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Composition, Chemical Fingerprinting and Antimicrobial Assessment of Costa Rican Cultivated Guavas (*Psidium friedrichsthalianum* (O. Berg) Nied. and *Psidium guajava L.*) Essential Oils from Leaves and Fruits

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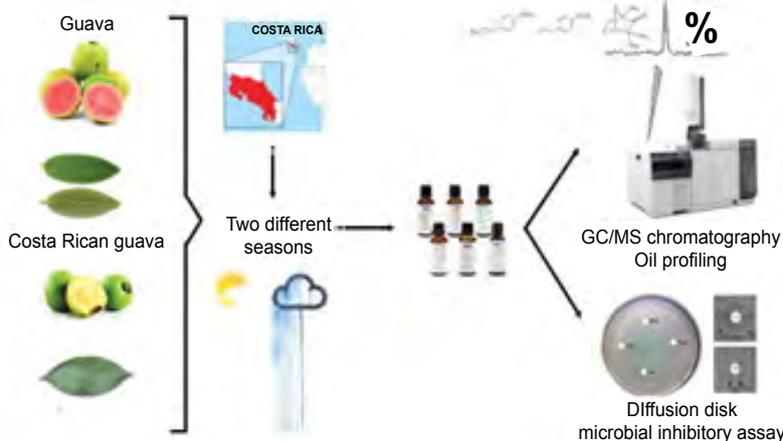
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Abstract

The essential oil of two related tree species, *P. friedrichsthalianum* and *P. guajava,* where obtained. A total of six different oil samples were recovered including leaves in dry/rainy season and fruits of both plant species. Oil yields ranged between 0.128% (*P. friedrichsthalianum* leaves during dry season)-0.743% (*P. guajava* leaves during rainy season). All extracts were subjected to a GC/MS analysis using, during the chromatographic separation, a polyethylene glycol column. In general terms, we recognized three independent biosynthetic routes i. aromatic compounds ii. Terpenes and iii. Fatty acids derivatives. Several compounds were found to be preserved in the oils, such as 2,4-di-tert-butylphenol, α-terpineol and neointermedeol whereas Costa Rican guava fruit exhibit unique compounds such as 2H-pyran-2,6-(3H)-dione. Terpenes and fatty acids are among the most variable (*p*<0.005) in content when comparing dry season with rainy season leaves. Finally, based on profiling, a descriptive PCA analysis showed three related groups and that Costa Rican guava fruit oil as the most different in terms of composition. Herein we report more than 50 compounds for each species and relative percentages of major components (>0.1%) and trace compounds. In addition, we evaluated the antimicrobial activity of these essential oils against common foodborne and food-spoilage related bacteria. The rainy season *P. guajava* leafs' presented the highest antimicrobial activity against all the bacteria strains tested, with inhibition zones ranging from 31 to 52 mm. This study will help understand volatile composition of a fruit producing plant native from this geographic area and hints toward possible applications.

Keywords: *Psidium friedrichsthalianum*; *Psidium guajava*; Essential oil; Volatile compounds; GC/MS

Introduction

The Myrtaceae is a family of dicotyledonous plants which is comprised of at least 5650 species (ca. 130-150 genera) [1,2]. One group of trees and shrubs contained in this family, *Psidium*, are native to warmer parts of the Western Hemisphere [3]. Specifically, two economically and nutritionally relevant species in Costa Rica are *P. friedrichsthalianum* (found in Southern Mexico and Central America) commonly known as Costa Rican guava [4] and *P. guajava* (found in Central and South America, West Indies, Mexico, Florida, Louisiana, Arizona) [5].

Essential oils are usually by-products of fruits or fruit tree processing [6]; their importance reflects their industrial or bioactive properties [7]. Furthermore, as Costa Rica's tropical fruit production and exportation (estimated at 1600 million USD in 2014) has increased in the last several years [8], so has fruit processing to juice and pulp.

Extraction of essential oils from such species is not only feasible, but also represents a viable alternative to increase value from the fruit production industry [9]. In 2011 alone, the essential oil global industry was estimated to be ca. 24 billion USD [9].

Because the quality and composition of essential oils depends on different factors such as plant chemo type and biotype as well as the climatic conditions [10,11], a study of the influence of different periods of ripening on the chemical fingerprinting of guava and Costa Rican guava essential oil from leaves is, therefore, considered useful. A similar approach has been used to characterize essential oil of other plants. Despite the relevance of such *Psidium* species, the volatile compounds of both the leaves and fruits of *P. friedrichsthalianum* have only partially been described [12], whereas *P. guajava* volatiles have been described in regions where the tree is not native [13]. The complete chemical composition of essential oils from other *Psidium* species has been described elsewhere [14,15].

It is well known that many volatile compounds found in oil bearing plants are implicated in plant defense and cytotoxic activity against pathogens and/or fungi [16,17]. The chemical species related to the volatile components that may be responsible for this activity are seldom addressed.

To our knowledge there is no information regarding the composition of these trees grown in their native Central America region and no literature sources have compared both *Psidium* species leaves and fruits including leaf's oil composition changes with respect to any edaphoclimatic conditions. Herein, we describe the essential oils components of leaf (in dry and rainy seasons) and fruit from *P. friedrichsthalianum* and *P. guajava* cultivated in Costa Rica and explore their antimicrobial potential against common foodborne pathogens.

Materials and Methods

Plant material and extraction

P. friedrichsthalianum and *P. guajava* leaves were collected in two different weather conditions (dry and rainy) during the months of April (average of 11.3 mm precipitation and 4 days of rain) and July (average of 223.0 mm precipitation and 23 days of rain) respectively, from local areas of San José, Costa Rica. Only undamaged leaves were collected. Mature fruits were collected directly from the tree when in season. Specimens were identified and selected based on structural characteristics of their leaves and trunks by a biologist with botanical and taxonomical expertise and based on the guidelines previously described by Sharma et al. [18]. All samples were collected from adult trees and randomly from the tops. Each collection was formed by sampling three different specimens. The essential oil was extracted by the process of steam distillation using an all glass still and purified water. Briefly, crushed fruits and aerial parts of plant material (ca. 150 g in each case) were placed in a Clevenger type apparatus with 1000 mL flask, oil separator tube and condenser, 250 mL of purified water was added and the mixture was vapor distilled (at 96°C at a rate of 20°C/minute and then kept at 96°C for 180 minutes) into a 125 mL Erlenmeyer, which was used to collect the aqueous distillate. The receiving receptacle was kept cold (0°C, using acetone-ice mixture) during the extent of distillation. Finally, liquid-liquid extraction was performed, with diethyl ether as the organic solvent, in order to recover volatiles. The organic fraction was dried in a rotatory evaporator until an oily substance (invariably, mixtures of organic volatile compounds) was obtained. Only ripened, healthy (without visible scarring) and non-infested (by common pests such as members of the Tephritidae family) [19], fruits were processed. In the samples of fruits, paraffin was added to avoid foaming of the non-volatile material in the flask during processing. Type III water with a final conductivity of <10 µScm⁻¹ was obtained using a RiOS™ system (EMD Millipore, Billerica, MA, USA). Oil yields ranged between 0.13% (*P. friedrichsthalianum* leaves during dry season)-0.74% (*P. guajava* leaves during rainy season).

GC/MS analysis

Qualitative analyses of the volatile compounds were carried out by means of an Agilent gas chromatography (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Technologies J&W DB-WAX micro bore column of 10 m length, 0.1 mm diameter, 0.1 µm film thickness and Agilent 5977E mass spectrometer (MSD). The carrier gas was helium at a constant flow of 0.3 mL/min. The GC oven temperature was kept at 50°C for 0.34 minutes and programmed to 200°C at a rate of 72.51°C/minute, this temperature was kept constant 0.17 minutes and then programmed to 230°C at a rate of 8.7°C/minute, held for 7.9 minutes for a total run time of 13.93 min. The split ratio was adjusted at 30:1. The injector temperature was set at 250°C. The mass range was 50-450 m/z. Electron energy was set at 70 eV, 150°C. Constituents were identified by matching their spectra with those in NIST library 14. Only hits with a match factor above 80% were considered. In all cases geraniol (98%, 163333, Sigma-Aldrich, St Louis, Mo) was used as an internal standard. Additionally, *trans*-cinnamic acid$_{(7.28 \text{ min; M+ 149.0 m/z})}$, benzeneacetic acid$_{(5.64 \text{ min; M+ 135.2 m/z})}$, benzoic acid$_{(5.07 \text{ min; M+ 121.3 m/z})}$, 2,4-ditertbutylphenol$_{(4.69 \text{ min; M+ 207.3 m/z})}$, globulol$_{(4.07 \text{ min; M+ 223.4 m/z})}$, caryophyllene oxide$_{(3.81 \text{ min; M+ 220.1 m/z})}$, benzyl alcohol$_{(3.46 \text{ min; M+ 109.1 m/z})}$, α-terpineol$_{(2.92 \text{ min; M+ 155.3 m/z})}$, caryophyllene$_{(2.65 \text{ min; M+ 205.4 m/z})}$, linalool$_{(2.46 \text{ min; M+ 136.3 m/z})}$, benzylaldehyde$_{(2.37 \text{ min; M+ 107.1 m/z})}$, nerolidol$_{(2.32 \text{ min; M+ 222.2 m/z})}$, *p*-cymene$_{(1.67 \text{ min; M+ 135.2 m/z})}$, γ-terpinene$_{(1.52 \text{ min; M+ 137.2 m/z})}$, limonene$_{(1.43 \text{ min; M+ 137.2 m/z})}$, *n*-butanol$_{(1.40 \text{ min; M+ 75.1 m/z})}$, and thujone$_{(1.05 \text{ min; M+ 153.1 m/z})}$ standards (Sigma-Aldrich, St Louis, Mo) were injected separately for confirmation purposes. Tetradecanoic$_{(6.16 \text{min; M+ 227.6 m/z})}$, pentadecanoic$_{(6.72 \text{ min; M+ 243.4 m/z})}$, hexadecanoic$_{(7.58 \text{ min; M+ 256.3 m/z})}$, octadecanoic$_{(9.70 \text{ min; M+ 285.5 m/z})}$, *cis*-13-octadecanoic$_{(10.21 \text{ min; M+ 285.7 m/z})}$ and 9Z-octadecenoic$_{(7.78 \text{ min; M+ 284.1 m/z})}$, (Z,Z)-9,12-octadecadienoic$_{(10.86 \text{ min; M+ 280.0 m/z})}$ acids were obtained from (Nu-Chek Prep, Inc., Elysian, MN, USA). Analytes with ≥ 5% relative composition, and when available commercially, were simultaneously monitored by SIM mode (total dwell time 100 ms and cycles 8.3 Hz) using the ions and retention times specified above. For compounds with no analytical standard injection, identification should be considered as tentative.

In vitro antimicrobial activity

Each of the six essential oils were mixed 80:20 with dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, Mo) and homogenized. Aliquots of 10 µL of these extracts were pipetted onto sterile discs of 7 mm of diameter prepared with 934-AH Whatman glass micro fiber filters (Whatman International Ltd, Maidstone, UK), the discs were placed onto 4 mm height Mueller–Hinton agar plates containing 10⁴-10⁶ CFU mL⁻¹ of the following strains: *S. Choleraesuis* ATCC 10708, *S. typhimurium* ATCC 14028, *S. enteritidis* ATCC 13076, *E. coli* O157:H7 ATCC 43888, *S. aureus* subsp. Aureus ATCC 25923, *B. cereus* ATCC 13061, *B. subtillis* ATCC 11774, *P. aeruginosa* ATCC 27853, *P. mirabilis* ATCC 25933 and *E. coli* ATCC 25922. These experiments were performed by triplicate. In addition, discs impregnated with water were tested in parallel to confirm that the filter paper used in their manufacturing was not toxic to *E. coli* ATCC 25922. An aqueous 10 µg mL⁻¹ solutions of oxytetracycline and DMSO were used as positive and negative controls, respectively. Additionally, some essential oil standards were tested in parallel for comparison (i.e., limonene, myrcene, terpinene, eucalyptol, linalool, thujone, caryophyllene and cymene;purchased from Sigma-Aldrich, St Louis, Mo).

Statistical analysis

ANOVA analysis with post-hoc Tukey test was used to explore statistical differences in relative concentrations among major components in the six different essential oils. Likewise, a categorization

of components based on their structural similarities was as follows: aromatic compounds, terpenes, fatty acids (and derivatives) and linear aliphatic hydrocarbons. The same test was used to analyze differences between relative concentrations obtained for the aforementioned categories. Principal component analysis was performed to the chemical fingerprint of the six oils in order to assess correlation, if any, among compositions. Components considered relevant if values were above |0.4| in the rotated matrix. All assays carried out using IBM® SPSS® Statistics version 22 (SPSS, Inc., Armonk, NY, USA).

Results and Discussion

The chemical profile obtained for the essential oils resulted to be a complex mixture as evidenced by heavily signal-charged total ion chromatograms (TIC). The chromatogram of *P. guajava* fruit essential oil serves as an example and is presented in Table 1 and Figures 1 and 2.

In the case of *P. friedrichsthalianum* leaf oil, caryophyllene (Table 1) was found to be only a minor component of the mixture (1.87%); this result may be a biological response to direct sunlight perceived by the plant as it has been demonstrated in other oils in a tropical country [20-22] as well as other geochemical factors (e.g., soil type and precipitation) [23].

Based in the data gathered here, we recognized three independent biosynthetic routes i) aromatic compounds ii) Terpenes and iii) Fatty acids derivatives. Aromatic compounds (i.e., phenols, benzenoids and phenylpropenes) may be present to preserve antioxidant capacity in green leaves in case of mechanical shear, stress or injury [24]. The presence of these antioxidants in larger quantities, in Costa Rican guava leaves, may also be due to the fact that *Psidium* species are deciduous trees, hence the moment of the sampling may correlate with a mayor leaf shedding process which may be assisted by such a compound. Interestingly, these compounds are also present in guava fruit.

In the case of leaf oil, compounds containing a tert-butyl moiety (i.e., 2,4,6-Tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one; 2,3-dimethyl-5-oxohexanethioic acid S-t-butyl ester; 2,4-di-tert-butylphenol)occur repeatedly especially in phenol and quinone based structures. This may very well be part of a reaction blockage or protection (avoid premature reactivity) mechanism [25] to preserve synthesized compounds needed downstream from the biogenic pathway.

Among the sulfur containing compounds found, of special interest is 2-hydroxy-3-(thiophene-2-yl)methyl-5-methoxy-1,4-benzoquinone. In general, functionalized quinones have been already described as compounds of interest due to their potential as antimalarial drugs [26]. Synthesis should be pursued.

The major components (>3%) of dry season Costa Rican guava leaves essential oil were: 2,4-di-tert-butylphenol [1] (27.62%), α-terpineol [2] (10.53%), neointermedeol (9.96%), 2-Hydroxy-3-(thiophen-2-yl) methyl-5-methoxy-1,4-benzoquinone (6.80%) caryophyllene oxide (3.43%) and globulol (3.33%). 2,4-di-tert-butylphenol [1] isolated from other natural sources (for example sweet potato [27]) is known as a compound with oxidative stress protection capabilities. The presence of this substance, in considerable percentages in other Myrtacea essential oils, [28] has been described previously. The antioxidative efficiency of phenolic compounds is increased when t-butyl groups are located in positions 2,4 and 6 of the aromatic ring [29]. In an analogous manner, 2H-pyran-2,6-(3H)-dione [6] has been also extracted from other oil bearing fruits such as Triphala in considerable quantities [30].

Furthermore, α-terpineol already has been described as an NF-κB signaling suppressor [31] and gastroprotective activity in animal models [32]. This should be noted that α-terpineol has been found in all *P. friedrichstalianum* oils with invariably relative concentrations of ≥ 10% (Figure 3A).

On the other hand, the major components of dry season guava leaves essential oil were: neointermedeol [3] (19.5%), 7-epi-α-selinene [9] (17.0%), nerolidol [7] (9.5%); caryophyllene (9.3%), 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5β-ol (8.4%), benzaldehyde (5.5%), caryophyllene oxide (8.1%), benzyl alcohol (4.5%).

Benzoic acid [5] a relatively common compound in oils [29] and a product of shikimate aromatization pathway, was found to be a major component in *P. fridrichstalianum* dry season leaf essential oil, and may serve as a building block (through amination or hydroxylation) for biosynthesis of more significant compounds [33]. This is reinforced by the capability of benzoic acid to eventually form derivatives (e.g., esters, aldehydes, phenols). In fact, some of these derivatives may already be found (e.g., 4-benzyloxybenzoic acid, 2-hydroxyethyl benzoate, benzaldehyde, benzyl alcohol). In fact, these derivatives have been found to be even more common in essential oils that their parent compound [33]. This may hint towards a more biosynthetically active plant when in rainy season.

As expected, some similarities do arise between both species when grown in the same region, under similar conditions and when leaves are harvested in the same season. Though in different compositions, several compounds are found in both tree leaves such as linalool, caryophyllene [8] and its oxide, neointermedeol [3] and α-terpineol [2] to name just a few (Table 1). It would appear these biosynthetic compounds are conserved and hence their synthetic routes [34]. For example, invariably, α-terpineol [2] was a constant compound recovered from *Psidium* essential oils. Interestingly, 2,4-di-tert-butylphenol [1] was also found in guava leaves collected in dry season, however its amount was negligible. Costa Rican Guava leaves extract exhibited, under the same extraction conditions, a mixture of fewer compounds (Table 1).

Further research may be focused on the characterization of these essential oils in order to determine biological activity such as antifungal, antimicrobial or antioxidant capability and exploit these characteristics, if any, by means of an application for animal or human nutrition, especially since this oils are generally regarded as safe (GRAS) [35]. For example, other researchers have evaluated *Psidium* leaves potential as forage [36], tried to incorporate the leaf meal or crude extract of *P. guajava* into broiler chicken diets [37] and even have introduced them into rat diets [38].

In all cases, the four primary compounds found in the extract described invariably >40% of the composition (Figures 3A and 3B). The repeated major components found among the six oils showed significantly different percentages (Figures 3A and 3B). Furthermore, when the chemical profiles are analyzed as four different groups of compounds, fruits of both species show rather similar compositions (Figures 4A and 4B). Marked differences are however evident when comparing chemical families among leaf essential oil from both species. The more interesting characteristics found were a dramatic drop ($p<0.05$) in terpenes in contrast to an increase in fatty acids when comparing *P. guajava* leaves in dry and rainy season (Figure 4B). Evidence of higher amount of terpenes in essential oil from leaves collected in dry season for guava is reinforced by the presence of the tertiary sesquiterpene alcohol nerolidol [7] in important concentrations (9.5%). Interestingly, this compound has been associated with ripening in other fruits [39]. The compounds 7-epi-α-selinene and caryophyllene and its oxide suffer from a decrease in

P. friedrichsthalianum		
Leaf (dry season)	**Leaves (rainy season)**	**Fruit**
Major components		
2,4-di-tert-butylphenol (4.69) [27.6%]	2,4-di-tert-butylphenol (4.62) [23.2%]	2H-pyran-2,6-(3H)-dione (3.86) [26.4%]
α-terpineol (2.92) [10.5%]	α-terpineol (2.83) [18.4%]	cis-13-octadecenoic acid (10.21) [14.0%]
neointermedeol (4.59) [10.0%]	Tetra decanoic acid (6.16) [9.5%]	α-terpineol (2.86) [11.7%]
2-hydroxy-3-(thiophen-2-yl)methyl-5-methoxy-1,4-benzoquinone (4.63) [6.8%]	benzoic acid (5.07) [9.2%]	n-hexadecanoic acid (7.58) [9.6%]
globulol (4.07) [3.3%]	methyl formate (2.41) [4.7%]	ammonium acetate (2.50) [5.64%]
caryophyllene oxide (3.81) [3.4%]	oleic acid (7.78) [4.4%]	octadecanoic acid (9.79) [5.3%]
spathulenol (4.19) [2.5%]	1-nonadecene (3.89) [3.4%]	benzeneacetic acid (5.64) [5.2%]
elemicin (5.03) [2.2%]	1-docosene (3.30) [2.9%]	N-phenylacetamide (5.64) [4.8%]
methyl octadecyl ether (5.68) [2.2%]	cetene (2.45) [2.4%]	1-butanol (1.40) [4.5%]
γ-cadinene (4.41) [2.1%]	nerolidol (2.32) [2.3%]	trans-cinnamic acid (7.22) [3.8%]
ledene oxide-(II) (4.97) [1.5%]	2-nonadecene (4.48) [2.1%]	2-furancarboxylic acid (5.12) [2.6%]
isoaromadendrene epoxide (5.16) [1.4%]	oleic acid 3-hydroxypropyl ester (9.16) [2.0%]	hexanoic acid (3.31) [1.5%]
nerolidol (3.89) [1.3%]	ledene (4.20) [1.9%]	octanoic acid (3.96) [1.2%]
2-((2R,4aR,8aS)-4α-methyl-8-methylenedecahydronaphthalen-2-yl) prop-2-en-1-ol (5.21) [1.0%]	1-octadecanol (5.67) [1.8%]	palmitoleic acid (7.80) [0.9%]
2,6-dimethylnaphthalene (5.20) [1.0%]	β-selinene (2.75) [1.8%]	Z-3-hexen-1-ol (1.99) [0.8%]
(E)-hexen-3-ol (2.04) [0.9%]	terpinen-4-ol (2.52) [1.6%]	3-metil-1-butanol (1.55) [0.7%]
(1R,7S, E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol (4.26) [0.9%]	2,6-di-tert-butylbenzoquinone (3.16) [1.3%]	4-hidroxi-α,α,4-trimetylcyclohexane methanol (4.11) [0.5%]
7,9-di-tert-butyl-1-oxaspiro (4,5)-deca-6,9-diene-2,8-dione (6.49) [0.8%]		vanillin (5.72) [0.3%]
cembrene (4.84) [0.8%]	(Z)-3-hexen-1-ol (1.97) [1.3%]	1,1-dimethoxy-2-propanone (3.43) [0.3%]
1-octadecanol (3.93) [0.8%]	(1S,4S,4αS)-1-isopropyl-4,7-dimethyl-1,2,3,4,4α,5-hexahydronaphthalene (4.34) [1.1%]	tetradecanoic acid (6.20) [0.3%]
n-hexadecanoic acid (7.46) [0.6%]	benzoyl benzyl disulfide (5.85) [1.0%]	hydroquinone (8.78) [0.3%]
phytol (5.82) [0.5%]	2-(4α,8-dimethyl-2,3,4,5,6,7-hexahydro-1H-naphthalen-2-yl) propan-2-ol (4.26) [0.9%]	
1-tridecene (2.66) [0.4%]		
(E)-4-oxohex-2-enal (3.17) [0.4%]		
1-nonadecene (3.30) [0.4%]		
myrtenol (3.21) [0.3%]		
octadecanoic acid (9.56) [0.3%]		
7-methoxycoumarin (7.88) [0.3%]		
Trace compounds (i.e., <0.1%)		
isobutyl ether (2.13)	thujene (1.08)	4-methyl-2-oxetanone (1.12)
di-tert-butyldicarbonate (2.57)	methylclooctane (1.47)	3-penten-2-one (1.33)
trans-linalool oxide (6) acetate (2.71)	(Z)-7-tetradecene (1.86)	γ-terpinene (1.45)
2,3-dimethyl-5-oxohexanethioic acid, S-t-butyl ester (2.79)	1,2-ethanediol, monoformate (2.51)	2-methyl-aziridine (1.55)
trans-pinocarvyl acetate (2.81)	methoxy-phenyl oxime (3.02)	4-carene (1.63)
phosphinic acid, diethyl-, methyl ester (3.47)	1,2,4,5-tetrazin-3-amine (3.17)	1-methyl-3-(1-methylethyl) benzene (1.64)
2,4,6-tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one (3.50)	2,4,6-tris(1,1-dimethylethyl)-4-methylcyclohexa-2,5-dien-1-one (3.47)	Methyl vinyl ketone (1.66)
dimethyl sulfone (3.60)	1H-pyrazolo[3,4-d]pyrimidin-4-amine (3.64)	1,3-dioxol-2-one (1.73)
τ-caudinol (4.32)	3-methylpiridazine (3.76)	propylcyclopropane (1.89)
isospathulenol (4.50)	(E)-tetradec-2-enal (3.84)	acetate 4-hexen-1-ol (1.93)
phenylethyl alcohol (4.50)	1,2-dimethyl-azetidine (4.29)	α-methylstyrene (2.35)
2-hydroxy-3-(thiophen-2-yl) methyl-5-methoxy-1,4-benzoquinone (4.63)	N-tert-butylhydroxylamine (4.36)	4-methyl-1-(1-methylethyl)-R-3-cyclohexen-1-ol (2.52)
N-(2,6-dimethylphenyl)-N-[(2E)-3-methyl-1,3-thiazinan-2-ylidene] amine (5.40)	N-methoxymethamine (4.76)	3-hydroxypropanenitrile (2.52)
bis-benzenamine, 4,4'-[(1-methylethylidene) bis(4,1- phenyleneoxy)] (5.95)	3-(1'-pyrrolidinyl)-2-butanone (4.92)	1-methyl-4-(1-methylethenyl) cyclo hexanol (2.64)
1-hexadecanol (5.97)	isoelemicin (4.96)	benzeneacetaldehyde (2.69)
1,7-dimethyl-4-(1-methylethyl)cyclodecane (7.08)	3,5-di-tert-butyl-4-hydroxybenzaldehyde (5.34)	diphenyl propanetrione (2.73)
45 compounds	N-phenylacetamide (5.70)	N-propargyloxycarbonyl L-alanine hexyl ester (2.83)

	7,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione (6.48)	2-oxopentanedioic acid (2.97)
	7-hexyl-2-oxepanone (6.81)	(1S-cis)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) naphtalene (3.05)
	7-azabicyclo[4,2,0]octan-8-one (10.90)	dihydro-3-methylene-2,5-furandione (3.07)
	45 compounds	3-hydroxy-3-phenylbutan-2-one (3.08)
		5-ethyldihydro-2-(3H)-furanone (3.09)
		2-(5H)-furanone (3.22)
		5-acetylpyrimidine (3.25)
		2,2'-oxbis-ethanol (3.45)
		ammonium acetate (3.55)
		phenylethyl alcohol (3.58)
		succinic anhydride (4.13)
		4,5-dimethyl-1,3-dioxol-2-one (4.22)
		1,6-dimethyl-4-(1-methylethyl)-naphthalene (4.43)
		pentanoic acid 2,4-di-t-butylphenylester (4.57)
		propylenen oxonyde (4.59)
		triethylene glycol (4.59)
		4',6'-dimethoxy-2',3'-dimethylacetophenone (4.65)
		1,2-benzenedicarboxylic acid (4.80)
		E-1,2,3-trimethoxy-5-(1-propenyl)-benzene (5.02)
		benzoic acid (5.14)
		tetraethylenglycol (5.57)
		2-dodecanol (5.68)
		1,2,3-trimethoxy-propane (5.75)
		hydrocinnamic acid (5.92)
		cathecol (6.30)
		niacin (6.39)
		4-(3-hydroxybuthyl)- 3,5,5-trimethyl-2-cyclohexane-1-one (6.48)
		3-phenyl-2-propenoic acid (6.62)
		butyrovanillone (6.95)
		2-pirimidinamine (7.02)
		(3S, 3aS, 6R, 7R, 9aS)-1,1,7-trimethyldecahydro-3a,7-methano cyclopenta[8]annulene-3,6-diol (8.05)
		2-methoxy-1,4-benzenediol (8.12)
		anhydride 2-methyl-propanoic acid (8.15)
		tridecanoic acid (8.44)
		pentaethylene glycol (9.02)
		3,4,5-trimethoxy-phenol (9.13)
		4-acetate-2-methyl-1,4-benzenediol (9.39)
		75 compounds

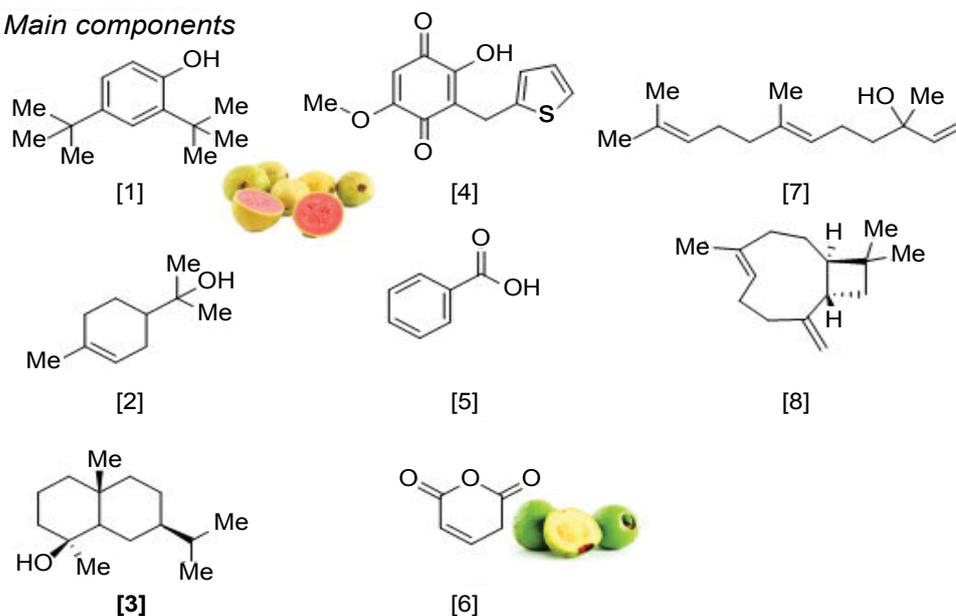| *P. guajava*		
Leaves (dry season)	**Leaves (rainy season)**	**Fruit**
Major components		
neointermedeol (4.66) [19.5%]	neointermedeol (4.52) [20.4%]	octadecanoic acid (9.74) [33.5%]
7-epi-α-selinene (3.05) [17.0%]	2-hydroxy-2-methyl-butanoic acid methyl ester (1.77) [14.0%]	2,4-di-tert-butylphenol (4.66) [12.7%]
nerolidol (3.93) [9.5%]	1-ethyl-2,4-dimethylbenzene (1.60) [8.6%]	(Z,Z)-9,12-octadecadienoic acid (10.86) [11.8%]
caryophyllene (2.63) [9.3%]	n-hexadecanoic acid (7.51) [8.2%]	neointermedeol (4.57) [9.0%]
10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0] undecan-5β-ol (4.77) [8.4%]	benzaldehyde (2.37) [6.5%]	methyl octadecyl ether (5.68) [5.7%]
benzaldehyde (2.43) [5.5%]	urea (2.00) [5.9%]	pentadecanoic acid (6.72) [3.8%]
caryophyllene oxide (3.84) [8.1%]	oleic acid (10.10) [5.9%]	1-nonadecene (3.93) [2.6%]
benzyl alcohol (3.46) [4.5%]	cryptomeridiol (6.45) [4.6%]	n-hexadecanoic acid (7.56) [2.4%]
p-mentha-1(7),8-dien-2-ol (3.20) [2.1%]	7-epi-α-selinene (2.99) [4.1%]	1-octadecanol (3.33) [2.0%]
D-limonene (1.44) [2.0%]	octadecanoic acid (9.70) [3.9%]	(E)-3,7,11-trimethyl-1,6,10-dodecatrien-3-ol (3.88) [1.9%]
isoaromadendrene epoxide (4.87) [1.5%]	propanoic acid, propyl ester (2.57) [2.1%]	1-docosene (4.51) [1.8%]
eucalyptol (1.53) [1.5%]	D-limonene (1.42) [2.1%]	tetradecanoic acid (6.15) [1.8%]
muurola-4,10(14)-dien-1.β.-ol (4.27) [1.1%]	benzyl alcohol (5.00) [1.9%]	(Z)-3-hexen-1-ol (2.04) [1.6%]
α -terpineol (2.92) [1.1%]	trans-muurola-3,5-diene (4.30) [1.5%]	ethyl oleate (5.27) [1.5%]
(Z)-3-hexen-ol, benzoate (4.19) [1.1%]	benzyl benzoate (5.84) [1.2%]	heptadecanoic acid (8.35) [1.3%]

methyl salicylate (3.15) [1.0%]	9,12-octadecadienoic acid (Z,Z)- (10.80) [1.1%]	oleic acid (7.77) [1.3%]
(1Z,4Z,7Z)-1,5,9,9-tetramethyl-cycloundecatriene (2.84) [1.0%]	11,11-dimethyl-4,8-dimethylenebicyclo [7.2.0]undecan-3-ol (4.61) [0.9%]	2,3,3-trimethyl-cyclobutanone (3.16) [0.8%]
carveol (3.31) [0.9%]	crotonic anhydride (4.51) [0.7%]	[4αR-(4aα, 7α, 8aβ)]-decahydro-4α-methyl-1-methylene-7-(1-methylethenyl)- naphthalene (2.85) [0.8%]
cis-Z-α-bisabolene epoxide (6.73) [0.8%]	1,3-dioxolane-2-methanol (1.15) [0.7%]	glycerin (4.80) [0.7%]
(Z)-3-hexen-1-ol (2.03) [0.7%]	1-methyl-4-(1-methylethenyl)-cyclohexanol, (2.75) [0.7%]	2-methyl-1-undecanol (2.62) [0.7%]
l-calamenene (3.33) [0.6%]	acetophenone (2.82) [0.7%]	cetene (2.57) [0.6%]
11-octadecenoic acid, methyl ester (5.16) [0.4%]	4α,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4α,5,6,7-octahydronaphthalene (2.85) [0.6%]	2,6-bis(1,1-dimethylethyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (4.06) [0.6%]
α -limonene diepoxide (6.44) [0.4%]	(1S-cis)-1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl) naphthalene (3.27) [0.6%]	methyl (Z)-N-hydroxybenzenecarboximidate (3.05) [0.5%]
hexadecanoic acid, methyl ester (4.36) [0.3%]	terbutol (3.30) [0.6%]	octadecanoic acid, ethyl ester (5.19) [0.5%]
phenol, 3,5-bis(1,1-dimethylethyl)- (4.70) [0.3%]	caryophyllene oxide (4.86) [0.6%]	
n-hexadecanoic acid (7.48) [0.3%]	tetradecanoic acid (6.12) [0.5%]	
δ-cadinene (3.11) [0.3%]	N-methyl-N-nitro-methanamine (2.64) [0.4%]	
benzyl benzoate (5.95) [0.2%]	methyl salicylate (3.11) [0.3%]	
oleic acid (10.00) [0.2%]	α-calacorene (3.48) [0.3%]	
4,4,8-trimethyltricyclo [6.3.1.0(1,5)] dodecane-2,9-diol (7.97) [0.2%]	(Z)-3-hexen-1-ol, benzoate (4.06) [0.2%]	
benzoic acid (5.12) [0.2%]	4-benzyloxybenzoic acid (6.65) [0.2%]	
Trace compounds (i.e., <0.1%)		
o-cymene (1.62)	oxalic acid, allyl isobutyl ester (1.68)	2,3-pyridinedicarboxylic anhydride (1.78)
6-methyl-5-hepten-2-one (1.85)	eucalyptol (1.84)	4-hexen-1-ol, acetate (1.98)
2-oxo-4-phenyl-6-(4-chlorophenyl)-1,2-dihydropyrimidine (1.87)	1,3-dioxolan-2-one (1.92)	benzaldehyde (2.40)
1-methyl-3-(1-methylethenyl) benzene (2.08)	1,1-diethoxy-ethane (2.03)	4α,8-dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4α,5,6,7-octahydronaphthalene (2.69)
propanoic acid, 2-methylpropyl ester (2.11)	2-Pentyn-4-one (2.22)	dimethyl-silanediol (2.70)
4-hexen-1-ol, acetate (2.17)	3-acetoxy-2-butanone (2.28)	azulene (2.72)
α-copaene (2.27)	3-amino-butanoic acid (2.35)	2,3,3-trimethyl-cyclobutanone (3.22)
ammonium acetate (2.32)	propanoic acid, propyl ester (2.63)	2,6-bis(1,1-dimethylethyl)-2,5-Cyclohexadiene-1,4-dione (3.23)
linalool (2.44)	acetic anhydride (2.71)	acetic anhydride (3.63)
benzoic acid, 2-hydroxy-, ethyl ester (3.25)	tetrahydro-2-(methoxymethyl)-furan (3.47)	1H-pyrazolo[3,4-d]pyrimidin-4-amine (3.70)
phenylethyl alcohol (3.56)	butanoic acid, ethyl ester (4.04)	3-methylpyridazine (3.79)
α-calacorene (3.57)	2-(formyloxy)-1-phenyl-ethanone (5.25)	neointermedeol (4.21)
β-calacorene (3.70)	2-(4α,8-Dimethyl-1,2,3,4,4α,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol (5.32)	nonanoic acid (4.24)
methyl octadecyl ether (5.69)	N-phenyl-acetamide (5.62)	[1R-(1α,4β,4aβ,8aβ)]-1,2,3,4,4α,7,8,8α-octahydro-1,6-dimethyl-4-(1-methylethyl)-1-naphthalenol (4.38)
isospathulenol (5.73)	pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester (5.74)	triethylene glycol (4.85)
bicyclo[2.2.2]octane, 1,2,3,6-tetramethyl- (5.77)	phenanthrene (6.27)	1-tetradecanol (4.88)
3-methyl-hexane (5.82)	3-phenyl-1-propanol, acetate (7.14)	benzoic acid (5.10)
4-propylphenol (6.94)	(Z)-11-hexadecenoic acid (7.74)	1-octadecanol (5.17)
1-nonadecene (7.11)	(4αS,7R)-7-(2-hydroxypropan-2-yl)-1,4α-dimethyl-4,4α,5,6,7,8-hexahydronaphthalen-2(3H)-one (7.78)	benzophenone (5.32)
oxalic acid, allyl octadecyl ester (8.89)	4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol (7.91)	3,5-di-tert-butyl-4-hydroxybenzaldehyde (5.37)
octadecanoic acid (9.59)	heptadecanoic acid (8.34)	vanillin (5.66)
	2-(3H)-furanone, dihydro-5-tetradecyl- (8.90)	2-ethyl-1-dodecanol (6.05)
		benzene, 1,1'-[1,2-ethanediylbis(oxy)]bis- (6.14)
		7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (6.48)
		oleic acid, 3-hydroxypropyl ester (9.15)
		hexanamide (11.05)
		1-methylene-2-vinylcyclopentane (11.94)
53 compounds	*53 compounds*	*50 compounds*

Table 1: Description and relative composition of fruit essential oils from two *Psidium* species and seasonal effect over the leaves' essential oil.
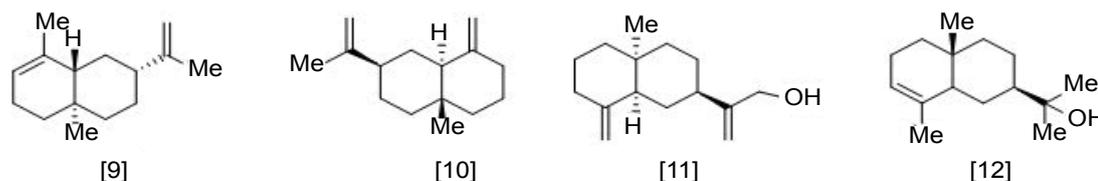
guava leaves during seasonal change as well. This further hint towards a general reduction in terpenes during rainy season (Table 1 and Figure 3B). Noteworthy, β-selinene [10] and (E)-β-caryophyllene [8] both share the same synthetic route as they appear to from the farnesyl cation forming thereafter the (E,E)-germacradenyl and (E, E)-humulyl cations, respectively [40]. We hypothesize that selinene isomers may be transformed into other important sesquiterpenes (e.g., Ref. [11] and Ref. [12]) during seasonal change. A similar phenomenon is observed as well in the case of *P. friedrichsthalianum* leaves (Figure 4A), in the latter, however linear aliphatic hydrocarbons are also increased
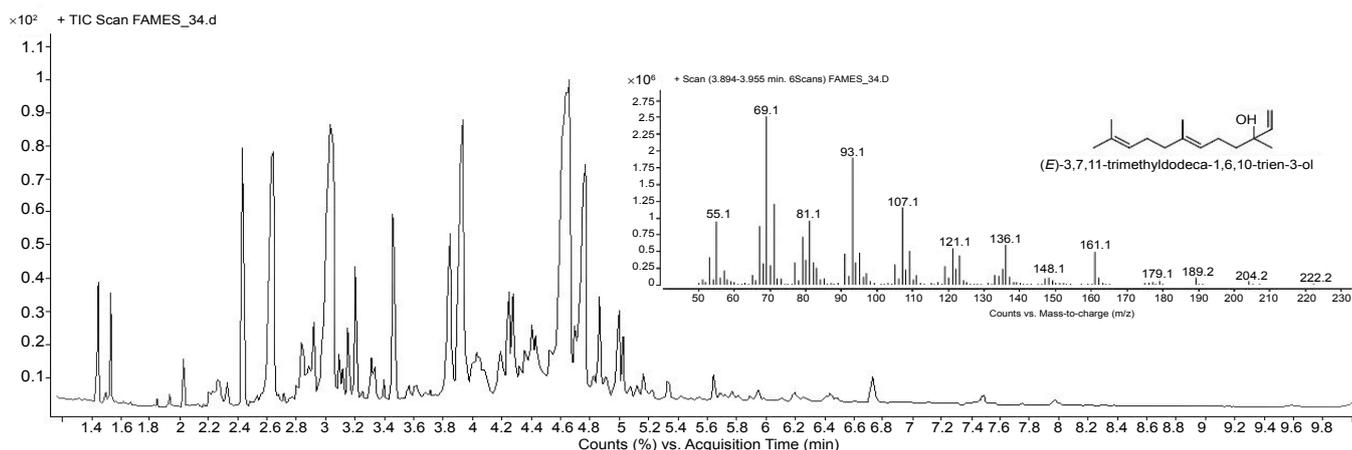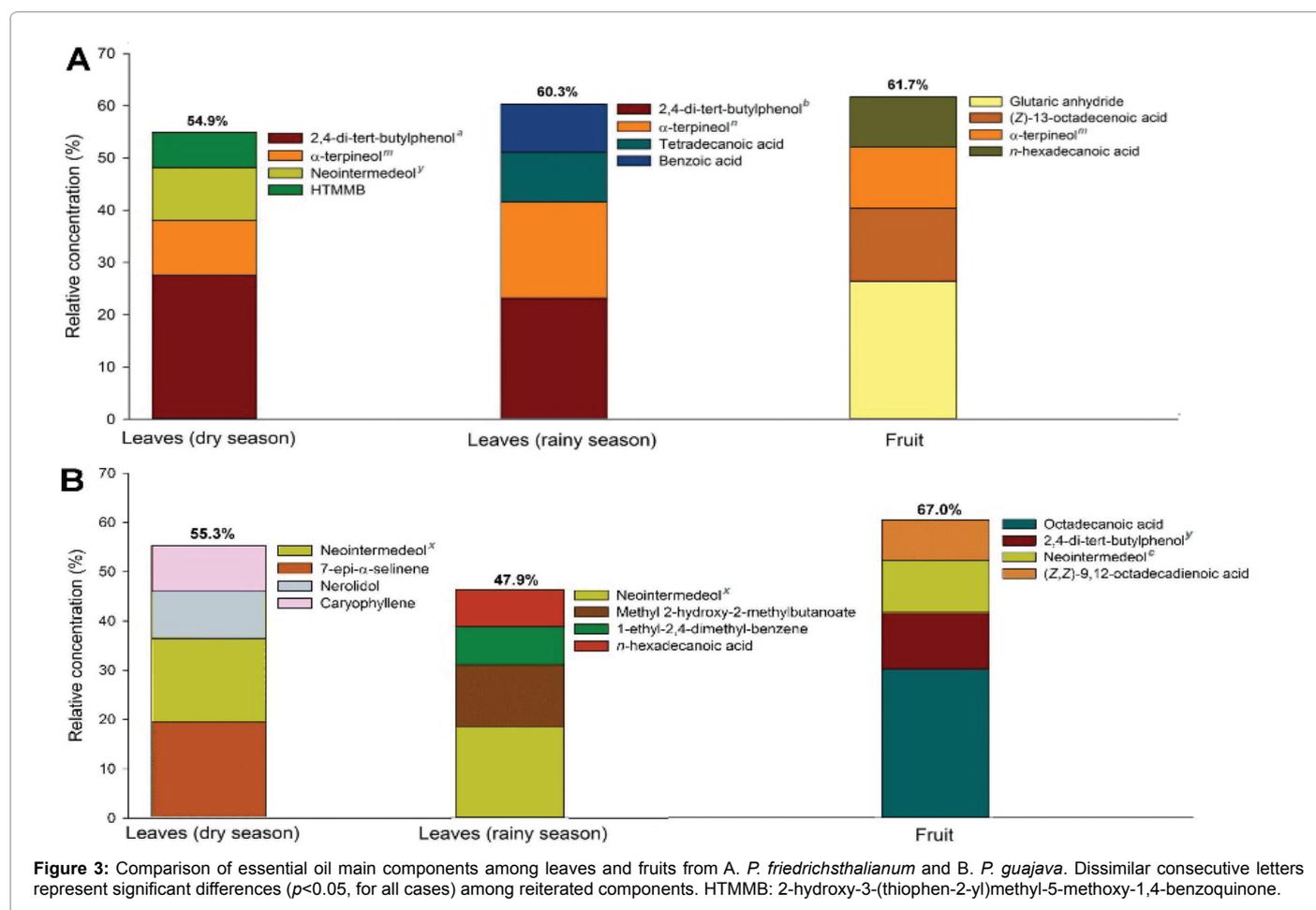
Figure 1: Chemical structure of main components and noteworthy terpenoids from *P. friedrichsthalianum* and *P. guajava* fruits and leaves essential oil. Me used as abbreviated notation for the methylene moiety.

Figure 2: *P. guajava* fruit essential oil chromatogram and mass spectrum extraction for signal from 3.894 to 3.955 min corresponding to β-Nerolidol.

significantly ($p<0.05$, Figure 4A). Worth mentioning is an increase in the concentration of β-selinene in the rainy season, that may be related to a growing insecticidal activity in the plant leaves [41,42] due, in turn, to a variation of insect population dynamics.

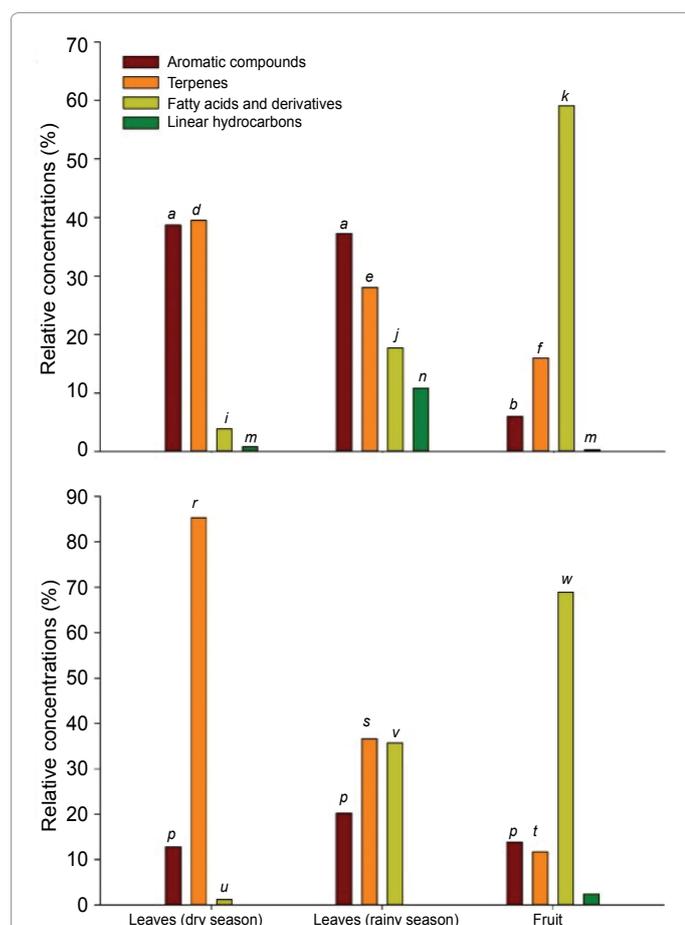Principal component analysis demonstrated three clearly segregated subsets grouped by similarities in composition (Figure 5). Costa Rican guava (fruit) oil composition is the least similar from the rest of extracts (with main components including 2H-pyran-2,6-(3H)-dione, *cis*-13-octadecanoic acid, α-terpineol and *n*-hexadecanoic acid). In fact, these specific oils exhibited not only a stronger contrast, with respect of the rest of the study objects, but also display the highest diversity and number (i.e., 75 hits) of chemical identifiable compounds (Figure 5).
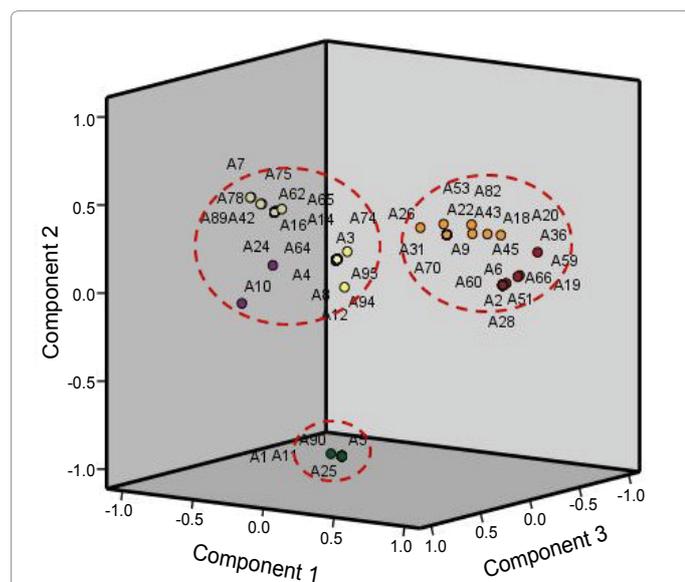
Figure 3: Comparison of essential oil main components among leaves and fruits from A. *P. friedrichsthalianum* and B. *P. guajava*. Dissimilar consecutive letters represent significant differences (*p*<0.05, for all cases) among reiterated components. HTMMB: 2-hydroxy-3-(thiophen-2-yl)methyl-5-methoxy-1,4-benzoquinone.

Strain (UFC mL^{-1})/Oil[a]	*P. friedrichsthalianum* leaves rainy season	*P. friedrichsthalianum* leaves dry season	*P. guajava* leaves rainy season	*P. guajava* leaves dry season	*P. friedrichsthalianum* fruit	*P. guajava* fruit	Oxytetracycline, 10 µg mL^{-1}
Inhibition zone ± SD (mm)							
S. Choleraesuis (1.2 × 10⁵)	-	-	44.7 ± 0.5	-	9.8± 0.4	-	21.7 ± 1.7
S. typhimurium (4.0 × 10⁴)	-	-	38.7 ± 0.9	-	9.8± 0.7	-	19.0 ± 0.8
S. enteritidis (7.60 × 10⁵)	-	-	42.7 ± 1.9	-	7.7 ± 1.7	-	20.7 ± 0.5
E. coli O157:H7 (7.30 × 10⁵)	-	-	44.5 ± 1.9	-	10.0 ± 0.5	-	29.0 ± 0.8
S. aureus (7.00 × 10⁴)	-	-	47.2 ± 2.2	8.3 ± 0.4	10.2± 1.4	-	33.7 ± 1.2
B. cereus (2.70 × 10⁵)	7.7 ± 0.3	-	37.3 ± 4.1	10.3 ± 0.6	10.0 ± 1.4	-	32.7 ± 2.1
B. subtillis (1.49 × 10⁶)	-	8.2 ± 0.6	34.0 ± 2.2	10.0 ± 0.8	9.7± 1.7	9.7 ± 0.2	14.3 ± 0.5
P. aeruginosa (1.07 × 10⁶)	-	-	36.8 ± 1.4	-	10.5± 0.4	-	9.0 ± 1.6
P. mirabilis (7.60 × 10⁵)	-	-	36.8 ± 2.8	-	10.7± 0.8	-	9.0 ± 0.8
Strain (UFC mL^{-1})/Oil[a]	Limonene	Myrcene	Linalool	Eucalyptol	Thujone	Caryophyllene	
Inhibition zone ± SD (mm)							
S. choleraesuis (1.2 × 10⁵)	-	-	12.0 ± 0.9	9.7 ± 0.3	10.7 ± 0.4	-	
S. typhimurium (4.0 × 10⁴)	4.2 ± 2.2	-	20.3 ± 0.7	-	9.0 ± 0.8	-	
S. enteritidis (7.60 × 10⁵)	-	-	21.0 ± 2.1	10.0 ± 0.5	11.0 ± 0.8	-	
E. coli O157:H7 (7.30 × 10⁵)	-	-	22.3 ± 0.7	8.2 ± 0.2	14.2 ± 0.7	-	
S. aureus (7.00 × 10⁴)	5.3 ± 1.5	10.0 ± 0.5	15.0 ± 1.2	-	18.3 ± 2.4	10.3 ± 1.5	
B. cereus (2.70 × 10⁵)	-	-	38.3 ± 1.2	-	14.0 ± 1.6	12.7 ± 0.4	
B. subtillis (1.49 × 10⁶)	4.0 ± 1.9	8.7 ± 0.4	26.8± 1.1	9.8± 0.3	12.0 ± 1.5	10.7 ± 0.2	
P. aeruginosa (1.07 × 10⁶)	-	-	-	-	-	-	
P. mirabilis (7.60 × 10⁵)	-	-	20.3 ± 0.6	9.3 ± 0.6	10.0 ± 1.2	11.7 ± 0.8	

[a]DMSO (used as a negative control), terpinene and cymene oils did not exhibit any inhibition zones (i.e. 0 mm). [b]Inhibition zones reported as the median of three replicates.
Table 2: Antimicrobial activity for the recovered essential oils and some terpene standards.

Figure 4: Comparison of essential oil grouped by structural families among leaves and fruits from A. *P. friedrichsthalianum* and B. *P. guajava*. Dissimilar consecutive letters represent significant differences ($p<0.05$, for all cases) among reiterated components.

Figure 5: Component plot in rotated space for principal component analysis for the comparison of the six essential oils. *P. guajava* leaves rainy season , *P. guajava* leaves dry season , *P. guajava* fruit , *P. friedrichsthalianum* leaves rainy season , *P. friedrichsthalianum* leaves dry season , *P. friedrichsthalianum* fruit .

As one may expect there are similarities among extracts from leaves obtained during dry and rainy season. Though, interestingly enough, important compositional differences are sufficient to distinguish them as well (Figure 5). The retention of some main compounds of importance such as 2,4-di-tert butylphenol [1] or α-terpineol [2] may indicate some plant synthetic routes are conserved during normal climatic changes. Interestingly, in all cases leaf oils exhibited nearly the same amount of compounds (from 46 to 54 different hits, Table 1). Several of the terpenes and sesquiterpenes listed here-in have been reported in other tropical trees including other Myrtacea species [43].

Finally, the rainy season *P. guajava* leafs' oil is the most effective against the bacteria assayedexhibiting inhibition zones that ranged from 31 (*B. subtilis*) to 52 mm (*S. aureus*, Table 2) and was effective against both Gram-negative and Gram-positive bacteria which in turn, are food-spoilage related. This activity was significantly higher ($p<0.05$) than that of the oxytetracycline 10 μg mL^{-1} solution (Table 2). Overall, *B. cereus* seems to be the more sensitive strain against all the essential oils producing inhibition zones from 0 to 42 mm (Table 2). A linalool oil standard exhibited a significantly stronger ($p<0.05$) activity compared with the other essential oils tested producing inhibition zones from 0 (*P. aeruginosa*) to 40 mm (*B. cereus*, Table 2).

Conclusion

Both Costa Rican guava and guava major components may be segregated within families and, based on structural characteristics alone, seem to possess potential bioactive capacity. Climatic or seasonal changes seem to affect the overall composition of the leaf essential oil in both species, though some major components seem to prevail and only are modified concentration-wise. Similarities during chemical fingerprinting do arise when both species are compared. Furthermore, the tert-butyl moiety seems to be a conserved and extended throughout the volatile compounds in both species (present together in fruits and leaves). Finally, the effective antibacterial activity of the rainy season *P. guajava* leafs' oil, demonstrated here, should be further investigated to assess its potential as an alternative to conventional antibiotics. As part of future work, compounds responsible for eliciting bioactivity may be purified by analytical separation of the mixtures obtained.

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