and γ-tocotrienol have unique antioxidant and anti-inflammatory properties that are superior to α-tocopherol in prevention and therapy against chronic diseases [64]. Likewise, at the supranutritional levels, γ-tocopherol and δ-tocopherol seem to be cancer preventive, but α-tocopherol is not effective [62].

Among pentacyclic terpenoids lupeol (RT 61.10±0.01) and lupeol acetate (RT 62.02±0.01) seem being important constituents in *A. absinthium* and *C. chayamansa*, respectively. These two compounds have exhibited important biological activities, including anticancer, antiprotozoal, chemopreventive and anti-inflammatory properties [66]. Isolated lupeol from the stem bark of *Zanthoxylum gilletii* (De Wild) Waterman exhibited collateral sensitivity against a panel of cancer cell lines with drug-sensitive, multidrug-resistant phenotypes and normal AML12 hepatocytes, via multiple mechanisms with marginal or no effect to normal cells at similar doses [67]. This compound has also been isolated from steam bark of *Diospyros mespiliformis* Horsch, and it exhibited strong antimicrobial activity against ten microbial clinical isolates [68]. Lupeol acetate has been isolated from the root bark of *Ficus sycomorus* Linn, exhibiting antimicrobial activity against *Samonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus* [69].

Finally, clionasterol (γ-sitosterol, RT 59.40±0.01 min) appears to be an important component in *A. absinthium* and *M officinalis* extracts among the phytosterols found in Ecuadorian plants. While, elasterol (59.36±0.02 min), β-sitosterol (59.40±0.02 min), fucosterol (59.63±0.01 min) and stigmasterol (59.33±0.63 min) were important constituents in *C. scolymus*, *C. chayamansa*, *M. oleifera*, and *B. spectabilis* extracts, respectively. Plant sterols or phytosterols, are a group of bioactive terpenes that have gained much attention in reducing blood cholesterol levels in humans, as well as the risk of heart disease [70, 71]. Through other properties reported for phytosterols, anticancer and anti-inflammatory activities seem being the most investigated [72, 73, 74].

5. Conclusions

Almost the entire constituents found in Ecuadorian plants have shown pharmacological applications, which can justify their ethnobotanical uses. However, more studies must carry out to improve the chemotaxonomic knowledge of these and other plants from Ecuador regions. For this reason, it is recommended to obtain extracts with solvents of different polarities, and assess their composition and bioactivity, trying to isolate and elucidate those secondary metabolites responsible of biological activity. Furthermore, many of the edible plant studied here could be considered as nutraceuticals, due to beneficil effects of their constituent in human health.

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7. References

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