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Chemical composition and selective BuChE inhibitory activity of the essential oils from aromatic plants used to prepare the traditional Ecuadorian beverage *horchata lojana*

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Carvone

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Myrcene (Beta-Myrcene)

PubChem CID: 31253

Methyl eugenol

PubChem CID: 7127

Geraniol

PubChem CID: 637566

Isomenthone

PubChem CID: 6986

Citronellol

PubChem CID: 8842

(*E*)-Anethole

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Pulegone

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ABSTRACT

Ethnobotanical and ethnomedicinal relevance: In southern Ecuador, *horchata lojana* is a popular aromatic and refreshing beverage that is prepared from an aqueous infusion of different mixtures of local medicinal and aromatic plants. The drink is considered a traditional anti-inflammatory agent and brain tonic; due these properties, it has been drunk since Colonial Times. Several pharmacological studies have evaluated the effects of *horchata* aqueous infusion. However, the aromatic profile and the contribution of the volatile components to the biological activity of the drink have not been investigated so far. For these reasons, we have determined the chemical composition of the essential oils (EOs) distilled from five mixtures of aromatic plants commonly used for the preparation of this traditional drink. Moreover, to support the curative properties of the aromatic plants, the anticholinesterase activity of the EOs was examined.

Material and methods: Different bunches of fresh mixed medicinal and aromatic plants, called *tongos*, are sold at local markets in the province of Loja for the preparation of different types of *horchata*. In this research we have purchased plant bunches sold at five popular markets of Loja province. Subsequently, aromatic plants in each bunch were separated from medicinal plants and were then hydrodistilled to give the corresponding EOs. Subsequently, the chemical composition of each EO was determined by GC-MS/GC-FID techniques, whereas the cholinesterase inhibitory activity *in vitro* was determined against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes.

Aims of the study: i) to contribute to the chemical and pharmacological study of the aroma components of the traditional Ecuadorian drink *horchata lojana*; ii) to identify botanically the mixtures of aromatic plants used to make the drink; iii) to establish, on the basis of the chemical composition of the EOs, the compounds mainly responsible for the characteristic beverage flavor; iv) to establish the possible existence of an aromatic pattern characteristic of each *horchata* preparation; v) to test the anticholinesterase activity of the EOs against AChE and BuChE in order to support the traditional consume of the drink as an effective brain tonic.

Results: A total of 23 botanical families and 32 species of plants used for the preparation of five different variants of the traditional *horchata lojana* beverage, have been identified. Fourteen aromatic species were determined to be responsible for the characteristic flavor of the drink. All the analyzed EOs belong to the monoterpene type. A total of 88 compounds have been identified in the different EOs, twenty-four of which are common components of the oils.

Conclusions: According to the main components of the EOs distilled from the five groups of *horchata lojana* plants, four aromatic profiles have been defined: (i) neral + geranial + carvone, (ii) neral + geranial + myrcene; (iii) geranial + methyl eugenol + isomenthone + neral + citronellol; (iv) (*E*)-anethole + geranial + pulegone. Moreover, according to the literature, several aromatic plants and individual EOs components exhibit a wide range of biological activities. This finding as well as the significant BuChE inhibitory activity exhibited *in vitro* by

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the EOs give scientific support to the use of identified aromatic plants in the traditional preparation of *horchata*, that is considered a natural analgesic and anti-inflammatory remedy, and an effective brain tonic.

1. Introduction

Horchata lojana (Fig. 1A) is a traditional beverage with supposed medicinal properties that is consumed in Loja province in southern Ecuador. The adjective *lojana* is added to differentiate this aromatic drink with a vivid ruby color from other types of beverages consumed across the Spanish-speaking world and known by the same name, for example, the creamy, rice-based, cinnamon-studded Mexican *horchata*. The *lojana* tea-like drink is made from a mixture of more than 20 medicinal and aromatic herbs (for the parts used, see Table 1) and flowers. It is prepared by adding a mixture of herbs and flowers to a cup of hot water, then let brew for about 5 min, and finally served cold or hot. The

drink can be consumed directly without other ingredients; however, sometimes a few drops of lemon, some sugar, solid cane sugar (*panela*), honey, stevia, or *savila* (*Aloe vera*) are added to adjust the taste and flavor. Moreover, flowers are added primarily to enhance the healing effect of the drink on the nervous system.

This refreshing and medicinal beverage with hydrating properties is consumed daily by all social classes in Loja province, as a common companion of meals and foods.

Rios et al. (2017) have reported that *horchata* has been prepared in the Southern Ecuador since Colonial Times. According to some historians, *horchata*'s journey to Ecuador began in Spain where in the eighth century the Moors imported the plant *Cyperus esculentus* from the Arabic



Fig. 1. A) *Horchata lojana*; B–F) *Horchateras* at different Loja markets: B) La Tebaida market; C and F) Gran Colombia market; D) Centro Commercial market; E) Saraguro market.

Table 1

Aromatic plants used for the preparation of the HC, HL, HÑ, HQ, HS horchata drinks and their therapeutic applications.

N°	Scientific name/ Vernacular name	Herbarium voucher	Geographical origin	Part of plant used	Type of horchata lojana ^a	Therapeutic applications
AMARANTHACEAE						
1	<i>Iresine herbstii</i> Hook. <i>Escancel, tigrecillo</i>	PPN-am-001	Native	Leaves, stalks	HÑ, HQ, HS, HL, HC	Anti-inflammatory, anti-influenza, analgesic, diuretic, sedative, toning and healing. Intestinal infections, injuries, liver and kidney problems (Andrade et al., 2017; Bailón et al., 2017; Rios et al., 2017).
2	<i>Amaranthus cruentus</i> L. <i>Ataco, sangorache</i>	PPN-am-002	Native	Inflorescences, leaves	HQ, HS, HL, HC	Anti-inflammatory, astringent, anti-influenza, antihemorrhagic, diuretic and tonic, blood circulation, carminative, emmenagogue, hepatic and stimulant (Bailón et al., 2017; Rios et al., 2017)
ASTERACEAE						
3	<i>Matricaria recutita</i> L. <i>Manzanilla</i>	PPN-as-016	Introduced	Flowers, leaves, stalks	HÑ, HQ, HS, HL, HC	Anti-inflammatory, sedative, anti-flatulence, anthelmintic, analgesic, carminative, digestive, febrifuge, cramps, treat insomnia, wounds, stomach pain, burns and stimulating tonic (Bailón et al., 2017; Tene et al., 2007)
APIACEAE						
4	<i>Foeniculum vulgare</i> Mill. <i>Hinojo</i>	PPN-ap-004	Introduced	Leaves	HL, HC	Anti-inflammatory, digestive, conjunctivitis, diabetes and in lactation. Anticancer, gastritis, and relaxant (Tene et al., 2007)
BORAGINACEAE						
5	<i>Borago officinalis</i> L. <i>Borraja</i>	PPN-bo-001	Introduced	Flowers, leaves, stalks	HÑ, HQ, HS, HL, HC	Anti-inflammatory, anti-influenza, expectorant, febrifuge, blood circulation, sudorific, astringent, diuretic, anti-hypercholesterolemia, analgesic, antidiarrheal, antitussive and emmenagogue, hepatic pain, conjunctivitis, burnings, and headache (Bailón et al., 2017; Rios et al., 2017; Tene et al., 2007)
BEGONIACEAE						
6	<i>Begonia x tuberhybrida</i> Voss. <i>Begonia rosada</i>	PPN-ba-001	Native	Flowers	HÑ, HQ, HS	Constipation, sedative and tonic (Rios et al., 2017)
CAPRIFOLIACEAE						
7	<i>Sambucus nigra</i> L. <i>Tilo, sauco tilo</i>	PPN-ca-001	Introduced	Flowers	HQ, HS, HL	Anti-influenza, anti-bronchitis, febrifuge, antidiarrheal, sedative, antitussive, for curing nerves, cold, cough and headache (Bailón et al., 2017; Rios et al., 2017; Tene et al., 2007)
CARYOPHYLLACEAE						
8	<i>Dianthus caryophyllus</i> L. <i>Clavel</i>	PPN-cd-001	Introduced	Flowers	HÑ, HS	Anti-inflammatory, anti-influenza, analgesic, anti-cough, sedative, cardiostimulant, restorative, to cure nerves and stomach pain (Andrade et al., 2017; Bailón et al., 2017; Rios et al., 2017)
GERANIACEAE						
9	<i>Pelargonium graveolens</i> L'Hér. ex Aiton. <i>Esencia de rosas</i>	PPN-ge-004	Introduced	Flowers, leaves, stalks	HÑ, HS, HL, HC	Anti-inflammatory, analgesic, febrifuge, antidiabetic, antidiarrheal, to treat gallbladder and liver problems, digestive, to cure gastric ulcers, wounds, burns, respiratory diseases, jaundice, infertility, and urinary stones (Boukhris et al., 2012)
10	<i>Pelargonium odoratissimum</i> (L.) L. Hér. <i>Malva olorosa</i>	PPN-ge-001	Introduced	Flowers, leaves, stalks	HÑ, HQ, HS, HL, HC	Anti-inflammatory, analgesic, carminative and tonic, diuretic, antidiarrheal, to cure colic, nerves and heart problems (Bailón et al., 2017; Rios et al., 2017)
EQUISETACEAE						
11	<i>Equisetum bogotense</i> Kunth. <i>Cola de caballo</i>	PPN-eq-001	Native	Leaves, stalks	HQ, HS, HL, HC	Anti-inflammatory, antiseptic, depurative, diuretic, hepatic, febrifuge, anticancer, anti-cough, anti-parasite and to cure kidney problems (Bailón et al., 2017; Rios et al., 2017; Tene et al., 2007)
LAMIACEAE						
12	<i>Clinopodium brownei</i> (Sw.) Kuntze <i>Poleo chico, warmi poleo</i>	PPN-la-024	Native	Whole plant	HC	Digestive and to relieve the discomfort of menstrual colic, expectorant agent, and a remedy to cure colds, flu, cough, bronchitis, asthma (Andrade et al., 2017), and allergic symptoms; to relieve respiratory problems related to flu (Matailo et al., 2019)
13	<i>Clinopodium nubigenum</i> (Kunth) Kuntze. <i>Tipo del cerro</i>	PPN-la-018	Native	Whole plant	HÑ	Against cold and flu (Andrade et al., 2017), for treating stomach ache, digestion, dysentery problems.
14	<i>Melissa officinalis</i> L. <i>Toronjil</i>	PPN-la-004	Introduced	Leaves	HÑ, HQ, HS, HL, HC	Anti-inflammatory, anti-influenza, analgesic, antispasmodic, diuretic, antiulcer, digestive, sedative, cardiostimulant, sudorific, tonic, to treat colds, colic, flatulence, allergies, insomnia, hemorrhoids, nerves, headache, and hemorrhages (Abdellatif et al., 2014; Andrade et al., 2017; Tene et al., 2007)
15	<i>Mentha piperita</i> L. <i>Menta</i>	PPN-la-006	Introduced	Leaves, stalks	HQ, HL, HC	Analgesic, antidiarrheal, anti-influenza, anti-inflammatory, antitussive, carminative, digestive, tonic and against stomach pain (Rios et al., 2017)
16	<i>Mentha spicata</i> L. <i>Menta</i>	PPN-la-004	Introduced	Leaves	HQ	Anti-inflammatory, anti-influenza, analgesic, digestive, antitussive, carminative, febrifuge (Rios et al., 2017)
17	<i>Ocimum basilicum</i> L. <i>Albahaca</i>	PPN-la-017	Introduced	Flowers, leaves	HL	Anti-inflammatory, antispasmodic, anti-flatulence, analgesic, febrifuge, digestive, stimulant of lactation, relaxant, to treat headache, cough, heart problems, nerves, gastritis, high blood pressure, internal infections (Bailón et al., 2017; Tene et al., 2007)
LINACEAE						
18		PPN-li-001	Introduced		HS, HL, HC	

(continued on next page)

Table 1 (continued)

N°	Scientific name/ Vernacular name	Herbarium voucher	Geographical origin	Part of plant used	Type of <i>horchata</i> <i>lojana</i> ^a	Therapeutic applications
	<i>Linum usitatissimum</i> L. <i>Linaza</i>			Leaves, stalks, seeds		Anti-inflammatory, digestive, hepatic, diuretic, to treat stomachache and kidney problems (Bailón et al., 2017; Rios et al., 2017; Tene et al., 2007)
MALVACEAE						
19	<i>Malva arborea</i> (L.) Webb & Berthel. <i>Malva altea, malva</i> <i>blanca</i>	PPN-ma-009	Introduced	Flowers	HQ, HS, HC	Anti-inflammatory, depurative, tonic, (Rios et al., 2017; Bailón et al., 2017), antidiarrheal, febrifuge, diuretic, digestive, to treat obesity, constipation, insect bites
20	<i>Alcea rosea</i> (L.) <i>Malva goma, malva</i> <i>rosa</i>	PPN-ma-001	Introduced	Flowers, bark	HÑ, HQ, HS, HC	Anti-inflammatory, diuretic, analgesic, depurative (Rios et al., 2017)
ONAGRACEAE						
21	<i>Fuchsia magellanica</i> Lam. <i>Pena – pena, pena -</i> <i>pena morada</i>	PPN-on-004	Introduced	Flowers	HÑ, HS	Sedative, disinfectant, wound healer, relaxant (Bailón et al., 2017; Rios et al., 2017; Tene et al., 2007)
22	<i>Oenothera rosea</i> L'Her. ex Aiton <i>Shullo</i>	PPN-on-011	Native	Flowers, leaves, stalks	HL	Anti-inflammatory, digestive, diuretic, to treat hepatic and kidney problems (Bailón et al., 2017; Tene et al., 2007)
ORCHIDACEAE						
23	<i>Epidendrum</i> <i>fimbriatum</i> Kunth <i>Flor de cristo</i>	PPN-or-001	Native	Flowers	HQ	To treat internal tumors (Andrade et al., 2017)
PLANTAGINACEAE						
24	<i>Plantago major</i> L. <i>Llantén</i>	PPN-pn-001	Introduced	Whole plant	HÑ, HQ, HL, HC	Anti-inflammatory, antihemorrhagic, digestive, wound healer, diuretic, to treat liver problems, insomnia, insect bites (Bailón et al., 2017; Tene et al., 2007)
PIPERACEAE						
25	<i>Peperomia congona</i> Sodirol <i>Congona</i>	PPN-pi-003	Native	Leaves flowers, stalks	HL, HC	To treat nervous anxiety, stomach and hepatic pain, anti-parasitic, antiperspirant, analgesic, cardiotoxic, diuretic, sedative, to treat headache and insomnia (Bailón et al., 2017; Tene et al., 2007)
POACEAE						
26	<i>Cymbopogon citratus</i> (DC.) Stapf. <i>Hierba Luisa</i>	PPN-po-001	Introduced	Leaves	HÑ, HQ, HS, HL, HC	Anti-flatulence, analgesic, digestive, sedative, expectorant, spasmolytic, relaxant and diuretic, anti-inflammatory, to treat high pressure, nerves, gastritis, diarrhea, jaundice, insomnia and cold (Avoseh et al., 2015; Tene et al., 2007)
RUTACEAE						
27	<i>Citrus sinensis</i> (L.) Osbeck. <i>Hojas de naranja</i>	PPN-rt-003	Introduced	Leaves	HL	Antispasmodic, relaxant, antidiarrheal. Hair tonic, to treat insomnia, cold and kidney problems (Tene et al., 2007).
PROTEACEAE						
28	<i>Oreocallis grandiflora</i> (Lam.) R. Br. <i>Cucharillo</i>	PPN-pe-001	Native	Inflorescences	HL, HC	Anti-inflammatory, digestive, hepatic, astringent and diuretic, to treat headache, fever, diabetes, kidney problems and cough (Bailón et al., 2017; Tene et al., 2007)
ROSACEAE						
29	<i>Poterium sanguisorba</i> L. <i>Pimpinela</i>	PPN-ro-008	Introduced	Whole plant	HÑ, HQ, HS	To treat nerve problems (Andrade et al., 2017)
SOLANACEAE						
30	<i>Solanum americanum</i> Mill. <i>Mortño</i>	PPN-so-007	Native	Leaves, fruits	HL, HC	Anti-inflammatory, analgesic, digestive, febrifuge, sedative, to treat respiratory diseases, fever, cold, pneumonia, internal infections and kidney problems (Bailón et al., 2017; Tene et al., 2007)
VERBENACEAE						
31	<i>Aloysia triphylla</i> (L'Hér.) Britton. <i>Cedrón</i>	PPN-ve-002	Native	Flowers, leaves, stalks	HÑ, HQ, HL, HC	Anti-inflammatory, antispasmodic, anti-neuralgic, analgesic, cardiotoxic, digestive, chest and stomach tonic. Diuretic, to cure fever, headache, colds and colics (Andrade et al., 2017; Bailón et al., 2017; Tene et al., 2007)
VIOLACEAE						
32	<i>Viola tricolor</i> L. <i>Pensamiento</i>	PPN-vi-003	Introduced	Flowers	HÑ, HQ, HS, HC	Analgesic, antidiarrheal, anti-influenza, anti-inflammatory, antiseptic, diuretic, febrifuge, hoarseness, to heal kidney, skin conditions, heart problems, and headache (Rios et al., 2017; Tene et al., 2007)

^a Abbreviations, HÑ: Namarín; HQ: Quisquinchir; HS: Saraguro; HC: Chuquiribamba; HL: Loja.

world. With the tuber of this plant, called *tigernut* in English and *chufa* in Spanish, they prepared a beverage that became known as *horchata*. The Spaniards later brought the beverage to the Americas. Espinoza Gálvez (2016) has reported that in Loja province the name *horchata* derives from the two indigenous words “*hampikyaku*”, which mean medicinal water or healing water.

Traditionally, the preparation of *horchata lojana* starts with rural women, who collect herbs and flowers from the Andean countryside or grow them in their gardens. These women are called *horchateras* (Fig. 1,

B–F) and have historically fulfilled an important role for the cultural conservation and persistence of the consumption of this traditional drink in the Southern region of Ecuador. Moreover, they transmit the ancestral knowledge about traditional plants and their mixtures to new generations.

The herbs for preparing the *horchata* are dried or sold fresh to street vendors, who in turn brew glasses of the drink. Moreover, locals purchase the herbs directly from traders or in different markets of Loja province, where mixtures of fresh medicinal and aromatic plants and

Table 2
Physical properties of the EOs isolated from the 5 groups of *horchata* aromatic plants.

EO	Yield (%)	Density (g/ml)	Refraction Index	Optical Rotation	Color/Aroma
HL	0.172 ± 0.003	0.881 ± 0.014	1.485 ± 0.001	-2.212 ± 0.002	Slightly yellow/herbal, sweet, soft mentholated
HS	0.047 ± 0.011	0.895 ± 0.006	1.421 ± 0.001	-16.871 ± 0.003	Clear yellow/floral, sweet, slightly mentholated
HC	0.073 ± 0.004	0.982 ± 0.004	1.503 ± 0.003	+5.163 ± 0.009	Slightly blue/floral, sweet, slightly mentholated and aniseed
HÑ	0.044 ± 0.001	0.964 ± 0.002	1.502 ± 0.001	-5.979 ± 0.010	Yellowish/herbal, sweet, soft, and mentholated
HQ	0.947 ± 0.016	0.941 ± 0.009	1.488 ± 0.002	-7.782 ± 0.012	Pale yellow/herbal, slightly mentholated and citric

flowers are sold in bunches, called *tongos*, *atados*, or *manojos* in the indigenous language, or are placed inside plastic bags (Fig. 1C). Alternatively, herb bunches and flower bags are sold separately (Fig. 1D). The drink is then prepared at home.

In the rest of Ecuador, *horchata lojana* is marketed in the form of whole dehydrated herbs or tea bags. Moreover, it can be found in shopping centers and micro-markets in the form of a stabilized liquid beverage. Recently, the fame of the drink has gone internationally and dehydrated *horchata* is exported to the United States, England and Spain. More than 200 families in the Chuquiribamba area and in other places of Loja province such as at Malacatos or Vilcabamba, plant, grow, harvest, and sell fresh plants to local drying industries. In this way, an innovative *lojana* industry is rescuing a characteristic Ecuadorian tradition and promotes its knowledge and value in different countries abroad.

Peasant farmers in the Chuquiribamba Parish of Cantón Loja (see map in Fig. 2) believe that the original formula of *horchata*, which is the parent of all the other types, was invented in this part of Loja province. Indeed, there's not a single or an exclusive recipe to prepare the drink, and thus several types of *horchata* exist, depending on the types of available medicinal and aromatic herbs and flowers used for the preparation. Moreover, the herb combinations may depend on the plant season and weather conditions.

Each drink is believed by locals to have specific medicinal benefits. The inhabitants of Chuquiribamba Parish tell that *horchata* was drunk by ancient warriors before fighting, due to the energizing properties. Nowadays, *horchata* is consumed to relieve stomach pain, to treat infections, hepatic and kidney diseases, and as a natural anti-inflammatory, analgesic, and diuretic remedy. Actually, Rios et al. (2017) have reported a total of 32 therapeutic effects of *horchata*, among which the anti-inflammatory, refreshing, diuretic, anti-stress, brain tonic, moisturizing, energizing, sedative, and digestive properties are considered the most important ones. Moreover, Rios et al. (2017) have affirmed that the therapeutic effects of *horchata* are due to a well-balanced mixture of plants traditionally called *calientes* (warm) and *frías* (cold).

Twelve plants are most frequently used for the preparation of *horchata lojana*, that are believed to be necessary in order to give the drink its characteristic aroma, flavor, color, and medicinal properties. Specifically, *Amaranthus cruentus* (*sangorache* or *ataco* in the indigenous language) and *Iresine herbstii* (*escancel*) give the drink the pink-red color, whereas *Cymbopogon citratus* (*yerba Luisa*), *Mentha spicata* (*menta*), and *Aloysia triphylla* (*cedrón*) are responsible for the aroma and flavor. On the other hand, *Equisetum bogotense* (*cola de caballo*), *Plantago major* (*llanten*), *Borago officinalis* (*borraja*), *Linum usitatissimum* (*linaza*), *Poterium sanguisorba* (*pimpinela*), *Oenothera rosea* (*shullo*), and *Oreocallis grandiflora* (*cucharillo*) are used as medicinal plants. Identified flowers used for the *horchata* preparation include rose geranium, petals of *Rosa canina*, violets, begonias, carnations, and various flowers of Malvaceae species. In addition, several flowers were present in fragments in the bags and thus could not be identified botanically.

Some authors have reported a few biological activities exhibited in vitro by *horchata lojana* infusions. For example, Bailón et al. (2017) described the cytotoxic, antioxidant, genotoxic, and antigenotoxic effects of the drink, whereas Guevara et al. (2019) determined the anti-inflammatory activity of the infusion against LPS-induced cytotoxic damage in RAW 264.7 macrophages. In contrast, the chemical

components and bioactivity of the aromatic fraction of the drink have not been characterized so far.

In our opinion, the study of the aromatic plants used to prepare the drink *horchata lojana* is of great ethnobotanical importance. In fact, the pleasant organoleptic characteristics of the infusion stimulate the consumption of the drink by people of all ages. In addition, the contribution of the aromatic volatile fraction to the biological activity of the drink is highly plausible.

To define the characteristic aromatic profile of *horchata lojana*, the essential oils (EOs) distilled from the mixtures of aromatic plants used for the preparation of five types of the drink, were analyzed by GC-FID and GC-MS for the first time.

A search in the literature of the biological activities of aromatic plants used for the *horchata* preparation and the main EOs components could thus sustain some traditional properties attributed to the beverage, such as the anti-inflammatory, analgesic, and antioxidant effects, with scientific evidence. Moreover, we hypothesized that the traditional use of *horchata* as a brain tonic might indicate that this drink is effective for improving cognitive functions. Indeed, it is well-known that several EOs have cholinesterase (ChE) inhibitory activity and thus exert efficacious neuroprotective properties (Ayaz et al., 2017). ChE inhibitors act on the enzymes that hydrolyze acetylcholine (ACh) and butyrylcholine (BuCh). In the healthy brain acetylcholinesterase (AChE) predominates (80%) and butyrylcholinesterase (BuChE) is considered to play a minor role in regulating brain ACh levels. In the Alzheimer disease (AD) brain, BuChE activity rises, while AChE activity remains unchanged or declines (Mushtaq et al., 2014). Therefore, both enzymes are likely to be involved in regulating choline levels and represent legitimate therapeutic targets to ameliorate the cholinergic deficit. In fact, a decreased cholinergic activity in the brain is considered to be responsible for the declines in cognitive, behavioral, and global functioning characteristic of AD and other neurodegenerative disorders (McGleenon et al., 1999; Greig et al., 2002; Howes and Houghton, 2003). Moreover, it is now considered that the immune system and inflammation play a key role in the development of AD (Heppner et al., 2015). Via the $\alpha 7$ subunit of the nicotinic acetylcholine receptor ($\alpha 7$ nAChR) the cholinergic anti-inflammation pathway exerts a central role in the control of cytokine production and thus on AD evolution (Rosas-Ballina and Tracey, 2009). For these reasons, ChE inhibitors are regarded nowadays as the most promising drugs for the symptomatic management of cognitive disorders (Gyengesi and Münch, 2020).

ChE inhibitors as well as selective BuChE inhibitors derived from nature are actively searched in different laboratories worldwide. Therefore, we decided to test the potential cholinesterase (ChE) inhibitory activities of the EOs isolated from the aromatic plants used for the preparation of different variants of *horchata lojana*.

This research is part of our ongoing program on the study and valorization of aromatic plants traditionally used to prepare ancestral foods and drinks in Southern Ecuador (Armijos et al., 2018; Matailo et al., 2019).

2. Materials and methods

2.1. Plant material

The plant material used for this research are *horchata* bunches (*tongos*

Table 3

Chemical constituents of the EOs distilled from *horchata* plants collected at Ñamarín (HN), Loja (HL), Saraguro (HS), Chuquiribamba (HC), and Quisquinchir (HQ) markets, respectively.

N°	Compound	DB5-ms		HP-INNOWax		Compound average content ^c									
						ÑAMARIN (HN)		LOJA (HL)		SARAGURO (HS)		CHUQUIRIBAMBA (HC)		QUISQUINCHIR (HQ)	
						DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax
		LRI ^d	LRI ^b	LRI ^c	LRI ^d	% ^e									
1	α-Pinene	932	932	1055	1039 ¹	0.2 ± 0.06	–	0.1 ± 0.03	0.2 ± 0.04	0.1 ± 0.02	–	2.8 ± 0.02	5.4 ± 0.17	0.3 ± 0.06	0.4 ± 0.08
2	Sabinene	971	969	1120	1132 ²	0.2 ± 0.01	–	0.3 ± 0.09	0.4 ± 0.01	–	–	1.1 ± 0.01	1.2 ± 0.01	0.2 ± 0.00	0.3 ± 0.01
3	β-Pinene	976	974	1108	1113 ³	0.2 ± 0.01	–	–	–	–	–	0.4 ± 0.00	0.6 ± 0.00	–	0.1 ± 0.00
4	6-Methyl-5-heptene-2-one	–	–	1341	1348 ²	–	–	–	0.9 ± 0.01	–	0.3 ± 0.01	–	0.9 ± 0.00	–	1.5 ± 0.04
5	Myrcene	988	988	1163	1165 ³	6.4 ± 0.24	5.9 ± 0.12	12.5 ± 0.07	13.0 ± 0.19	6.1 ± 0.08	6.9 ± 0.12	5.2 ± 0.03	–	14.5 ± 0.17	14.5 ± 0.63
6	dehydro-1,8-Cineole	–	–	1190	1195 ¹	–	–	–	0.1 ± 0.00	–	0.1 ± 0.00	–	–	–	–
7	3-Octanol	997	988	1398	1393 ⁴	0.3 ± 0.01	0.4 ± 0.00	–	–	–	–	0.2 ± 0.00	–	–	–
8	α-Phellandrene	1006	1002	1163	1150 ⁵	–	–	0.2 ± 0.00	–	0.4 ± 0.01	–	2.5 ± 0.01	7.4 ± 0.06	–	–
9	ρ-Cymene	1023	1020	1270	1278 ⁵	–	–	–	–	0.1 ± 0.00	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	–	–
10	Limonene	1028	1024	1199	1198 ³	2.3 ± 0.12	2.6 ± 0.06	0.6 ± 0.02	0.7 ± 0.03	0.4 ± 0.01	0.4 ± 0.01	1.8 ± 0.01	1.6 ± 0.02	3.5 ± 0.05	3.8 ± 0.17
11	β-Phellandrene	1029	1025	1207	1209 ⁶	–	–	–	–	0.2 ± 0.01	0.2 ± 0.03	–	–	–	–
12	1,8-Cineole	1031	1026	1206	1204 ³	2.6 ± 0.36	2.1 ± 0.25	0.5 ± 0.04	0.5 ± 0.23	–	–	1.1 ± 0.01	1.5 ± 0.20	0.8 ± 0.00	1.3 ± 0.34
13	(Z)-β-Ocimene	1035	1032	1236	1246 ²	–	0.6 ± 0.01	0.5 ± 0.28	0.6 ± 0.01	1.9 ± 0.21	2.0 ± 0.02	0.7 ± 0.01	2.4 ± 0.01	1.2 ± 0.31	0.9 ± 0.04
14	(E)-β-Ocimene	1045	1044	1253	1266 ²	1.1 ± 0.04	1.2 ± 0.01	0.5 ± 0.01	0.6 ± 0.01	0.5 ± 0.01	0.5 ± 0.00	0.7 ± 0.01	0.9 ± 0.00	1.8 ± 0.03	2.0 ± 0.07
15	γ-Terpinene	1057	1054	1244	1241 ⁷	–	–	–	–	–	–	0.5 ± 0.00	0.1 ± 0.00	–	0.1 ± 0.00
16	Artemisia ketone	1061	1056	–	–	0.1 ± 0.13	–	–	–	0.2 ± 0.01	–	–	–	–	–
17	Fenchone	1087	1083	1391	1389 ⁸	–	–	0.2 ± 0.00	0.1 ± 0.00	–	–	0.5 ± 0.00	0.7 ± 0.00	–	–
18	cis-Rose oxide	1110	1106	1351	1362 ²	–	–	0.1 ± 0.00	–	0.2 ± 0.00	0.2 ± 0.09	–	–	0.22 ± 0.00	0.1 ± 0.00
19	α-Cubebene	–	–	1482	1466 ⁵	–	–	–	–	–	–	–	–	–	0.3 ± 0.01
20	β-Bourbonene	–	–	1508	1501 ⁹	–	0.4 ± 0.00	–	–	–	–	–	–	–	0.3 ± 0.00
21	Linalool	1100	1095	1554	1553 ²	0.6 ± 0.00	0.9 ± 0.00	1.5 ± 0.01	2.0 ± 0.06	1.0 ± 0.02	1.2 ± 0.05	0.4 ± 0.00	4.0 ± 0.02	0.9 ± 0.01	1.3 ± 0.00
22	exo-Isocitral	1147	1140	–	–	0.2 ± 0.01	–	0.3 ± 0.01	–	0.1 ± 0.01	–	–	–	0.3 ± 0.01	–
23	trans-α-Necrodol	1151	1144	–	–	0.2 ± 0.00	0.2 ± 0.00	–	–	–	–	–	–	0.2 ± 0.00	–
24	Citronellal	1156	1148	–	–	0.2 ± 0.00	–	0.2 ± 0.00	0.2 ± 0.00	0.3 ± 0.01	0.5 ± 0.01	–	–	0.3 ± 0.00	–
25	Menthone	1155	1148	1457	1460 ¹⁰	–	2.0 ± 0.01	–	–	0.6 ± 0.01	0.3 ± 0.01	1.9 ± 0.00	1.6 ± 0.01	–	0.2 ± 0.00
26	trans-Chrysanthemal	1148	1151	–	–	–	–	0.2 ± 0.00	0.2 ± 0.00	0.6 ± 0.01	0.1 ± 0.00	–	–	–	–
27	Isomenthone	1163	1158	1484	1492 ¹¹	1.9 ± 0.00	2.3 ± 0.01	0.6 ± 0.00	0.7 ± 0.00	12.9 ± 0.18	13.3 ± 0.14	1.8 ± 0.00	1.0 ± 0.00	0.2 ± 0.00	0.6 ± 0.00
28	(Z)-Isocitral	1165	1160	–	–	0.8 ± 0.01	–	1.1 ± 0.01	0.4 ± 0.01	–	0.2 ± 0.09	–	–	–	–
29	(E)-Isocitral	1179	1177	–	–	0.2 ± 0.00	–	1.6 ± 0.03	1.0 ± 0.00	0.9 ± 0.01	0.5 ± 0.01	0.8 ± 0.00	–	1.5 ± 0.01	–
30	Neoisomenthol	1187	1184	1629	1626 ¹¹	–	–	–	–	0.3 ± 0.01	–	–	0.5 ± 0.00	–	–
31	Methyl chavicol	1196	1195	1671	1655 ¹²	–	–	0.5 ± 0.00	0.5 ± 0.08	0.2 ± 0.00	0.2 ± 0.04	1.0 ± 0.00	1.3 ± 0.00	–	–
32	Citronellol	–	–	1772	1772 ¹³	–	3.8 ± 0.03	4.0 ± 0.02	3.3 ± 0.02	11.6 ± 0.17	11.1 ± 0.06	–	0.4 ± 0.00	5.1 ± 0.03	5.3 ± 0.09
33	Nerol	1224	1227	1805	1804 ¹³	4.6 ± 0.02	1.3 ± 0.00	–	1.0 ± 0.03	–	0.8 ± 0.02	1.6 ± 0.00	0.7 ± 0.01	–	0.7 ± 0.02
34	Pulegone	1237	1233	1638	1637 ¹⁰	6.9 ± 0.05	15.3 ± 0.07	–	–	–	–	8.6 ± 0.01	5.6 ± 0.04	–	0.4 ± 0.01
35	Neral	–	–	1681	1680 ¹	23.2 ± 0.13	15.0 ± 0.06	22.7 ± 0.13	22.3 ± 0.01	11.7 ± 0.14	11.3 ± 0.10	6.5 ± 0.03	4.2 ± 0.03	18.7 ± 0.20	17.8 ± 0.28
36	Carvone	1242	1239	1727	1722 ¹	7.8 ± 0.04	8.6 ± 0.38	0.6 ± 0.00	–	–	–	3.5 ± 0.00	4.1 ± 0.08	1.2 ± 0.01	1.1 ± 0.00
37	Geraniol	–	–	1854	1857 ¹³	2.7 ± 0.00	3.0 ± 0.01	6.8 ± 0.05	7.4 ± 0.00	3.7 ± 0.06	3.9 ± 0.02	–	1.5 ± 0.01	2.6 ± 0.04	2.8 ± 0.05
38	Linalool acetate	1249	1254	1561	1559 ¹⁴	–	–	–	–	–	–	1.4 ± 0.00	2.7 ± 0.03	–	–
39	Geranial	1267	1264	1732	1730 ¹	20.2 ± 0.12	19.6 ± 0.27	31.6 ± 0.82	31.8 ± 0.02	16.5 ± 0.21	16.0 ± 0.06	9.2 ± 0.00	5.5 ± 0.01	26.3 ± 1.54	25.5 ± 0.25
40	Citronellylformate	1273	1271	1624	1628 ¹³	1.4 ± 0.10	1.6 ± 0.00	1.1 ± 0.08	1.3 ± 0.00	1.3 ± 0.02	1.4 ± 0.01	0.3 ± 0.00	0.2 ± 0.00	1.8 ± 0.00	2.2 ± 0.03
41	2-Tridecanone	1496	1495	1808	1815 ¹⁴	–	0.1 ± 0.00	–	–	–	0.1 ± 0.00	–	–	0.2 ± 0.01	0.2 ± 0.01
42	(E)-Anethole	1286	1282	1829	1845 ²	–	–	0.8 ± 0.01	0.8 ± 0.01	–	–	22.8 ± 0.01	32.2 ± 0.25	–	–
43	2-Undecanone	1292	1293	–	–	0.2 ± 0.01	–	0.2 ± 0.00	–	–	–	0.4 ± 0.00	–	–	–
44	Geranyl formate	1301	1298	1709	1715 ¹³	0.4 ± 0.02	0.4 ± 0.02	0.5 ± 0.03	0.5 ± 0.01	0.6 ± 0.01	0.6 ± 0.02	–	–	0.4 ± 0.00	0.5 ± 0.01
45	Piperitenone	–	–	1911	1927 ¹⁰	0.1 ± 0.00	–	–	–	–	0.3 ± 0.01	–	0.2 ± 0.00	–	–
46	Citronellic acid	1312	1312	2264	2240 ¹⁵	–	–	–	–	1.7 ± 0.04	1.6 ± 0.02	–	–	1.2 ± 0.05	–
47	δ-Elementene	1334	1335	1466	1469 ⁶	–	–	–	–	0.1 ± 0.01	0.1 ± 0.00	–	–	–	–
48	Citronellyl acetate	1350	1350	–	–	–	–	0.2 ± 0.06	–	0.3 ± 0.02	–	–	–	–	–
49	Decanoic acid	1367	1364	2284	2288 ¹⁶	–	–	–	–	0.7 ± 0.05	0.6 ± 0.04	–	–	–	–
50	α-Copaene	1373	1374	–	–	–	–	0.1 ± 0.02	–	0.3 ± 0.01	–	–	–	0.4 ± 0.00	–

(continued on next page)

Table 3 (continued)

N°	Compound	DB5-ms		HP-INNOWax		Compound average content ^c											
						ÑAMARIN (HN)		LOJA (HL)		SARAGURO (HS)		CHUQUIRIBAMBA (HC)		QUISQUINCHIR (HQ)			
						DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax	DB5-FID	HP-INNOWax
		LRI ^a	LRI ^b	LRI ^c	LRI ^d	% ^e											
51	(E)-Methyl cinnamate	1382	1376	2075	2096 ¹⁷	–	–	0.6 ± 0.00	0.5 ± 0.00	–	–	–	–	–	–	–	
52	Geranyl acetate	1377	1379	1761	1765 ¹³	0.5 ± 0.00	0.4 ± 0.00	0.9 ± 0.00	1.0 ± 0.03	0.3 ± 0.01	0.3 ± 0.00	0.3 ± 0.00	0.5 ± 0.00	–	–	0.4 ± 0.01	
53	β-Bourbonene	1381	1387	1509	1501 ⁹	0.4 ± 0.00	–	–	0.1 ± 0.00	0.3 ± 0.01	0.3 ± 0.00	0.3 ± 0.00	0.2 ± 0.00	–	–	–	
54	Methyl eugenol	1398	1403	2021	2030 ²	1.4 ± 0.01	1.5 ± 0.00	1.2 ± 0.03	1.2 ± 0.01	13.4 ± 0.20	13.0 ± 0.04	5.5 ± 0.00	3.5 ± 0.03	4.7 ± 0.02	–	4.8 ± 0.14	
55	(Z)-Caryophyllene	1416	1408	–	–	–	–	–	–	–	–	2.4 ± 0.01	–	–	–	–	
56	(E)-Caryophyllene	1422	1417	1586	1577 ⁹	2.0 ± 0.02	1.9 ± 0.01	0.6 ± 0.01	0.6 ± 0.00	1.6 ± 0.02	1.5 ± 0.01	–	1.1 ± 0.01	2.5 ± 0.02	–	2.3 ± 0.04	
57	α-trans-Bergamotene	1432	1432	1580	1576 ⁶	–	–	0.1 ± 0.01	0.2 ± 0.01	0.1 ± 0.00	0.3 ± 0.00	–	–	–	–	–	
58	6,9-Guaiadiene	1440	1442	1598	1617 ¹	–	–	0.8 ± 0.00	1.0 ± 0.13	1.0 ± 0.01	1.2 ± 0.00	–	–	1.3 ± 0.02	–	–	
59	Aromadendrene	1447	1439	1613	1620 ⁵	–	–	0.2 ± 0.11	–	–	0.1 ± 0.01	–	–	–	–	0.2 ± 0.04	
60	Menthol	–	–	1629	1630 ⁶	–	–	–	–	–	0.3 ± 0.01	–	–	–	–	–	
61	α-Humulene	–	–	1658	1650 ¹⁴	–	–	–	0.1 ± 0.02	–	0.2 ± 0.01	–	0.1 ± 0.00	–	–	0.2 ± 0.00	
62	(E)-β-Farnesene	1451	1454	1668	1673 ⁵	–	–	0.1 ± 0.09	–	0.3 ± 0.23	0.6 ± 0.02	3.1 ± 0.02	0.6 ± 0.01	0.2 ± 0.00	–	0.5 ± 0.01	
63	Alloaromadendrene	1462	1458	–	–	–	–	–	–	0.1 ± 0.00	0.2 ± 0.01	–	–	0.2 ± 0.00	–	–	
64	cis-Cadina-1 (6), 4-diene	1459	1461	–	–	0.4 ± 0.01	–	–	–	–	–	0.3 ± 0.00	–	–	–	–	
65	Geranyl propanoate	1469	1476	1823	1816 ⁶	–	–	0.1 ± 0.00	0.2 ± 0.01	0.2 ± 0.11	0.7 ± 0.00	–	–	0.2 ± 0.01	–	–	
66	γ-Murolene	1484	1478	–	–	3.1 ± 0.05	–	–	–	–	–	–	–	2.6 ± 0.01	–	–	
67	Germacrene D	1478	1480	1697	1700 ¹⁸	–	2.6 ± 0.03	0.8 ± 0.00	0.7 ± 0.08	1.8 ± 0.02	1.9 ± 0.16	2.3 ± 0.02	1.6 ± 0.01	–	–	2.0 ± 0.00	
68	Bicyclogermacrene	1492	1500	1723	1727 ¹⁸	2.1 ± 0.00	2.0 ± 0.00	0.8 ± 0.02	0.3 ± 0.38	1.0 ± 0.01	1.1 ± 0.01	1.8 ± 0.02	1.1 ± 0.01	–	–	1.7 ± 0.02	
69	(E,E)-α-Farnesene	1502	1505	1751	1740 ¹	0.5 ± 0.00	–	–	–	0.2 ± 0.00	–	–	0.7 ± 0.01	0.3 ± 0.00	–	–	
70	β-Curcumene	1507	1514	–	–	0.4 ± 0.00	–	–	–	–	–	0.2 ± 0.00	–	–	0.3 ± 0.00	–	
71	δ-Cadinene	1515	1522	1750	1761 ¹⁴	0.2 ± 0.00	0.6 ± 0.00	–	0.3 ± 0.03	0.2 ± 0.00	0.4 ± 0.00	0.1 ± 0.00	–	0.2 ± 0.00	–	0.4 ± 0.01	
72	Myristicin	1517	1517	2272	2261 ⁶	–	–	0.2 ± 0.00	0.2 ± 0.02	–	–	–	–	–	–	–	
73	Elemicin	1546	1555	2240	2231 ⁶	–	–	0.4 ± 0.00	0.4 ± 0.00	–	–	–	–	–	–	–	
74	Germacrene B	1555	1559	1814	1814 ¹⁹	–	–	–	–	0.6 ± 0.01	0.1 ± 0.00	0.8 ± 0.00	0.1 ± 0.00	–	–	0.3 ± 0.01	
75	Caryophyllene oxide	–	–	1967	1979 ¹⁹	0.4 ± 0.00	0.3 ± 0.00	–	–	–	0.1 ± 0.00	–	–	0.4 ± 0.00	–	0.2 ± 0.03	
76	(E)-Nerolidol	–	–	2047	2050 ¹³	0.5 ± 0.00	0.5 ± 0.00	–	0.1 ± 0.00	–	–	–	0.2 ± 0.00	0.5 ± 0.00	–	0.4 ± 0.02	
77	Spathulenol	1573	1577	2118	2125 ¹⁹	0.7 ± 0.01	0.5 ± 0.00	–	–	–	0.1 ± 0.00	0.2 ± 0.00	0.1 ± 0.00	0.5 ± 0.01	–	0.4 ± 0.02	
78	2-Phenyl ethyl tiglate	1581	1584	2191	2210 ⁶	–	–	0.1 ± 0.03	0.1 ± 0.02	0.5 ± 0.02	0.4 ± 0.02	–	–	–	–	–	
79	10-epi-γ-Eudesmol	1620	1622	–	–	–	–	0.1 ± 0.06	–	–	–	–	–	0.2 ± 0.01	–	–	
80	epi-α-Cadinol	1639	1638	2167	2170 ⁶	–	–	–	0.1 ± 0.01	0.3 ± 0.10	0.2 ± 0.05	–	–	0.1 ± 0.00	–	–	
81	α-Cadinol	1658	1652	2229	2239 ²⁰	0.2 ± 0.01	0.1 ± 0.00	–	–	–	–	–	–	–	–	–	
82	Citronellyltiglate	1661	1666	2210	2214 ²¹	–	–	–	–	0.1 ± 0.08	0.1 ± 0.01	–	–	–	–	–	
83	epi-α-Bisabolol	1683	1683	2218	2214 ⁶	–	–	–	–	0.3 ± 0.01	0.3 ± 0.00	–	–	–	–	–	
84	α-Bisabolol	1686	1685	2221	2214 ⁶	–	–	0.2 ± 0.01	0.2 ± 0.00	–	–	–	–	–	–	–	
85	α-Bisabolol oxide B	–	–	2126	2156 ²²	–	–	–	–	–	–	–	0.1 ± 0.00	–	–	–	
86	Geranyl tiglate	1695	1696	2113	2099 ⁶	–	0.2 ± 0.01	0.2 ± 0.00	0.2 ± 0.00	0.2 ± 0.06	0.2 ± 0.00	–	–	0.3 ± 0.01	–	–	
87	α-Bisabolol oxide A	–	–	2419	2408 ²²	–	–	–	–	–	–	–	0.1 ± 0.00	–	–	–	
88	(Z)-Spiroether	1881	1879	–	–	–	–	–	–	–	–	2.2 ± 0.08	–	–	–	–	
	Oxygenated monoterpenes (%)					70.9	73.4	74.1	72.7	62.7	62.5	60.0	65.0	64.6		57.2	
	Monoterpene hydrocarbons (%)					10.2	10.1	14.9	15.3	9.6	10.2	15.7	19.8	18.6		22.0	
	Oxygenated sesquiterpenes (%)					1.9	1.7	0.6	0.6	0.9	1.0	0.2	0.5	2.0		1.0	
	Sesquiterpene hydrocarbons (%)					9.8	8.4	3.5	3.3	8.2	8.0	11.9	5.2	7.3		8.2	
	Others (%)					5.3	4.5	5.0	6.1	16.9	17.0	10.3	7.9	5.6		9.7	
	TOTAL (%)					98.1	98.1	98.1	98.0	98.3	98.7	98.1	98.4	98.1		98.1	

^a Calculated linear retention index on a DB-5MS capillary column.

^b Linear retention indices on a DB-5MS column from reference (Adams, 2009).

^c Calculated linear retention indices on a HP-INNOWax capillary column.

^d Linear retention indices on a HP-INNOWax column from references. (¹Demirci et al., 2003; ²Schepetkin et al., 2015; ³Kan et al., 2015; ⁴Ali et al., 2016; ⁵De Falco et al., 2013; ⁶Babushok et al., 2011; ⁷Agostini et al., 2009; ⁸Cozzani et al., 2005; ⁹Rodríguez et al., 2018; ¹⁰Kang et al., 2012; ¹¹Yasa et al., 2012; ¹²Heydari et al., 2018; ¹³Kürkçüoğlu et al., 2013; ¹⁴Khan et al., 2016; ¹⁵Mann et al., 2013; ¹⁶Kundakovic et al., 2007; ¹⁷Sezik et al., 2005; ¹⁸Padalia et al., 2013; ¹⁹Ruiz et al., 2010; ²⁰Solis et al., 2016; ²¹Kiran et al., 2005; ²²Kaya et al., 2018).

^e Average percentage from three GC-FID analyses and relative standard deviation less than 10%.

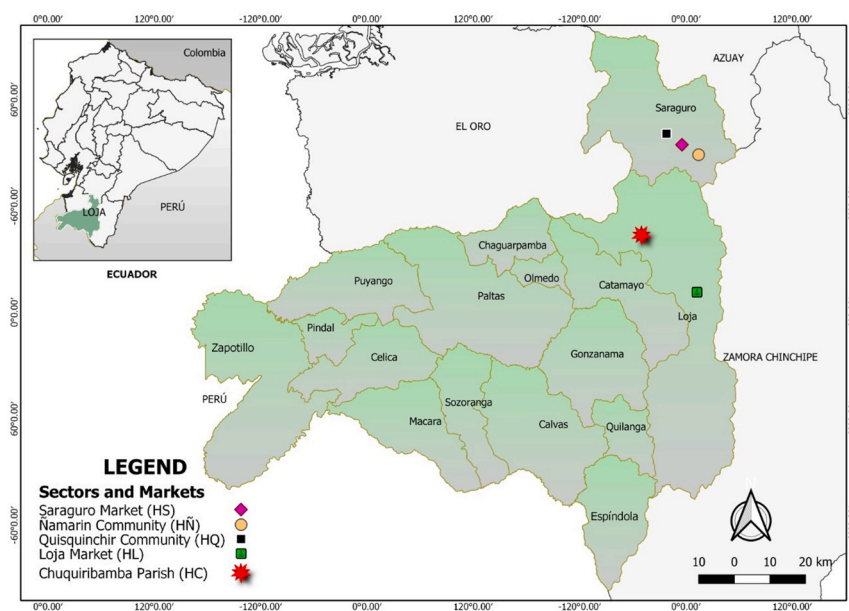


Fig. 2. Locations of traditional markets in Loja province in Southern Ecuador.

or *atados*) or bags of fresh plants purchased at five different places of Loja province (Fig. 2): (i) at the area of Namarin (HN); (ii) at the *Mercado Mayorista* of Loja (HL); (iii) at the Saraguro market (HS); (iv) at the Chuquiribamba market (HC); and (v) at the Quisquinchir sector (HQ) (Fig. 2). These places where selected according to the following criteria: (i) the existence of a deep-rooted cultural tradition for the local consumption of *horchata*; (ii) an intense cultivation of medicinal and aromatic plants used to prepare the drink; (iii) the preservation of the same drink formulation, due to the transfer of knowledge between generations; (iv) an easy differentiation, in terms of number and types, of medicinal and aromatic herbs occurring in the same bunch or bag.

We were well-aware of the possible seasonal variation of aromatic plants used for the *horchata* preparations, and the consequent changes in the EOs aromatic profiles. Therefore, all plants were purchased in the same vegetative period, from January to March 2019. Moreover, in order to reduce the variability of the vegetable material investigated, the plants were always purchased from the same vendors (*horchateras*) at the selected places; additionally, sellers were instructed to maintain the same mixtures of plants (number and types) during the entire period of the research.

The plants were identified by Bolivar Merino, curator of the Herbarium Loja at the Universidad Nacional de Loja, by comparison with reference samples stored in the Herbarium. The plant scientific names are based on the Catalogue of the Vascular Plants of Ecuador (Jørgesen and León-Yáñez, 1999) and on the database of The Plant List <http://www.theplantlist.org> (accession date: 3 March 2019). A voucher sample of each species was deposited in the Herbarium of the Universidad Técnica Particular de Loja.

This research was carried out with the authorization of the Ministry of Environment of Ecuador (MAE-DNB-CM-2016-0048).

2.2. Criteria for the distillation of the vegetable material

Aromatic plants (AP) occurring in each *horchata* bunch (*tongo*) or bag collected at the different selected locations (Fig. 2) were separated from non-aromatic medicinal plants (NAP) and flowers. The two groups were weighed; subsequently, the AP plants were chopped to a size suitable to steam-distillation. It must be noted that the highest amounts of petal fragments occurred in HS, HQ and HN vegetable materials, which are sold in bags (Fig. 3B, D, E).

2.3. Essential oil preparation

HL, HC, HS, HQ and HN mixtures of aromatic plants were separately hydrodistilled for 4 h using a Clevenger-type apparatus. Subsequently, each EO was dried over anhydrous sodium sulfate and kept in a sealed amber glass vial at 4 °C, shielded from light until analysis. This procedure was repeated three times for each EO.

2.3.1. Physical properties of the essential oils

The relative density of each oil was determined at 20 °C according to the international standard method AFNOR NF T 75–111 (ISO 279:1998). The refractive index was measured at 20 °C on an ABBE refractometer according to the AFNOR NF 75–112 (ISO 280:1998) international standard method. The specific rotation was determined on an automatic polarimeter Hanon P-810, according to the international standard ISO



Fig. 3. *Horchata* plants collected at different markets: A) Loja (HL); B) Namarin (HN); C) Chuquiribamba (HC); D) Saraguro (HS); E) Quisquinchir (HQ).

592–1998 guidelines. Each test was performed in triplicate and an average value was calculated.

2.3.2. Gas chromatography/flame ionization (GC/FID) analyses

GC-FID analyses were performed on an Agilent Technologies instrument (6890N series) equipped with a flame ionization detector (FID). Two types of chromatographic columns were used; a non-polar capillary column (DB-5MS, 5%-phenyl-methylpolysiloxane stationary phase, 30 m × 0.25 mm i.d. × 0.25 µm of film thickness) and a polar capillary column (HP-Innowax, 30 m × 0.25 mm i.d. × 0.25 µm of film thickness), GC analysis on stationary phases of opposite polarity guarantees that the identification of EOs components on the basis of the linear retention indices is more reliable, and facilitates separation of overlapped peaks (Marriot et al., 2001). In both analyses helium was the carrier gas (0.9 mL/min in constant flow mode). An automatic injector (series 7683) in split mode was used. A solution (1 µL) of each EO in dichloromethane (1:100 v/v) was injected with a split ratio of 1:50. Oven temperature was held initially at 50 °C for 3 min then it was raised to 210 °C with a gradient of 2.5 °C/min, maintained at 210 °C for 3 min. Injector and detector temperatures were 210 °C and 250 °C, respectively. Compound linear retention indices (LRI) were determined, according to Van Den Dool and Kratz (1963), on the basis of a homologous series of standard *n*-alkanes C₁₀–C₂₅ (TPH-6RPM of CHEM SERVICE), which were injected after each EO under the same chromatographic conditions. The percentage content of each identified compound in each EO was calculated by comparing the area of the corresponding GC-FID peak to the total area of identified peaks (Table 3), without applying any correction factor. Average values and standard deviations were calculated from the results of three injections.

2.3.3. Gas chromatography/mass spectrometry (GC/MS) analyses

GC-MS analyses were performed using an Agilent Technologies chromatograph (6890N series) coupled to a mass spectrometer detector (model Agilent series 5973 inert). The spectrometer operated at 70 eV, the electron multiplier was set at 1600 eV, the scan rate was 2 scan/s, and the mass range *m/z* 40–350. The instrument was provided with a computerized system MSD-Chemstation D.01.00 SP1. The same columns described for the GC/FID analyses were used. The ion source temperature was set at 250 °C. EO components were identified by comparing their MS data and their relative retention indices with the literature (Adams, 2009; NIST 05, 2005 and NIST, 2017).

2.4. Determination of cholinesterase (ChE) inhibitory activity

Cholinesterase inhibitory activity of each EO was determined separately against acetylcholinesterase (AChE) from *Electrophorus electricus* (SIGMA-ALDRICH, C3389, St Louis MO.) and butyrylcholinesterase (BuChE) from equine serum (SIGMA-ALDRICH, SRE020, St Louis MO.), according to Ellman et al. (1961). A typical 200 µL inhibition assay volume contained phosphate buffered saline solution (pH 7.4), DTNB (1.5 mM), tested sample in DMSO (1% v/v final). Both AChE (Type V–S, lyophilized powder, 744 U/mg solid, 1272 U/mg protein) and BuChE (lyophilized powder, 900 U/mg protein) were dissolved in PBS pH 7.4 and used at 25 mU/mL for the assay. After 10 min of pre-incubation, the enzyme substrate acetylthiocholine iodide (1.5 mM) was added to start the reaction. During 1 h of incubation at 30 °C, 96-well microtiter multiplate were read on a PherastarFS (BMG Labtech) detection system. Enzymatic activities were tested in the presence of 0.05–250 µg/mL of each EO dissolved in DMSO, whose concentration was kept constant and expressed versus DMSO alone. Donepezil was used a reference ChE inhibitor for both enzymes (McGleenon et al., 1999). IC₅₀ values were determined from three individual experiments. Activity results were expressed as the mean ± SD of the three replicates. IC₅₀ values were determined from a nonlinear regression model by using the online GNUPLOT package version 5.2.8 (www.ic50.tk, www.gnuplot.info). One-way ANOVA simple or multiple comparison as well as

Mann-Whitney tests were run with GraphPadPrim software.

3. Results and discussions

3.1. Medicinal and aromatic plants used to prepare horchata lojana

Considering the 5 groups of plants used to prepare the different types of horchata, a total of 23 botanical families and 32 species were identified. The family with the highest number of identified species was Lamiaceae with 6 species, followed by Amaranthaceae, Geraniaceae, Malvaceae, and Onagraceae with 2 species each. Only one species was identified for each of the following families: Asteraceae, Apiaceae, Boraginaceae, Begoniaceae, Caprifoliaceae, Caryophyllaceae, Equisetaceae, Linaceae, Orchidaceae, Plantaginaceae, Piperaceae, Poaceae, Rutaceae, Proteaceae, Rosaceae, Solanaceae, Verbenaceae, Violaceae.

In general, 4–9 medicinal plants were used for the preparation of each type of horchata investigated in this research, whereas the number of aromatic species ranged from 5 to 11. Aromatic species were directly related to the traditional aroma of the drink.

In the case of flowers, a number varying between 1 and 7 was roughly estimated for each bunch of aromatic herbs; however, botanical identification of petal fragments was impossible.

The voucher numbers of the plants used to prepare the five types of horchata, their vernacular and scientific names, the part of plant used, the geographical origin, and the traditional therapeutic applications reported in the literature are listed in Table 1.

As regards the origin of the plants used to prepare the five types of horchata examined in this study, 12 are native to Ecuador and 20 have been introduced. Four of the native species were aromatic and include *Clinopodium brownei* (poleo chico or warmi poleo), *Clinopodium nubigenum* (tipo del cerro), *Peperomia congona* (congona), and *Aloysia triphylla* (cedrón). The high number of introduced plants used for the horchata preparation strongly indicates that the drink has been consumed since the time of the Colony. In fact, a great number of plants and animals as well as new food recipes were introduced from Europe to South America at that time, thereby causing an important cultural, gastronomic and medicinal hybridization between local and introduced habits. In this way, the populations of South America, after having experienced the benefits of European natural remedies and their synergistic activities with indigenous cures, included the new agents in their traditional medicinal systems.

Regarding the composition of the mixtures (tongos or bags) used to prepare the five types of horchata investigated, 16 species were identified in the bags purchased at the Namarin sector (HN). Four were medicinal (*I. herbstii*, *B. officinalis*, *P. major*, *P. sanguisorba*) and seven aromatic herbs (*M. recutita*, *P. graveolens*, *P. odoratissimum*, *M. officinalis*, *C. citratus*, *A. triphylla*, *C. nubigenum*), whereas the remaining were flowers (*B. x tuberhybrida*, *D. caryophyllus*, *A. rosea*, *F. magellanica*, *V. tricolor*). In the bags collected at the Quisquinchir sector (HQ), 5 of the 19 identified species were medicinal (*I. herbstii*, *A. cruentus*, *B. officinalis*, *E. bogotense*, and *P. major*) and 7 were aromatic herbs (*M. recutita*, *P. odoratissimum*, *M. officinalis*, *M. piperita*, *M. spicata*, *C. citratus*, *A. triphylla*), whereas 7 were flowers (*B. x tuberhybrida*, *S. nigra*, *M. arborea*, *A. rosea*, *E. fimbriatum*, *P. sanguisorba*, *V. tricolor*). In the bags purchased at Saraguro (HS) a total of 18 species were identified, among which 6 were medicinal (*I. herbstii*, *A. cruentus*, *B. officinalis*, *E. bogotense*, *L. usitatissimum*, *P. sanguisorba*) and 5 were aromatic herbs (*M. recutita*, *P. graveolens*, *P. odoratissimum*, *M. officinalis*, *C. citratus*), whereas 7 were flowers (*B. x tuberhybrida*; *S. nigra*; *D. caryophyllus*; *M. arborea*; *A. rosea*; *F. magellanica* and *V. tricolor*). A total of 21 species were identified in the mixture purchased at Loja (HL), comprising 9 medicinal (*I. herbstii*, *A. cruentus*, *B. officinalis*, *E. bogotense*, *L. usitatissimum*, *O. rosea*, *P. major*, *O. grandiflora*, *S. americanum*) and 11 aromatic herbs (*M. recutita*, *F. vulgare*, *P. graveolens*, *P. odoratissimum*, *M. officinalis*, *M. piperita*, *O. basilicum*, *P. congona*, *C. citratus*, *C. sinensis*, *A. triphylla*). In addition, the flowers of *S. nigra* were used in the Loja preparation. It is worth

Table 4Butyrylcholinesterase (BuChE) inhibitory activity of the EOs distilled from *horchata* plants collected at different locations, compared with donepezil.

IC ₅₀ best fit value (µg/ml)						
Sample ^a	EO aromatic profile	Experiment 1	Experiment 2	Experiment 3	Mean	deviation
HL	Neral, geranial, myrcene	30.4	31.1	23.5	28.3	3.2
HS	Geranial, isomenthone, methyl eugenol, neral, citronellol	29.9	35.7	51.6	39.1	8.3
HÑ	Neral, geranial, carvone	35.8	38.6	50.9	41.8	6.1
HQ	Neral, geranial, myrcene	32.9	46.3	32.9	37.4	5.9
HC	(<i>E</i>)-Anethole, geranial, pulegone	42.9	37.0	40.0	40.0	2.0
Donepezil	–	3.4	3.5	3.8	3.6	0.2

^a The acronyms HL, HS, HÑ, HQ and HC correspond to the groups of plants collected at Loja, Saraguro, Ñamarin, Quisquinchir, and Chuquiribamba, respectively.

noting that the selected vendor at Loja sells other mixtures of flowers (*Fuchsia loxensis*, *Fuchsia harlingii*, *Fuchsia hybrida*, *Pimpinella anisum*, *Ludwigia nervosa*, *Viola odorata*, *Tagetes filifolia*, *Matthiola incana*, *Malva parviflora*, *Peperomia inaequalifolia*, *Triumfetta semitriloba*) in separate bags, that may occasionally be used for the preparation of the local *horchata*. Twenty-one species were determined in the bunches purchased at Chuquiribamba (HC), comprising 9 medicinal (*I. herbstii*, *A. cruentus*, *B. officinalis*, *E. bogotense*, *L. usitatissimum*, *A. rosea*, *P. major*, *O. grandiflora*, *S. americanum*), 10 aromatic herbs (*M. recutita*, *F. vulgare*, *P. graveolens*, *P. odoratissimum*, *C. brownei*, *M. officinalis*, *M. piperita*, *P. congona*, *C. citratus*, *A. triphylla*), and two types of flowers (*M. arborea* and *V. tricolor*).

Aromatic plants outnumbered medicinal ones in each of the blend of herbs that has been investigated. This finding clearly indicated the importance of aromatic plants for the traditional preparation of different *horchata* drinks. The highest number of aromatic plants (11) was found in HL, followed by HC where 10 aromatic species were used. Aromatic plants used for all types of *horchata* preparations included *M. recutita*, *P. odoratissimum*, *M. officinalis*, and *C. citratus*. It is worth noting that *C. nubigenum* and *C. brownei* were only used for the HN and HC preparations, respectively, whereas the remaining aromatic species were used in the recipes for more than two types of *horchata*.

3.2. Chemical composition of the essential oils

The yield, relative density, refraction index, and optical rotation of each EO distilled from the 5 groups of aromatic plants examined in the study, are reported in Table 2. For each EO the average values measured on three distillates are shown. It is evident from Table 2 that each EO exhibited specific chemical-physical properties and aroma characteristics, depending on the mixture of aromatic plants used to prepare the drink.

The chemical components of each EO, their retention indices (LRI) and contents (%) are listed in Table 3.

The EOs from the five groups of plants belonged to the monoterpene type, independently from the collection places, since monoterpenoids largely predominated over sesquiterpenoids. Between monoterpenoids, oxygenated compounds predominated over hydrocarbons whereas, in the case of sesquiterpenoids, hydrocarbons exceeded oxygenated compounds. A total of 88 compounds have been identified in the different EOs examined; only 24 occurred in all the EOs. Moreover, some components were detected in only one of the two GC chromatograms.

The chemical compositions of the five EOs were quite similar qualitatively, while the content of each component varied significantly, thus considerably affecting the aroma of each type of *horchata*. In this regard, it must be considered that the amount of the same plant in the bunches could vary significantly, depending on the location where it was collected.

The most abundant components of the EO from Ñamarin plants were neral (23.2%) and geranial (20.2%), followed by smaller amounts of carvone (7.8%), myrcene (6.4%), nerol (4.6%), γ -muurolene (3.1%). The EO of the plants purchased at Loja (HL) contained neral (22.7%), geranial (31.6%), and myrcene (12.5%) as predominant compounds and

minor percentages of geraniol (6.8%) and citronellol (4.0%). The most abundant components of the EO distilled from plants collected at Saraguro (HS) were geranial (16.5%), methyl eugenol (13.4%), isomenthone (12.9%), neral (11.7%) and citronellol (11.6%), whereas myrcene (6.1%) and geraniol (3.7%) occurred in minor amounts. In the EO distilled from the plants collected at Chuquiribamba (HC), the main constituent was (*E*)-anethole (22.8%), followed by minor amounts of geranial (9.2%), pulegone (8.6%), neral (6.5%), methyl eugenol (5.5%), myrcene (5.2%), and carvone (3.5%). Finally, neral (18.7%), geranial (26.3%), and myrcene (14.5%) predominated in the EO distilled from plants purchased at Quisquinchir (HQ), whereas minor percentages of citronellol (5.1%), methyl eugenol (4.7%), and limonene (3.5%) were determined.

Considering the main components, the five EOs can be characterized by four different aromatic profiles, namely: (i) neral + geranial + carvone; (ii) neral + geranial + myrcene; (iii) geranial + methyl eugenol + isomenthone + neral + citronellol; (iv) (*E*)-anethole + geranial + pulegone.

3.3. Cholinesterase inhibitory activity assay

The anti-AChE and anti-BuChE activities in vitro of the five EOs were evaluated according to Matailo et al. (2019). The IC₅₀ (µg/ml) values for each EO tested (three replicates) are shown in Table 4, compared with the activities of donepezil, that was used as the reference compound. All the EOs were inactive as AChE inhibitors with IC₅₀ > 250 µg/ml. However, the five oils showed interesting inhibitory activity against BuChE with IC₅₀ values ranging between 28.3 and 41.8 µg/ml. However, no statistical difference could be deduced from the various sources of *horchata*.

Agents that display selective inhibitory activity higher against BuChE than against AChE may have potential therapeutic benefits in the treatment of AD and other related dementia diseases (Greig et al., 2002; Ahmad et al., 2016). Indeed, it has been demonstrated that selective inhibition of BuChE not only increases the acetylcholine level significantly, but also improves memory in elderly rats (Ayaz et al., 2015).

4. Conclusions

Horchata lojana is a popular beverage, made through infusion of different medicinal and aromatic plants, that has been prepared throughout the centuries as part of the cultural tradition of the people living in Loja province, in Southern Ecuador. Indeed, still today, this province remains the main production area of *horchata* in the entire Ecuador.

A single standardized or preferred recipe to prepare this traditional drink does not exist, although local people select the plants for the different preparations following some important criteria that have been mentioned above.

The wide variety of *horchata* preparations thus complicates any systematic chemical and pharmacological study performed on this beverage. As an example of contrasting results, we have found that *Clinopodium brownei* is used for the aromatization of an atypical *horchata*

(HC); in contrast, the use of this plant is not mentioned in previous studies (Rios et al., 2017; Tinitana et al., 2016). For these reasons, we have focused our investigation on plants collected in a restricted vegetative period of the year and in a limited number of locations in the province of Loja. To complete the study, plant collection should be extended to more locations and the entire year. Even with these limitations, however, this inaugural study may serve as a model for future investigations.

We have shown that the pleasant flavor of *horchata lojana* is due to several organic compounds, mostly terpenoids, occurring in the volatile fractions (EOs) isolated from the aromatic plants used to prepare the drink. Each plant gives its individual contribution to the characteristic aroma of the drink. Specifically, we have identified four aromatic profiles characterizing five different types of *horchata*, which can serve as reference data for studies on other preparations of the drink.

Aromatic plants used for the preparation of *horchata lojana* have been identified botanically in this study. A search of the literature has revealed that they exhibit a wide range of biological activities, including antibacterial, analgesic, and anti-inflammatory properties (see Table 1). Moreover, several individual components identified in the EOs, such as neral, geraniol, geraniol, carvone, and pulegone have showed potent anti-inflammatory, anti-oxidant, and other biological effects in vitro and in vivo (Edris, 2007; Bakkali et al., 2008; Adorjan and Buchbauer, 2010; Wojtunik-Kulesza et al., 2019).

We have demonstrated that all the EOs showed selective BuChE inhibitory activity (Table 4); the most promising BuChE activity was observed for the EO isolated from the HL blend of plants. It is worth noting that the BuChE inhibitory activity has been claimed to have therapeutic potential for the treatment of AD and other neurodegenerative disorders. *Horchata lojana* is a cheap and easily prepared traditional beverage, and the longstanding daily consume by the people of Loja province clearly demonstrates the edibility and absence of side-effects. Therefore, this beverage has great potential to be developed as an effective and save botanical dietary supplement for preventing cognitive disorders, although further in vitro as well as in vivo tests must be carried out to fully support the efficacy and action mechanism.

In conclusion, our findings suggest a plausible scientific explanation for the use of the aromatic plants used to prepare *horchata lojana*. First of all, they impart a pleasant aroma to the drink, making its assumption more attractive. Moreover, they contribute to the beverage medicinal properties, justifying the traditional consume as brain tonic and anti-inflammatory, analgesic, and diuretic remedy, and for the prevention of neurodegenerative disorders.

Dehydrated forms of *horchata* are increasingly commercialized abroad. We think that this research can help establish criteria for assuring product quality based on the chemical composition of volatile fractions. On the other hand, besides the potential as dietary supplements, the EOs isolated from *horchata* aromatic plants can be used as raw materials to prepare new perfumes or aromatized foods.

Authors' contributions

C. A., G. A., and N. D. collected the vegetable material and performed the hydro distillation. G. A., N. S. and C. Lu, performed the physical analysis of the EOs studied. A. M., M. S., G. A., N. S., C. A. J. C., and G. V. share contributions in data analysis; C. A., and G. V. prepared the manuscript; N. B., and C. L. performed the biological assays. All authors have read and approved the final manuscript.

Declaration of competing interest

The authors declare to have no actual or potential conflict of interest.

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References

- Abdellatif, F., Boudjella, H., Zitouni, A., Hassani, A., 2014. Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian *Melissa Officinalis* L. *Excli J.* 13, 772–781.
- Adams, R.P., 2009. Identification of Essential Oil Components by Gas Chromatography/mass Spectrometry, fourth ed. Allured Business Media. Carol Stream, IL, USA.
- Adorjan, B., Buchbauer, G., 2010. Biological properties of essential oils: an updated review. *Flavour Fragrance J.* 25, 407–426.
- Agostini, F., Atti, A., Rossato, M., Pansera, M., Dos Santos, P., Atti, L., Molon, R., Moyna, P., 2009. Essential oil yield and composition of Lamiaceae species growing in Southern Brazil. *Braz. Arch. Biol. Technol.* 52, 473–478.
- Ahmad, S., Ullah, F., Sadiq, A., Ayaz, M., Imran, M., Ali, I., Zeb, A., Ullah, F., Shah, M.R., 2016. Chemical composition, antioxidant and anticholinesterase potentials of essential oil of *Rumex hastatus* D. Don collected from the North West of Pakistan. *BMC Compl. Alternative Med.* 16, 29. <https://doi.org/10.1186/s12906-016-0998-z>.
- Ali, A., Tabanca, N., Demirci, B., Blythe, E., Can-Baser, K.H., Khan, I., 2016. Chemical composition and biological activity of essential oils from four *Nepeta* species and hybrids against *Aedes aegypti* (L.) (Diptera: Culicidae). *Record Nat. Prod.* 10, 137–147.
- Andrade, J.M., Lucero, H., Armijos, C., 2017. Ethnobotany of indigenous saraguros: medicinal plants used by community healers “Hampiyachakuna” in the san Lucas parish, southern Ecuador. *BioMed Res. Int.* <https://doi.org/10.1155/2017/9343724>.
- Armijos, C., Valarezo, E., Cartuche, L., Zaragoza, T., Vita Finzi, P., Mellerio, G.G., Vidari, G., 2018. Chemical composition and antimicrobial activity of *Myrcianthes fragrans* essential oil, a natural aromatizer of the traditional Ecuadorian beverage colada morada. *J. Ethnopharmacol.* 225, 319–326.
- Avoseh, O., Oyedeji, O., Rungqu, P., Nkeh-chungag, B., Oyedeji, A., 2015. *Cymbopogon* species; ethnopharmacology, phytochemistry and the pharmacological importance. *Molecules* 20, 7438–7453. <https://doi.org/10.3390/molecules20057438>.
- Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Khan, M.A., Ahmad, W., Shah, M.R., Imran, M., Ahmad, S., 2015. Comparative chemical profiling, cholinesterase inhibitions and anti-radicals properties of essential oils from *Polygonum Hydropiper* L: a preliminary anti-Alzheimer's study. *Lipids Health Dis.* 14, 141–152.
- Ayaz, M., Sadiq, A., Junaid, M., Ullah, F., Subhan, F., Ahmed, J., 2017. Neuroprotective and anti-aging potentials of essential oils from aromatic and medicinal plants. *Front. Aging Neurosci.* 9, 168–183. <https://doi.org/10.3389/fnagi.2017.00168>.
- Babushok, V., Linstrom, P., Zenkevich, G., 2011. Retention indices for frequently reported compounds of plant essential oils. *J. Phys. Chem. Ref. Data* 40, 043101. <https://doi.org/10.1063/1.3653552>.
- Bailón, N., Tinitana, F., Martínez, R., Jaramillo, A., Palacio, A., Aguilar, J., Romero, J.C., 2017. Cytotoxic, antioxidative, genotoxic and antigenotoxic effects of horchata, beverage of South Ecuador. *BMC Compl. Alternative Med.* 1, 1–14. <https://doi.org/10.1186/s12906-017-2048-x>.
- Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils – a review. *Food Chem. Toxicol.* 46, 446–475.
- Boukhris, M., Simmonds, M.S.J., Sayadi, S., Bouaziz, M., 2012. Chemical composition and biological activities of polar extracts and essential oil of rose-scented geranium, *Pelargonium graveolens*. *Phytother Res.* 27, 1206–1213. <https://doi.org/10.1002/ptr.4853>.
- Cozzani, S., Muselli, A., Desjobert, J., Bernardini, A., Tomi, F., Casanova, J., 2005. Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. *Flavour Fragr. J. Res.* 20, 436–441.
- Demirci, B., Can, H., Yıldız, B., Bahçecioglu, Z., 2003. Composition of the essential oils of six endemic *Sabia* spp. from Turkey. *Flavour Fragrance J.* 18, 116–121.
- De Falco, E., Mancini, E., Roscigno, G., Mignola, E., Tagliatalata Scafati, O., Senatore, F., 2013. Chemical composition and biological activity of essential oil of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules* 18, 14948–14960.
- Edris, A.E., 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytother Res.* 21, 308–323.
- Ellman, G., Courtney, D., Valentino, A., Featherstone, R., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7, 88–95.
- Espinoza Gálvez, E.G., 2016. La tradicional horchata lojana: Entre iniciativas de desarrollo gubernamentales y comunitarias. FLACSO Ecuador. Quito, Ecuador. <http://hdl.handle.net/10469/9652>.
- Greig, N.H., Lahiri, D.H., Sambamurti, K., 2002. Butyrylcholinesterase: an important new target in Alzheimer's disease therapy. *Int. Psychogeriatr.* 14 (Suppl. 1), 77–91.
- Guevara, M., Tejera, E., Iturralde, G.A., Jaramillo-Vivanco, T., Granda-Albuja, M.G., Granja-Albuja, S., Álvarez-Suárez, J.M., 2019. Anti-inflammatory effect of the medicinal herbal mixture infusion, horchata, from southern Ecuador against LPS-induced cytotoxic damage in RAW 264.7 macrophages. *Food Chem. Toxicol.* 131–141, 110594. <https://doi.org/10.1016/j.fct.2019.110594>.
- Gyengesi, E., Münch, G., 2020. In search of an anti-inflammatory drug for Alzheimer disease. *Nat. Rev. Neurol.* 16, 131–132. <https://doi.org/10.1038/s41582-019-0307-9>.
- Heppner, F.L., Ransohoff, R.M., Becher, B., 2015. Immune attack: the role of inflammation in Alzheimer disease. *Nat. Rev. Neurosci.* 16, 358–372. <https://doi.org/10.1038/nrn3880>.
- Heydari, M., Zanfardino, A., Taleei, A., Shahnejat, A., Hadian, J., Maresca, V., Sorbo, S., Di Napoli, M., Varcamonti, M., Basile, A., Rigano, D., 2018. Effect of heat stress on yield, monoterpene content and antibacterial activity of essential oils of *Mentha x*

- piperita* var. Mitcham and *Mentha arvensis* var. *piperascens*. *Molecules* 23, 1903–1915. <https://doi.org/10.3390/molecules23081903>.
- Howes, M.J., Houghton, P.J., 2003. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. *Pharmacol. Biochem. Behav.* 75, 513–527. [https://doi.org/10.1016/s0091-3057\(03\)00128-x](https://doi.org/10.1016/s0091-3057(03)00128-x).
- Jørgesen, P., León-Yáñez, S., 1999. Catalogue of the Vascular Plants of Ecuador. Missouri Botanical Garden Press, St. Louis, Mo, USA.
- Kan, A., Günhan, R., Celik, S., 2015. The chemical composition profile of *Dorystoehashastata* Boiss. & Heldr. ex Bentham cultivated in Turkey. *Record Nat. Prod.* 9, 135–145.
- Kang, W., Li, Y., Xu, Y., Jiang, W., Tao, Y., 2012. Characterization of aroma compounds in Chinese bayberry (*Myrica rubra* Sieb. et Zucc.) by gas chromatography mass spectrometry (GC-MS) and olfactometry (GC-O). *J. Food Sci.* 12, 1030–1035.
- Kaya, A., Demirci, B., Dinç, M., Dogu, S., 2018. A monotypic species from Turkey: characterization of the essential oil of *Berulaerecta* (Apiaceae). *NVEO-Nat. Volatiles Essent. Oils* 5, 7–10.
- Khan, M., Al-Saleem, M., Alkhatlan, H., 2016. A detailed study on chemical characterization of essential oil components of two *Plectranthus* species grown in Saudi Arabia. *J. Saudi Chem. Soc.* 20, 711–721.
- Kiran, G., Badu, D., Kaul, V., 2005. Variation in essential oil composition of rose-scented geranium (*Pelargonium* sp.) distilled by different distillation techniques. *Flavour Fragrance J.* 20, 222–231.
- Kundakovic, T., Fokialakis, N., Kovacevic, N., Chinou, I., 2007. Essential oil composition of *Achillea linguata* and *A. umbellata*. *Flavour Fragrance J.* 22, 184–187.
- Kürkçüoğlu, M., Abdel-Megeed, A., Başer, K.H.C., 2013. The composition of Taif rose oil. *J. Essent. Oil Res.* 25, 364–367. <https://doi.org/10.1080/10412905.2013.809322>.
- Mann, T.S., Kiran Babu, G.D., Guleria, S., Singh, B., 2013. Variation in the volatile oil composition of *Eucalyptus citriodora* produced by hydrodistillation and supercritical fluid extraction techniques. *Nat. Prod. Res.* 27, 675–679.
- Marriot, P., Shellie, R., Cornwell, C., 2001. Gas chromatographic technologies for the analysis of essential oils. *J. Chromatogr., A* 936, 1–22.
- Mataillo, A., Bec, N., Calva, J., Ramírez, J., José Miguel Andrade, J.A., Larroque, C., Vidari, G., Armijos, C., 2019. Selective BuChE inhibitory activity, chemical composition, and enantiomer content of the essential oil from the Ecuadorian plant *Clinopodium brownei*. *Rev. Bras. Farmacogn.* 29, 749–754.
- McGleeson, B., Dynan, B., Passmore, A., 1999. Acetylcholinesterase inhibitors in Alzheimer's disease. *Br. J. Clin. Pharmacol.* 48, 471–480.
- Mushtaq, G., Greig, N.H., Khan, J.A., Kamal, M.A., 2014. Status of acetylcholinesterase and butyrylcholinesterase in Alzheimer's disease and type 2 diabetes mellitus. *CNS Neurol. Disord. - Drug Targets* 13, 1432–1439. <https://doi.org/10.2174/1871527313666141023141545>.
- NIST 05, 2005. Mass Spectral Library (NIST/EPA/NIH). National Institute of Standards and Technology, Gaithersburg, MD.
- NIST/EPA/NIH Mass Spectral Library, 2017. NIST/Wiley Technology. Accessed on August 2019.
- Padalia, R., Verma, R., Chauhan, A., Sundaresan, V., Chanotiya, C., 2013. Essential oil composition of sixteen elite cultivars of *Mentha* from western Himalayan region, India. *Maejo Int. J. Sci. Technol.* 7, 83–93.
- Rios, M., Tinitana, F., Jarrín, P., Donoso, N., Romero, J., 2017. “Horchata” drink in Southern Ecuador: medicinal plants and people's wellbeing. *J. Ethnobiol. Ethnomed.* 13, 1–20. <https://doi.org/10.1186/s13002-017-0145-z>.
- Rodríguez, J.D.W., Peyron, S., Rigou, P., Chaliel, P., 2018. Rapid quantification of clove (*Syzygiumaromaticum*) and spearmint (*Mentha spicata*) essential oils encapsulated in a complex organic matrix using an ATR-FTIR spectroscopic method. *PloS One* 13 (11), e0207401. <https://doi.org/10.1371/journal.pone.0207401>.
- Rosas-Ballina, M., Tracey, K.J., 2009. Cholinergic control of inflammation. *J. Intern. Med.* 265, 663–679. <https://doi.org/10.1111/j.1365-2796.2009.02098.x>.
- Ruiz, S., Malagón, O., Zaragoza, T., Valarezo, E., 2010. Composition of the essential oils of *Artemisia sodiroi* hieron, *siparunaeggersii* hieron, *Tagetesfilifolia* Lag. And *Clinopodium nubigenum* (Kunth) Kuntze from Loja-Ecuador. *J. Essent. Oil-Bear. Plants* 13, 676–691.
- Schepetkin, I., Kushnarenko, S., Özek, G., Kirpotina, L., Utegenova, G., Kotukhov, Y., Danilova, A., Ozek, T., Baser, H., 2015. Inhibition of human neutrophil responses by essential oil of *Artemisia kotuchovii* and its constituents. *J. Agric. Food Chem.* 63, 4999–5007.
- Sezik, E., Kocakulak, E., Baser, K.H.C., Ozek, T., 2005. Composition of the essential oils of *Juniperus oxycedrus* subsp. *Macrocarpa* from Turkey. *Chem. Nat. Compd.* 41, 352–354.
- Solis, L., Tomaylla-Cruz, C., Callo-Choquelvica, Y., Solis-Quispe, A., Rodeiro, L., Hernández, I., Fernández, M., Pino, J., 2016. Chemical composition, antioxidant and antiproliferative activities of essential oil from *Schinusareira* L. and *Minthostachys spicata* (Benth.) Epl. grown in Cuzco, Peru. *J. Essent. Oil Res.* 28, 234–240.
- Tene, V., Malagón, O., Vita Finzi, P., Vidari, G., Armijos, C., Zaragoza, T., 2007. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. *J. Ethnopharmacol.* 111, 63–81. <https://doi.org/10.1016/j.jep.2006.10.032>.
- Tinitana, F., Rios, M., Romero-Benavides, J.C., de la Cruz, R.M., Pardo-de-Santayana, M., 2016. Medicinal plants sold at traditional markets in Southern Ecuador. *J. Ethnobiol. Ethnomed.* 12, 1–18.
- Van Den Dool, H., Kratz, P.A., 1963. Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chromatogr. Res.* 11, 463–471.
- Wojtunik-Kulesza, K.A., Kasprzaka, K., Oniszczuk, T., Anna Oniszczuka, A., 2019. Natural monoterpenes: much more than only a scent. *Chem. Biodivers.* 16, e1900434.
- Yasa, H., Onar, H.Ç., Yusufoglu, A.S., 2012. Chemical composition of the essential oil of *Mentha pulegium* L. from Bodrum, Turkey. *J. Essent. Oil-Bear. Plants* 15, 1040–1043.