Chemical composition and biological activity of *Myrcianthes fragrans* essential oil, a natural aromatizer of the traditional Ecuadorian beverage colada morada

Chabaco Armijos[1],* Eduardo Valarezo[1], Luis Cartuche[1], Tomas Zaragoza[2], Paola Vita Finzi[3], Giorgio Giacomo Mellerio, Giovanni Vidari[3]

[1]Departamento de Química y Ciencias Exactas, Universidad Técnica Particular de Loja / San Cayetano alto, calle Marcelino Champagnat, s/n. AP: 11 01 608, Loja, Ecuador.

[2]Departamento de misiones universitarias, Pontificia Universidad Católica del Ecuador sede Ibarra (PUCE-SI), Av. Jorge Guzmán Rueda y Av. Aurelio Espinosa Pólit. Ciudadela "La Victoria, Ibarra – Provincia de Imbabura – Ecuador

[3]Dipartimento di Chimica and Centro CEMEC, Università degli Studi di Pavia, Viale Taramelli 12, 27100, Pavia, Italy.

Abstract

Different essential oils of *Myrcianthes fragrans* (Sw.) McVaught were obtained by hydrodistillation of the aerial parts of the plant collected in Cerro Villonaco (Loja-Ecuador) at three different phenological growth stages, i.e., during foliation (Fo), flowering (Fl) and fruiting (Fr) stages. The chemical compositions of the essential oils were determined by GC-MS and GC–FID techniques. 37, 46. and 38 compounds, representing 96,5, 96,2, and 95,6% of the three essential oils, respectively, have been identified. Oxygenated monoterpenes (OM) were the major components with percentages of 63,1 (Fo), 49,4 (Fl), and 61,9% (Fr), respectively. The main constituents of the essential oils were the monoterpene aldehydes geranial and neral, the content of which varied, depending on the phenological development stage of the plant, spanning from 31,1 and 23,6% (Fo), to 23,6 and 17.8% (Fl), and 29.7 and 24,3% (Fr), respectively. The antimicrobial activities, determined by the broth microdilution method, showed that the essential oils from *M. fragrans* exhibited good activity against the *Gram-negative* bacteria, *K. pneumoniae* (ATCC 9997), and against the yeasts, *C. albicans* (ATCC 24433) and *S. cerevisiae* (ATCC 2601). A rational explanation of the traditional uses of *M. fragrans* is presented.

Key Words.

Myrtaceae, Ecuador, *Myrcianthes fragrans*, **c**olada morada, geranial, neral, GC-MS, antimicrobial activity.

1. Introduction

The genus *Myrcianthe*s belongs to the family Myrtaceae which includes about 30 genera and 1500 species growing in the neotropical region (León-Yánez et al., 2011). It comprises about 30 - 40 species of flowering plants distributed in the American continent, from southern Florida and Mexico to Bolivia and northern Argentina, Uruguay and north central Chile and the Caribbean area (Mc Vaugh, 1963; Turker et al., 1992, 2002; Chaverri & Ciccio, 2017). The species *Myrcianthes fragrans* (Sw.) McVaugh, is an aromatic shrub or tree about 3 m to 10 m high, whose distribution ranges from southern Florida, eastern Mexico, Mesoamerica, the Caribbean islands and northern South America (Mc Vaugh, 1963, Chaverri & Ciccio, 2017), including Ecuador (Jorgensen and León-Yánez, 1999).

M. fragrans grows in Ecuador between 1500-3500 m a.s.l (Jorgensen and León-Yánez, 1999) and can be found especially on the Cerro Villonaco (Villonaco hill) in the Loja Province (southern Ecuador). *M. fragrans* is known with the indigenous names of saco, arrayán or aromatic arrayán, and it has traditionally been used for preparing water infusions to treat respiratory problems, inflammations of the throat and the gums, tonsillitis, stomatitis and as a tonic. Moreover, according to Jiménez (2008), a leaf infusion of *M. fragrans* is used to combat vaginal diseases. In addition, Van Den Eyden et al. (1999, 2003) and De la Torre et al. (2008) have reported that in the southern highlands of Ecuador where it is known as guaguel, maceration of *M. fragrans* fruits gives an aromatic liquor.

In many places of Loja and Catamayo provinces (Southern Ecuador), the aromatic leaves of *M. fragrans* are used as important ingredients to obtain the characteristic aroma of a traditional Ecuadorian spiced drink called “colada morada” (Spanish for *purple strained*). This is a very well-known beverage which is typically drunk in the Day of the Dead or All Souls’ Day, on 2 November of each year. Indeed, this festive act is a pre-Hispanic celebration and for many authors it dates back thousand years ago in the Quitu-Cara culture, which flourished in areas at the slopes of the Pichincha volcano, near Quito (Gallardo de la Puente, 2014). The colada morada is typically served with sweet breads called ‘t'anta wawa’, which are filled with fruit jam (strawberry or guava) and are shaped in the form of dolls called ‘guaguas de pan’ – literally bread babies – and other human figures such as soldiers and warriors, different animals such as turtledoves, llamas, pigeons, horses, pigs, guinea pigs, and various sacred images. In indigenous Andean communities, especially in rural areas of the Chimborazo province, the ‘colada morada’ is consumed and offered in the cemetery next to the tomb of deceased relatives as part of the rite of reunion with ancestors. Actually, indigenous peoples believe that the drink represents the blood whereas the bread represents the body of their loved dead. The rite is a clear manifestation of what can be defined a religious syncretism between pre-Christian rituals and beliefs introduced in Ecuador by the Spaniards.

The traditional sacred drink colada morada, which is called *Yana Api* in the Quechua language, is made from purple (black) corn (*Zea mays*) flour, which cooked with water and a brown solid cane sugar mass, called panela or rapadura. This solid form of sucrose is derived from the boiling and evaporation of sugarcane juice. Added to this corn mixture is an infusion of cinnamon (*Cinnamomum* spp.), cloves (*Syzygium aromaticum*), sweet

pepper, ‘hierba luisa’ or lemongrass (*Cymbopogon citratus*) and cedrón (*Aloysia triphylla*). Finally, the juice of naranjilla (*Solanum quitoense*), blackberry (*Rubus* spp.) and mortiño (*Vaccinium* spp.) are added, together with orange (*Citrus sinensis*) and arrayán (*Myrcianthes spp.*) leaves (Van Den Eyden et al., 2003; Gallardo de la Puente, 2014).

Among the Andean communities of Ecuador, different species of arrayán (*Myrcianthes* spp.) are used as an aromatic additive to the drink colada morada, depending on the local availability of the plants. For example; in the Northern Sierra of the country (Pichincha, Chimborazo, Imbabura, Cotopaxi, Cañar, Tungurahua and others places), the leaves of *M. halli* are are used (Gallardo de la puente, 2014; Ulloa, 2006) , while in the Province of Loja, the leaves of *M. fragrans* are used. In other places of Ecuador such as in the Pichincha province (Quito), people add another native Ecuadorian aromatic spice called ishpingo (*Ocotea quixos*), that is collected in the Amazonian region, and was highly appreciated even by the Incas as a medicine and as a spice (Naranjo et al., 1981; Bruni et al., 2004).

The presence of “arrayan” in various sites of the Loja Province (Southern Ecuador) is documented in several botanical registers of Ecuador, including the Herbarium of the Universidad Nacional de Loja, the Herbarium of the Universidad Técnica Particular de Loja (HUTPL), the Herbarium Nacional del Ecuador (QCNE), the Herbarium of the Universidad Católica (QCA), the Herbarium of the Escuela Politécnica del Chimborazo, and the Herbarium of the Universidad del Azuay. However, according to these herbarium registers, only one single population of *M. fragrans* has been found in the locality of Cerro Villonaco, and this information has been confirmed by 105 individuals (Jiménez, 2008). In this locality several trees of *M. fragrans* are used as living fences or trees that shade livestock. The rare finding of *M. fragrans* trees in the native Andean forests of Southern

Ecuador may depend on an erroneous classification of other species or on the disappearance of the plant, due to an indiscriminate collection of the wood which, due to its good quality, have been used as firewood and in carpentry and construction.

The red-white flowers of *M. fragrans* plant emanate a pleasant fragrance that attracts many kinds of butterflies and various pollinating insects. The fruits of *M. fragrans* are round like berries and assume a black color upon ripening. They are appreciated as a tasteful food by men as well as by various kinds of birds that, like "Tartars", fly to the area of Cerro Villonaco during the fruiting period. Moreover, cattle grazing near the shading trees of *M. fragrans* eat the fruits attached to lower branches.

Despite the ethnobotanical importance of *M. fragrans*, there are no chemical or farmacologycal studies that validate the traditional uses and support its sustainable use in Ecuador. Our study, as a part of a project aimed at validating the native aromatic flora of southern Ecuador, wants to contribute to the knowledge of the composition and biological activity of the essential oils from *M. fragrans* and to explore the possible sustainable use of the plant in the region. Furthermore, we believe that the chemical composition of the essential oils can provide important chemotaxonomic data useful to distinguish *M. fragrans* from other species of the same genus growing in the Andean region, such as *M. halli*, *M. irregularis*, *M. limbata*, and *M. rhopaloides*, and thus to contribute to a correct classification of the different species of *Myrcianthes* occurring in Ecuador (De la Torre et al., 2008; León-Yánez et al., 2011; Chavez et al., 2016).

On the other hand, our study represents the first and till now unique description of the chemical composition of the essential oils from *M. fragrans* collected at three different phenological growth stages. The aromatic contribution of the oil to the flavor of the

traditional beverage colada morada in southern Ecuador, is described. Furthermore, the antimycotic activity and the potential use of the essential oil as an antifungal agent have been determined.

2. Methodology

2.1. *Plant Material*

Aerial parts of *Myrcianthes fragrans* were collected at three different phenological growth stages [foliation (Fo), flowering (Fl) and fruiting (Fr)] in the locality Cerro Villonaco, on the old road from Loja to Catamayo town (Figure 1), between the coordinates 692557N/ 9558326S at 2.610 m a.s.l. and 692582N/9558316S at 2.628 m a.s.l., from January to September 2016. The plant collections were authorized by a permit of the Ministry of Environment of Ecuador (MAE-DNB-CM-2016-0048).

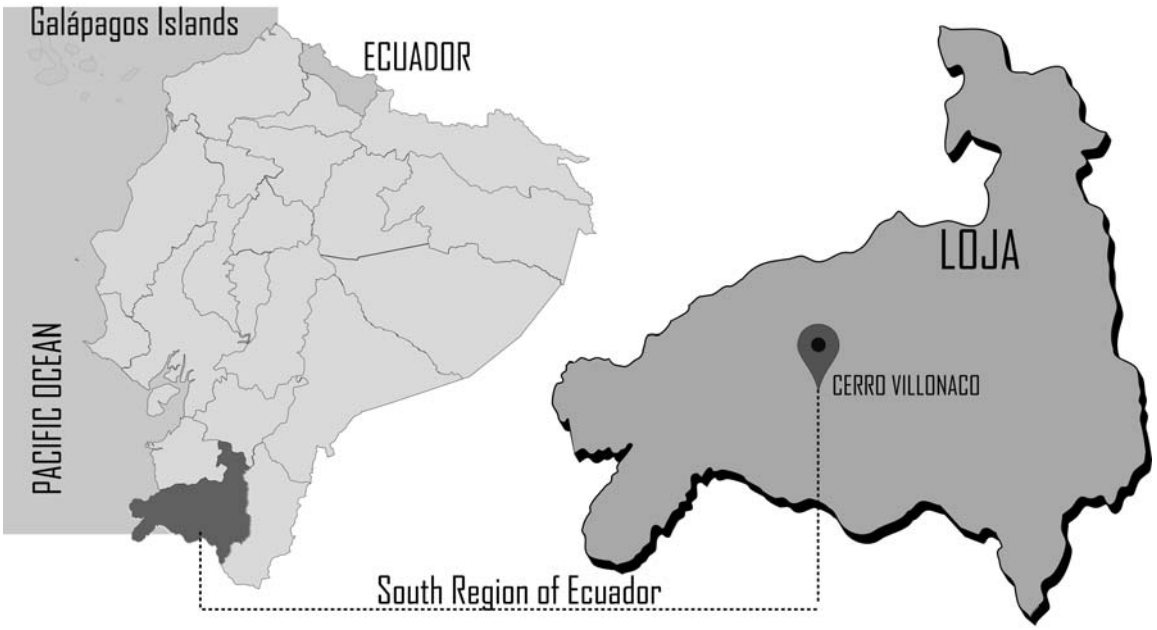

Figure 1. The sector Cerro Villonaco where is located the unique population of *Myrcianthes fragrans* (Sw.) McVaugh in the South region of Ecuador.

The plant was 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 scientific name is based on the Catalogue of the Vascular Plants of Ecuador (Jorgensen and León-Yánez, 1999) and on the database of Tropicos.org. Missouri Botanical Garden (http://www.tropicos.org). A voucher specimen of *M. fragrans* is conserved in the Herbarium HUTPL of the Universidad Técnica Particular de Loja) under the code PPN-my-008.

Criteria for the collection of the plant material

A total of 10 adult trees were harvested according to the following criteria: (i) a good phytosanitary status of the trees and leaves, flowers and fruits; (ii) central tree trunk diameter ≥15-20 cm and 4-10 m tall; (iii) vegetable material collected between 08 and 10 a.m. and (iv) from the most accessible trees.

During the flowering period (Fl), leaves and flowers were collected when the flowering stage was complete and was characterized by the reddish color of petals and stamens, corresponding to the maximum development of flowers, while the anthers continue maintaining the characteristic white and cream color. During this period there was a high presence of bees and other pollinators; as well as hummingbirds, in the area of collection.

During the fruiting period (Fr) of the plant, fully matured leaves and fruits were harvested, as indicated by the black color and fruit diameters of about 1.15 and 1.20 cm, which corresponded to the maximum fruit growth observed *in loco*.

During the foliation period (Fo), only leaves without flowers or fruits were collected (see Figure 2).

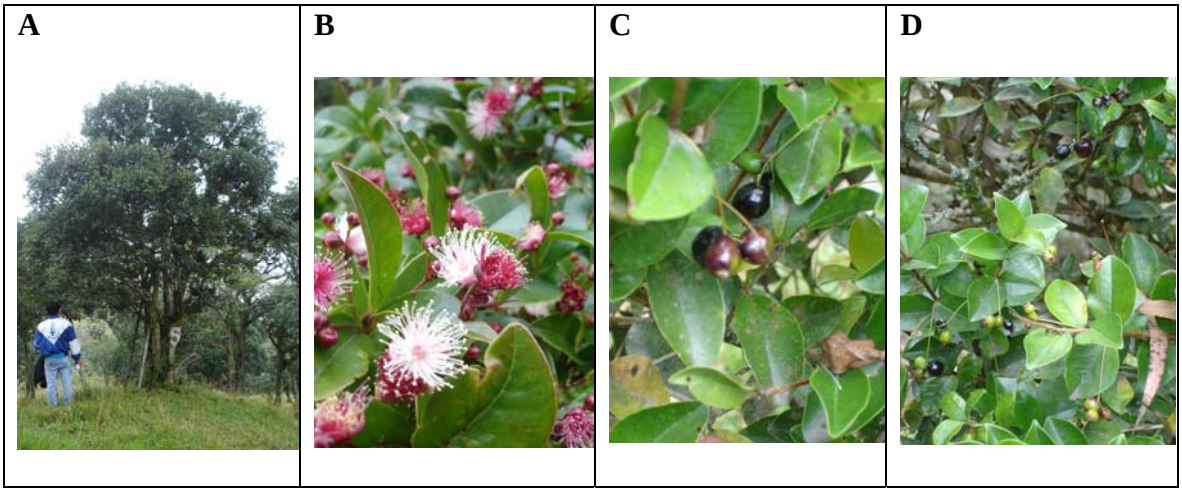

Figure 2. Cerro Villonaco area. **A.** Trees of *Myrciantes fragrans* (Sw.) McVaugh**. B.** *M. fragrans* collected at the flowering stage (Fl)**. C.** *M. fragrans* at the fruiting stage (Fr). **D.** *M. fragrans* at the foliation stage (Fo).

The raw plant material harvested from different trees at the same phenological stage, was then cleaned in our laboratory, mixed, and minced before distilling the essential oil.

2.2. Essential oil preparation

Plant material was hydrodistilled for four hours using a Clevenger-type apparatus. Subsequently, each essential oil sample was dried over anhydrous sodium sulfate and kept in sealed amber glass vials at 4°C, shielded from light until analysis.

Gas chromatography/flame ionization (GC/FID) analyses

GC-MS analyses were performed on an Agilent Technologies (6890N series) equipped with a flame ionization detector (FID). A nonpolar DB-5MS (5%-phenyl-methylpolyxilosane), 30 m x 0.25 mm, 0.25 μm film thickness, column was used. An automatic injector (series 7683) in split mode was used. Each sample [1 μL of a 1:100 (v/v) solution of essential oil/dichloromethane], was injected with a split ratio of 1:50. Helium was used as a carrier gas at 0.9 mL/min in constant flow mode. Oven temperature was held initially at 50 ºC for 3 min. then it was raised to 210 ºC with a gradient of 2.5 ºC/min, maintained eventually at 210 ºC for 3 min. Injector and detector temperatures were 210 ºC and 250 ºC; respectively. The compound retention indices were determined on the basis of a homologous series of standard *n*-alkanes C10-C25 (TPH-6RPM of CHEM SERVICE), which were injected after the oils under the same conditions.

2.3. Gas chromatography/Mass spectrometry (GC/MS) analyses

GC-MS analyses were performed using an Agilent chromatograph 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 ºC. The essential oil components were identified by comparing their MS data and their relative retention indices with the literature (Adams, 2009; NIST 05, 2005 and NIST, 2017). The relative amounts of individual

components were calculated on the basis of the GC peak areas (FID response) without using a correction factor.

2.4. Antimicrobial activity

2.4.1. Evaluation of the antibacterial activity

Antimicrobial activity was evaluated against five *Gram-negative* and two *Gram-positive* bacteria, as shown in Table 2. The bacterial strains were incubated at 37 ºC for 24 hours in Müeller-Hinton (MH) broth; subsequently, the cell suspension was adjusted with a sterile physiological solution to obtain an equivalent concentration of 0.5 in the McFarland scale (1.5 x 108 cells/mL). Another dilution was done in MH broth to adjust the concentration of inoculum to 2 x 106 CFU/mL. This solution was kept permanently at +4 ºC. Subsequently, a volume of 100 µL was dispensed into the wells, which contained an essential oil sample so to achieve a concentration of 5 x 105 CFU/mL.

Minimum inhibitory concentration (MIC) was determined by the microdilution method using 96-well microtitre plates (NCCLS, 2002; Rex et al., 2001). Each essential oil [100 µL of a 2:100 solution (v/v) in DMSO] was dissolved in 100 µL MH broth with bacterial inoculum; subsequently, a serial dilution was performed to achieve the required concentrations (1000 – 8 µg/mL). Gentamicin dissolved in DMSO (1 mg/mL) was used as the positive control for *Gram-positive* and *Gram-negative* bacteria. The microplates were incubated at 37 ºC for 24 h.

2.4.2. Evaluation of the antifungal activity

The antifungal activity of the essential oils against two fungal organisms was determined by the microdilution method as described for the antibacterial activity. A reserve solution (14 µL), diluted with Sabouroud broth (7 mL), was stored at 4 ºC until analysis.

MIC was determined using a final concentration of 5 x 104 cells/mL in 96-well microtitre plates. Essential oil was dissolved in Sabouroud broth with fungal inoculum to achieve the required concentrations 1000 – 0.5 µg/mL. Itraconazole was used as the positive antimycotic control. The microplates were incubated at 28 ºC for 72 h (17).

In the antibacterial and antifungal activity tests, a negative control with DMSO was also considered.

MIC was the lowest concentration of essential oil that prevented visible bacterial or fungal growth, respectively. All the experiments were performed in triplicated and the results are expressed as mean values.

3. Results and discussion

Chemical composition of the essential oils

The essential oil yields of *M. fragrans* were 0,28±0.04% at the foliation (Fo) growth stage of the plant, 0,38±0.02% at the flowering stage (Fl), and 0.36±0.02% at the fruiting stage (Fr). The relative average density of the *M. fragrans* essential oils was 0.8961±0.0051.

The chemical components of the essential oils, and their retention indices and percentages are listed in Table 1.

INSERT TABLE 1 HERE

37, 46, and 38 compounds, representing 96.5, 96.2, and 95,6%%, respectively, of the essential oils distilled from the plant material harvested at the foliation (Fo), flowering (Fl), and fruiting (Fr) growth stages, respectively, have been identified.

The essential oils of *M. fragrans* belong to the monoterpene type, since at all the three phenological stages (Fo, Fl and Fr), monoterpenoids largely predominated over

sesquiterpenoids. Oxygenated monoterpenes (OM) were the major components present in the essential oils, with percentages of 63,1% (Fo), 49,4% (Fl), and 61,9% (Fr), respectively, while the percentages of monoterpene hydrocarbons (HM) were 11% (Fo), 12,8% (Fl), and 18,8% (Fr), respectively. Monoterpenoids, due to their major volatility, can be released more easily by the plant than higher molecular weight terpenes and would thus function as pollination vectors or controllers of the water potential (Palá -Pul, 1997, 2000).

As regard the sesquiterpenoid fraction of the oils, oxygenated sesquiterpenes (OS) were present in greater abundance than sesquiterpene hydrocarbons (HS) at all the three development stages of *M. fragrans*, with percentages of 11.1 *vs* 6.0% (Fo), 18.7 *vs* 8.2% (Fl), and 7.2 vs 3,8% (Fr).

The three essential oils of *M. fragrans* were chemically characterized by the predominant presence of two well-known stereoisomeric monoterpene aldehydes, geranial (CAS C141274) (**1**) and neral (CAS C106263) (**2**), which together accounted for the 54.7, 41.4 and 54% of the essential oils at the plant foliation, flowering, and fruiting development stages, respectively. Geranial predominated over neral in all the three oils and the corresponding relative abundances were 31.1 and 23.6% in Fo, 23.6 and 17.8% in Fl, and 29.7 and 24.3% in the Fr oil. Citral is the name given to a mixture of the two geometric isomers, geranial (*trans*-citral, citral A) and neral (*cis*-citral, citral B), that occur in nature in many citrus fruit essential oils and in other herbs or spices (Lewinsohn et al., 1998). The Food and Drug Administration has approved the use of citral in foods (FDA, GRAS, 21 CFR 182·60) as generally safe for human and animal consumption (National Toxicology, 2003; Ress et al., 2003), though the International Fragrance Association recommends that citral only be used in association with substances that prevent a sensitizing effect. The citral

aroma is stronger and sweeter than that of lemon; moreover, geranial has a strong lemon (citrus) odor, while neral has a sweeter, yet less intense lemon odor. Citral has a great industrial importance (Dawson, 1994; Marques et al., 2013), and it is widely used in food and perfume industries as an aroma and flavor compound for its citrus effect; moreover, it is used in the synthesis of vitamin A, ionone, and methylionone. Thus, the *M. fragrans* essential oil is a new rich source of this important monoterpenoid substance.

Other important components of the essential oils at the three development stages, Fo, Fl, and Fr, were, respectively (relative abundances in brackets): α-pinene (2.8, 2.8, 5.9%), β-pinene (3.9, 5.7, 7.5%), myrcene (1.6, 1.5, 2%), α-terpinene (1.8, trace, 1.9%), citronellal (0.3, 1.5, 0.3%), terpinen-4-ol (2, 1.8, 2.2%), nerol (2.1, 1.4, 2.1%), geraniol (3.1, 2.5, 2.6%), methyl geranate (2.1, 0.9, 0.9%), neryl acetate (2.2, 2.1, 1.6%), geranyl acetate (0.5, 3.7, 0.5%), (*E*)-β-caryophyllene (1.3, 1.2, 0.8,%), (*E*)-β-farnesene (1.9, 1.3, 0.6%), (*E*)-nerolidol (1.2, 1.1, 0.3%), (2*Z*,6*E*)-farnesal (4.7, 6.7, 3%), (2*Z*, 6*E*)-farnesol (1.1, 2.1, 0.7%), and (2*E*,6*E*)-farnesal (3.8, 8, 3.2%).

In conclusion, the chemical compositions of the *M. fragrans* essential oils at the three phenological growth stages (Fo, Fl and Fr) were very similar qualitatively but not quantitatively. In fact, they showed the same pattern of mono and sesquiterpenoids; however, the corresponding relative percentages were significantly different. Terpenoids and more easily monoterpenes are released by evapotranspiration to avoid dehydration of the plant or to attract insects and other organisms such as pollinators or seed dispersers. This fact would explain the difference in the concentration of geranial and neral as well as other minor monoterpenes occurring in the essential oils at the three phenological stages.

In southern Ecuador people usually aromatize the traditional beverage colada morada using a symbiotic mixture of ‘arrayán’ (*M. fragrans*) and ‘hierba luisa’ or lemongrass (*Cymbopogon citratus*), which enhances the flavor of citral in the drink. Thus, citral is the main contributor to the characteristic aroma of the drink imparted by *M. fragrans*. Moreover, it is possible that, initially, indigenous people used plants from the native aromatic flora, such as *Mycianthe*s spp, and only more recently they started using other aromatic species such as ‘hierba Luisa’, that were introduced with the Spanish conquest.

It is worthy to note that the high content of geranial and neral in the essential oils distilled from *M. fragrans* collected at Cerro Villonaco (Ecuador) makes these oils chemically quite different from other essential oils distilled from *M. fragrans* collected in other countries, such the essential oils of the plant collected at Pinar del Río in Cuba (Pino et al., 2000), Monteverde in Costa Rica (Cole et al., 2008), Douglas Castle, St. Ann in Jamaica (Tucker et al., 1992), and at Táchira in Venezuela (Mora et al., 2009). In fact, the presence of geranial and neral has been reported in none of these essential oils. Traces of geranial and neral have, instead, been detected in the essential oil distilled from *M. fragrans* collected at San Luis de Santo Domingo, (Costa Rica) (Chaverri - Ciccio, 2017) that, therefore, may be considered the chemotype closest to the *M. fragrans* variety collected in Cerro Villonaco.

Antimicrobial composition.

The antimicrobial activity of *M. fragrans* essential oils were evaluated using the microdilution method. The values of the minimum inhibitory concentration (MIC, μg/ml) are shown in Table 2.

INSERT TABLE 2 HERE

Currently, there are no accepted standard criteria for defining the *in vitro* antimicrobial activity of natural products. According to Holetz et al. (2002), the antimicrobial activity of an extract is considered good if the MIC is <100 μg/ mL, moderate if the MIC is comprised between 100 and 500 μg/mL, low if the MIC is between 500 and 1000 μg/mL, null if the MIC is over 1000 μg/mL. Accordingly, the essential oil from *M. fragrans* showed moderate activity against the *Gram-negative* bacteria *Klebsiella pneumoniae* (ATCC 9997) with a MIC of 125 μg/mL at foliation (Fo) and at fruiting (Fr) growth stages, while at the flowering (Fl) the activity was low (MIC of 625μg/mL). Against the *Gram-negative* bacteria *Proteus vulgaris* (ATCC 8427), the, Fl oil showed moderate activity with a MIC of 625 μg/mL, while at foliation (Fo) and fruiting (Fr) stages the inhibitory activity was low. All the three oils were inactive against the *Gram-negative* bacteria *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922), as well as against the *Gram-positive* bacteria *S. aureus* (ATCC 25923).

On the other hand, *M. fragrans* essential oils exhibited higher activity against fungi than against bacteria. Of the three fungi studied; the yeasts *Candida. albicans* and *Saccharomyces.cerevisiae* were more sensitive to the essential oils than *A. niger*. The inhibitory activity against *Candida. albicans* was good for the Fr oil (MIC = 62.5 μg/mL), while was moderate for the Fo specimen (MIC = 250 μg/mL), and low (MIC = 625 μg/mL) for the oil distilled from the plant collected at the flowering stage. Against the yeast *Saccharomyces.cerevisiae* (ATCC 2601), the activity of the Fo essential oil was good (MIC

= 62,5 μg/mL), while those of Fl and Fr oils were only moderate (MIC = 321,5 and 250 μg/mL, respectively).

The different antimicrobial activities of the three *M. fragrans* essential oils (Fo, Fli and Fr) clearly depended on the different chemical composition of the oils, especially on the variation of monoterpenes and sesquiterpenes present and their percentages. The good activity against *C. albicans* is quite interesting because this dimorphic fungus commensally inhabits the human body and can cause opportunistic or pathogenic infections.

We believe that the interesting biological activities of *M. fragrans* essential oils were mainly due to the high contents of geranial and neral (citral) in synergy with other minor terpenoids present in the oils. Chemically, the inhibitory effects of these two monoterpenoids are due to the α, β-unsaturated aldehyde moiety that can act as a reactive alkylating agent able to readily bind cellular nucleophilic groups (Witz, 1989). Actually, the potential antifungal activity of citral and citral rich essential oils against molds and yeasts has been demonstrated by several studies (Yousef and Tawil, 1980; Wuryatmo et al., 2003; Belleti et al., 2004, 2007; Zhou et al., 2014; Tao et al., 2014; Caccioni et al., 1993, 1995, Tzortzakis and Economakis, 2007; Silva et al., 2008; Leite et al., 2014). Citral, selectively inhibits the mycelial growth of *C. albicans*, when applied in a concentration range of 25-250 ug/mL (Abe S et al., 2003). In general, the possible mechanism of action of anticandidal compounds from essential oils involves selective inhibition of filamentous growth, membrane leakage, respiratory chain inhibition, and uncoupling oxidative phosphorylation (Knobloch et al., 1988, McGeady et al., 2002; Abe S et al., 2003; Pauli, 2006).

Moreover, a recent work has showed that citral has potential to be developed as an alternative agent to mitigate the infections caused by *Cronobacter sakazakii* (a Gram-negative, opportunistic bacteria) that is a foodborne pathogen (Shi et al., 2017).

In addition, the ability of citral to induce cell death of breast cancer as well as leukemia cells has been described in several studies (Xia et al., 2013; Dudai et al., 2005; Liu et al., 2012), and a nanoparticle formulation of citral is effective in controlling growth of subcutaneously implanted 4T1 mouse breast tumors (Zheng et al., 2015).

In conclusion, the symbiotic use of two aromatic species, arrayán (*M. fragrans)* and 'hierba luisa' or lemongrass *(C. citratus*) in the traditional preparation of 'colada morada' in southern Ecuador, which results in an increase concentration of citral in this beverage, can have a rational on the results of the biological activity tests. In fact, besides being an aromatic additive mixed with the other essential oil components, citral plays antimicrobial effects against opportunistic yeasts such as *S. cerevisiae* and other wild yeasts, that are responsible for the fermentation of fruit-juices.

Analogously, the ethnobotanical use of an aqueous infusion of the leaves of arrayán (*M. fragrans*) in southern Ecuador to treat vaginal diseases (Jiménez, 2008), as well as for treating gum and oral diseases, can be explained by the high content of the anticandidal and antibacterial geranial and neral. An analogous explanation can be given to the traditional habit of several indigenous communities living on Ecuadorian Andean regions to chew arrayán (*Myrciantes* spp.) leaves, an old-established custom that dates back to the Inka period.

Conclusions

The importance given in Ecuador to the rescue of traditional knowledge and nutritional value of ancestral foods and drinks, has stimulate our investigation of the chemical composition and some biological activities of *M. fragrans* ('arrayán') essential oil. In fact, the leaves of this plant in conjunction with those of *C. citratus* ('hierba luisa' or lemongrass) are commonly used by peoples living in southern Ecuador as a natural aromatic additive in the preparation of the traditional fruit-juice 'colada morada', which still holds great importance in particular religious ceremonies. The oil is characterized by a high concentration of the monoterpene aldehydes geranial and neral (citral), that make the aroma of colada morada prepared in southern Ecuador quite different from the beverage made in other regions of the country, where other types of myrtles (Myrtaceae spp.) are used. Moreover, the oil may become a rich new source of the important industrial intermediate citral. In this regard, the highest content of citral was detected in the Fo essential oil. Therefore, to obtain the maximum yield in citral, *M. fragrans* should be harvested and distilled during this phenological stage, i.e., without flowers or fruits which it is better not to collect, so to avoid altering the stages of plant pollination (Fl stage) or seed dispersion (Fr stage). On the other hand, the plant distillation at the Fl and Fr stages does not afford significant changes in the composition of the essential oil.

Our study has also revealed that the arrayán oil have good *in vitro* activities against *Klebsiella pneumoniae*, *Candida albicans* and *Saccharomices. cerevisiae,* suggesting a possible scientific explanation for traditional uses of the plant as a natural anti-infective and anti-yeast agent.

The long history of *M. fragrans* in folk medicine and traditional foods, suggesting the relative safety of the plant, and the presence of many known antimicrobial compounds in

the essential oil, indicate that the plant or its essential oil can be used as a potential new antiseptic or antimycotic agent, and for food preservation (Lanciotti et al., 2004; Tyagi et al., 2014). Moreover, new anti-infective or antimycotic biomaterials could be designed through the incorporation of *M. fragrans* essential oil.

In conclusion, given the possible useful applications, in particular in food, cosmetic and wood industries, the potential of *M. fragrans* being used for future agro-industrial ventures in the region is high and an adequate sustainable management can guarantee a possible source of income. The inclusion of *M. fragrans* as a native species in the reforestation programs developed by different governmental agencies of Ecuador, would be an interesting strategy to achieve the sustainable use of this plant. In this context, the Reynaldo Espinosa Botanical Garden of the National University of Loja (https://es.wikipedia.org/wiki/Jard%C3%ADn_botánico_Reinaldo_Espinosa) is carrying out an interesting rescue project of *M. fragrans*, which has resulted in the successful *in situ* growth of several trees.

Acknowledgments

This study was carried out with a financial support from the 'Master in Science and Technology for sustainable uses of non-traditional biological resources' held at the University of Pavia (Italy) and the CISTRE collaboration. The project was part of the Master thesis of C. A. We deeply thank the Universidad Técnica Particular de Loja for the financial support of this study, and we are also grateful to the SENESCYT (Secreataría Nacional de Ciencia y Tecnología) of Ecuador.

References

1. León-Yánez S., Valencia R., Pitman N., Endara L., Ulloa Ulloa C., Navarrete H., editors. 2011. Libro Rojo Delas Plantas Endémicas del Ecuador. 2nd ed. Publicaciones del Herbario QCA, Pontificia Universidad Católica del Ecuador; Quito, Ecuador. pp. 432.

2. McVaugh R. 1963. Flora of Guatemala. Myrtaceae. Fieldiana Bot 24: 377 - 379.

3. Tucker, A. O., Maciarello, M. J., & Landrum, L. R. 1992. Volatile Leaf Oils of Caribbean Myrtaceae *Myrcianthes fragrans* (Sw.) Mc Vaugh of Jamaica. Journal of Essential Oil Research, 4(3), 313–314.

4. Tucker AO, Maciarello MJ, Landrum LR. 2002. Volatile leaf oil of *Myrcianthes coquimbensis* (Barnéoud) Landrum et Grifo (Myrtaceae) of Chile. J Essent Oil Res 14: 40 - 41

5. Chaverri, C., & Cicció, J. F. (2017). Essential oil from leaves of *Myrcianthes fragrans* (Myrtaceae) from Costa Rica. A new chemotype? . Boletin Latinoamericano y Del Caribe de Plantas Medicinales y Aromaticas, 16(4), 385–397.

6. Jørgesen P. and León-Yánez S. 1999. Catalogue of the Vascular Plants of Ecuador. Missouri Botanical Garden Press, St. Louis, Mo, USA.

7. Jiménez I. 2008. Distribución y fenología del arrayán (*Myrcianthes fragrans* (Sw.) McVaugh) en la Provincia de Loja. Tesis de grado de Ingeniería en Gestión Ambiental. Universidad Técnica Particular de Loja. Loja, Ecuador, 90 pp.

8. Van den Eynden,V., E. Cueva, and O. Cabrera. 1999. Plantas silvestres comestibles del sur del Ecuador —wild edible plants of southern Ecuador. Ediciones Abya-Yala, Quito.

9. Van den Eynden, V., Cueva, E. and Cabrero, O. 2003. Wild foods from southern Ecuador. Economic Botany 57(4): 576-603

10. De la Torre L., H. Navarrete, M.P. Muriel, M. Macia, H. Balslev. 2008. Enciclopedia de las plantas útiles del Ecuador. Herbario QCA de la Escuela de Ciencias Biológicas de la Pontificia Universidad Católica del Ecuador, Herbario AAU del Departamento de Ciencias Biológicas de la Universidad de Aarhus, Quito & Aarhus.

11. Gallardo de la Puente, C. 2014. Colada Morada y Guaguas de Pan. La esencia de celebrar nuestras memorias. Quito Universidad de las Américas UDLA. Quito, Ecuador. 173 pp.

12. Ulloa, C. 2006. Aromas y sabores. En: M. Moraes R., B. Øllgaard, L.P. Kvist, F. Borchsenius y H. Balslev (Eds.), Botánica económica de los Andes Centrales. Universidad Mayor de San Andrés. La Paz, Bolivia. Pp.: 313-328.

13. Naranjo, P., Kijjoa, A., Giesbrecht, A. M., & Gottlieb, O. R. 1981. Ocotea quixos, American cinnamon. Journal of Ethnopharmacology, 4, 233–236.

14. Bruni, R., Medici, A., Andreotti, E., Fantin, C., Muzzoli, M., Dehesa, M., et al. (2003). Chemical composition and biological activities of Ishpingo essential oil, a traditional Ecuadorian spice from *Ocotea quixos* (Lam.) Kosterm. (Lauraceae) flower calices. Food Chemistry, 85(3), 415–421.

15. Chavez Carvajal, P., Coppo, E., Di Lorenzo, A., Gozzini, D., Bracco, F., Zanoni, G., Daglia, M. (2016). Chemical Characterization and in Vitro Antibacterial Activity of *Myrcianthes hallii* (O. Berg) McVaugh (Myrtaceae), a Traditional Plant Growing in Ecuador. Materials, 9(6), 454. http://doi.org/10.3390/ma9060454

16. Pino, J. A., Rosado, A., Bello, A., Urquiola, A., & Garcia, G. (2000). Essential oil of *Myrcianthes fragrans* (Sw.) McVaug from Cuba. Journal of Essential Oil Research, 12(2), 225–226. https://doi.org/10.1080/10412905.2000.9699503

17. Cole, R. A., Haber, W. A., Lawton, R. O., & Setzer, W. N. (2008). Leaf essential oil composition of three species of *Myrcianthes* from Monteverde, Costa Rica. Chem Biodivers, 5(7), 1327–1334. https://doi.org/10.1002/cbdv.200890120

18. Mora, Flor D., Rojas, Luis B.; Usubillaga, Alfredo.; Carmona, Juan; Silva, Bladimiro (2009). Composición química del aceite esencial de *Myrcianthes fragrans* (Sw.) Mc Vaught de los Andes venezolanos. [Revista de la Facultad de Farmacia de la Universidad de Los Andes, Venezuela, Vól. 51 Núm. 1 Ene-Jun, Pág. 20-23

19. Tropicos.org. Missouri Botanical Garden. Accessed 12 Feb 2016, (http://www.tropicos.org).

20. Adams, R. P. (2009). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry (4th Ed.). Allured Business Media. Carol Stream, IL, USA

21. NIST 05, (2005). Mass Spectral Library (NIST/EPA/NIH). National Institute of Standards and Technology. Gaithersburg, MD.

22. NIST, National Institute of Standards and Technology, Libro del Web de Química del NIST, SRD 69, (https://webbook.nist.gov/chemistry/), Accessed on August 2017.

23. NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; approved standard, 2nd edn. NCCLS document M27-A2, NCCLS, Wayne, PA (2002).

24. Rex J. H., M.A. Pfaller, T.J. Walsh, V. Chaturvedi, A. Espinel-Ingroff, M.A. Ghannoum, L.L. Gosey, F.C. Odds, M.G. Rinaldi, D.J. Sheehan and D.W. Warnock, (2001). Anti- fungal susceptibility testing: Practical aspects and current challenges. Clin. Microbiol. Rev., 14, 643–658

25. Palá-Paúl, J. 1997. Variación fenológica del aceite esencial de *Santolina rosmarinifolia* L. ssp. *rosmarinifolia*. Tesis de Licenciatura (Inéd.). Facultad de Biología. Universidad Complutense de Madrid. España.

26. Palá-Paúl, J. 2000. Contribución al conocimiento de los aceites esenciales del género "*Eryngium*" L., en la península ibérica. Tesis de Doctorado (Inéd.). Facultad de Ciencias ciológicas. Departamento de Biología Vegetal I. Universidad Complutense de Madrid. España.

27. Lewinsohn E., Dudai N., Tadmor et al., 1998. "Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae)," Annals of Botany, vol. 81, no. 1, pp. 35–39

28. National Toxicology, P. NTP toxicology and carcinogenesiss studies of citral (microencapsulated) (CAS No. 5392-40-5) in F344/N rats and B6C3F1 mice (feed studies). National Toxicology Program technical report series, 1–268 (2003).

29. Ress, N. B. et al. Toxicology and carcinogenesis studies of microencapsulated citral in rats and mice. Toxicological sciences : an official journal of the Society of Toxicology 71, 198–206 (2003).

30. Dawson F. A., 1994. "The amazing terpenes," *Naval Stores Review*, vol. 104, pp. 6–12, 1994.

31. Marques A. M., Lima C. H. P., Alviano D. S., Alviano C. S., Esteves R. L., and Kaplan M. A. C. 2013. "Traditional use, chemical composition and antimicrobial activity of *Pectis brevipedunculata* essential oil: a correlated lemongrass species in Brazil," Emirates Journal of Food and Agriculture, vol. 25, pp. 798–808

32. Holetz F.B., G.L. Pessini, N.R. Sanches, D.A. Cortez Garcia, C.V. Nakamura and B.P. Dias Filho. 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem. Inst. Oswaldo Cruz, 97, 1027–1031

33. Cosentino, S.; Tuberoso, C. I. G.; Pisano, B.; Satta, M.; Mascia, V.; Arzedi, E.; Palmas, E. 1999. In-vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett. Appl. Microbiol. 1999, 29, 130-135.

34. Witz, G. 1989. Biological interactions of α,β-unsaturated aldehydes. Free Radical Biol. Med. 1989, 7, 333-349.

35. Shi, C., Sun, Y., Liu, Z., Guo, D., Sun, H., Sun, Z., … Xia, X. (2017). Inhibition of Cronobacter sakazakii Virulence Factors by Citral. Scientific Reports, 7. https://doi.org/10.1038/srep43243

36. Xia, H. et al. The *in vitro* study of apoptosis in NB4 cell induced by citral. Cytotechnology 65, 49–57, 10.1007/s10616-012-9453-2 (2013).

37. Dudai, N., Weinstein, Y., Krup, M., Rabinski, T. & Ofir, R. Citral is a new inducer of caspase-3 in tumor cell lines. *Planta Med* **71**, 484–488, 10.1055/s-2005-864146 (2005).

38. Liu, Y. et al. Terpenoids from *Zingiber officinale* (Ginger) induce apoptosis in endometrial cancer cells through the activation of p53. PloS one 7, e53178, 10.1371/journal.pone.0053178 (2012).

39. Zheng, S., Kapur, A., Patankar, M. S. & Xiong, M. Formulation, Characterization, and Antitumor Properties of *Trans*- and *Cis*-Citral in the 4T1 Breast Cancer Xenograft Mouse Model. Pharmaceutical research 32, 2548–2558, 10.1007/s11095-015-1643-0 (2015).

40. Wuryatmo E., Klieber A., and Scott E. S., 2003. "Inhibition of citrus postharvest pathogens by vapor of citral and related compounds in culture," Journal of Agricultural and Food Chemistry, vol. 51, no. 9, pp. 2637–2640

41. Belletti N., Ndagijimana M., Sisto C., Guerzoni M. E., Lanciotti R., and Gardini F.. 2004. "Evaluation of the antimicrobial activity of citrus essences on *Saccharomyces cerevisiae*," Journal of Agricultural and Food Chemistry, vol. 52, pp. 6932–6938

42. Belletti N., Kamdem S.S., Patrignani F., Lanciotti R., Covelli A., and Gardini F. 2007. "Antimicrobial activity of aroma compounds against *Saccharomyces cerevisiae* and improvement of microbiological stability of so drinks as assessed by logistic regression," Applied and Environmental Microbiology, vol. 73, no. 17, pp. 5580–5586

43. Zhou H., Tao N., and Jia L. 2014. "Antifungal activity of citral, octanal and α-terpineol against *Geotrichum citriaurantii*," Food Control, vol. 37, pp. 277–283

44. Tao N., OuYang Q.,and Jia L. 2014. "Citral inhibits mycelial growth of *Penicillium italicum* by a membrane damage mechanism," Food Control, vol. 41, pp. 116–121

45. Caccioni, D. R. L.; Deans, S. G. 1993. Action of citrus fruits essential oils on germination of *Penicillium digitatum* and *Penicillium italicum*. In International Symposium on Industrial Crops and Products, 22-24 November; Pisa, Italy.

46. Caccioni, D. R. L.; Deans, S. G.; Ruberto, G. Inhibitory effect of citrus fruits essential oil components on *Penicillium italicum* and *Penicillium digitatum*. Petria 1995, 5, 177-182.

47. Tzortzakis, N. G.; Economakis, C. D. (2007). Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. Innovative Food Science and Emerging Technology, 8, 253e258.

48. Silva, Cristiane de Bona da, Guterres, Sílvia S., Weisheimer, Vanessa, & Schapoval, Elfrides E.S. 2008. Antifungal activity of the lemongrass oil and citral against *Candida* spp.. Brazilian Journal of Infectious Diseases, 12(1), 63-66. https://dx.doi.org/10.1590/S1413-86702008000100014

49. Leite M. C. A., Bezerra A. P. B., Sousa J. P., Guerra F. Q., and Lima E. d. O. 2014. "Evaluation of antifungal activity and mechanism of action of citral against *Candida*

albicans," *Evidence-Based Complementary and Alternative Medicine*, vol. 2014, Article ID 378280, 9 pages, 2014.

50. Abe, S. S. Y., Inoue, S., Ishibashi, H., Maruyama, N., Takizawa, T., Oshima, H., et al. 2003. Anti-*Candida albicans* activity of essential oils including lemongrass (*Cymbopogon citratus*) oil and ts component, citral. Nihon Ishinkin Gakkai zasshi=Japanese Journal of Medical Mycology, 44, 285–291.
51. Knobloch K, Pauli A, Iberl B, Weis N, Weigand H. 1988. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests. In: Schreier P, editor. Bioflavor'87. Berlin-New York: W. de Gruyter; pp 287–299.
52. McGeady P, Wansley DL, Logan DA. Carvone and perillaldehyde interfere with the serum-induced formation of filamentous structures in *Candida albicans* at substantially lower concentrations than those causing significant inhibition of growth. J Nat Prod 2002;65:953–995.
53. Pauli A. 2006. "Anticandidal low molecular compounds from higher plants with special reference to compounds from essential oils," Medicinal Research Reviews, vol. 26, no. 2, pp. 223–268
54. Lanciotti, R.; Gianotti, A.; Patrignani, F.; Belletti, N.; Guerzoni, M. E.; Gardini, F. 2004. Use of natural aroma compounds to improve shelf life and safety of minimally processed fruits. Trends Food Sci. Technol. 2004, 15, 201-208.
55. Tyagi A. K., Gottardi D., Malik A., Guerzoni M. E. (2014). "Chemical composition, in vitro anti-yeast activity and fruit juice preservation potential of lemon grass oil". LWT Food Science and Technology, 57 (2), 731- 737.
56. Yousef, R.T.; Tawil, G.G. 1980. Antimicrobial activity of volatile oils. Die Pharmazie, 35, 698 701.
57. Reynaldo Espinosa Botanical Garden of the National University of Loja. (https://es.wikipedia.org/wiki/Jard%C3%ADn_botánico_Reinaldo_Espinosa)

Table 1. Chemical constituents of *Myrcianthes fragrans* essential oils at the foliation (Fo), flowering (Fl) and fruiting (Fr) growth stages.

Peak #	Compound[a]	RI	RI[ref]	*Fo*	*Fl*	*Fr*	Type	Methods of identification[b]

1	α-Thujene	930	924	-	tr	tr	MH	MS,RI
2	α-Pinene	932	932	2,8	4,1	5,9	MH	MS,RI
3	Sabinene	979	969	-	-	tr	MH	MS,RI
4	β-Pinene	980	974	3,9	5,7	7,5	MH	MS,RI
5	6-Metil-5-hepten-2-one	984	981	0,2	0,1	0,5	OT	MS,RI
6	Myrcene	996	988	1,6	1,5	2	MH	MS,RI
7	Limonene	1027	1024	0,3	0,5	0,6	MH	MS,RI
8	(*Z*)-β-Ocimene	1037	1032	0,4	0,4	0,5	MH	MS,RI
9	(*E*)-β-Ocimene	1047	1044	0,2	0,5	0,3	MH	MS,RI
10	α-Terpinene	1053	1054	1,8	tr	1,9	MH	MS,RI
11	Terpinolene	1089	1086	-	0,1	0,1	MH	MS,RI
12	Linalool	1100	1095	0,5	0,5	0,6	OM	MS,RI
13	(*E*)-p-Mentha-2,8-dien-1-ol	1119	1119	0,1	0,2	-	OM	MS,RI
14	Citronellal	1153	1148	0,3	1,5	0,3	OM	MS,RI
15	Terpinen-4-ol	1176	1174	2	1,8	2,2	OM	MS,RI
16	Nerol	1229	1227	2,1	1,4	2,1	OM	MS,RI
17	Neral	1244	1235	23,6	17,8	24,3	OM	MS,RI
18	Geraniol	1256	1249	3,1	2,5	2,6	OM	MS,RI
19	Geranial	1276	1264	31,1	23,6	29,7	OM	MS,RI
20	Methyl geranate	1324	1322	2,1	0,9	0,9	OT	MS,RI
21	α-Cubebene	1349	1345	0,3	0,3	0,2	SH	MS,RI
22	Eugenol	1357	1356	0,3	0,1	0,1	OM	MS,RI
23	Neryl acetate	1366	1359	2,2	2,1	1,6	OT	MS,RI
24	α-Copaene	1375	1374	0,2	0,2	0,3	SH	MS,RI
25	Geranyl acetate	1385	1379	0,5	3,7	0,5	OT	MS,RI
26	β-Elemene	1391	1389	0,2	0,2	-	SH	MS,RI
27	Methyl eugenol	1405	1403	0,3	0,3	0,4	OT	MS,RI
28	(*E*)-β-Caryophyllene	1418	1417	1,3	1,2	0,8	SH	MS,RI
29	γ-Elemene	1433	1434	-	0,1	-	SH	MS,RI
30	α-Humulene	1452	1452	0,6	0,7	0,6	SH	MS,RI
31	(*E*)-β–Farnesene	1458	1454	1,9	1,3	0,6	SH	MS,RI
32	Germacrene D	1480	1484	0,4	1	0,7	SH	MS,RI
33	β-Selinene	1484	1489	-	0,3	-	SH	MS,RI
34	α-Selinene	1493	1498	0,2	0,1	-	SH	MS,RI
35	Bicyclogermacrene	1495	1500	-	0,4	0,1	SH	MS,RI
36	α-Muurolene	1499	1500	-	0,6	-	SH	MS,RI
37	δ–Cadinene	1523	1522	0,4	0,4	0,2	SH	MS,RI
38	Germacrene B	1555	1559	-	0,9	-	SH	MS,RI
39	(E)-Nerolidol	1564	1561	1,2	1,1	0,3	OS	MS,RI
40	Spathulenol	1576	1577	0,1	tr	tr	OS	MS,RI
41	Caryophyllene	1581	1582	0,5	0,5	0,3	SH	MS,RI
42	α-Muurolol	1641	1644	-	0,3	-	OS	MS,RI
43	α-Cadinol	1653	1652	0,2	0,3	tr	OS	MS,RI

44	(2*Z*, 6*E*)-Farnesal	1716	1715	4,7	6,7	3	OS	MS,RI
45	(2*Z*, 6*E*)-Farnesol	1722	1722	1,1	2,1	0,7	OS	MS,RI
46	(2*E*, 6*E*)-Farnesal	1744	1740	3,8	8	3,2	OS	MS,RI
47	(2*E*, 6*E*)-Farnesyl acetate	1842	1845	-	0,2	-	OS	MS,RI
Monoterpene hydrocarbons (MH)				11,0	12,8	18,8		
Oxygenated monoterpenes (OM)				63,1	49,4	61,9		
Sesquiterpene hydrocarbons (SH)				6,0	8,2	3,8		
Oxygenated sesquiterpenes (OS)				11,1	18,7	7,2		
Other compounds (OT)				5,3	7,1	3,9		
Total identified compounds				96,5	96,2	95,6		

tr, trace (< 0.05%); -, not detected.
[a]Compounds are ordered according to the elution order in the column DB5-MS.
[b]Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley 7, Adams (2009) and NIST 05 (2005); RI, by comparison of RI with the values reported in the literature [Adams (2009), NIST 05 (2005) and NIST (2017)].
RI, retention indices on the apolar column (DB5-MS).

Table 2. Antibacterial and antifungal activities of *Myrcianthes fragrans* essential oils at the foliation (Fo), flowering (Fl) and fruiting (Fr) growth stages, expressed as minimal inhibitory concentration (MIC, μg/mL).

Microorganism	**MIC (μg/mL)[a]**			
	Fo	**Fl**	**Fr**	**Positive control[b]**
Gram-negative bacteria				
*Pseudomona aeruginosa (*ATCC 27853)	>2500	1250	>2500	10
Klebsiella pneumoniae (ATCC 9997)	125	625	125	10
*Proteus vulgaris (*ATCC 8427)	>2500	625	>2500	10
*Escherichia coli (*ATCC 25922)	>2500	>2500	>2500	10
Gram-positive bacteria				
*Staphylococcus aureus (*ATCC25923)	>2500	1250	>2500	10
Fungi				
Aspergillus niger (9642-U)	>2500	321.5	>2500	10
Candida albicans (24433)	250	625	62.5	10
Saccharomices cerevisiae (2601)	62.5	321.5	250	10

Note. [a] Mean of nine determinations. [b] Gentamicin as the positive control against *P. aeruginosa, K. pneumonia, P. vulgaris, E. coli, S. aureus*, and ketoconazole against fungi.