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

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Abstract

The essential oil of two related tree species, P. friedrichstalianum 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. friedrichstalianum 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 recognize 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 leaves' 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 friedrichstalianum; 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. friedrichstalianum (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].

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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 ripe 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 with the appropriate oil standards were tested in parallel for comparison (i.e., limonene, 

**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 7.25°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 (1.41 min; M+ 137.2 m/z), benzoic acid (1.47 min; M+ 121.2 m/z), 2,4-ditertbutyolphthalein (4.68 min; M+ 207.3 m/z), benzy l alcohol (1.46 min; M+ 109.1 m/z), thujone (4.66 min; M+ 205.4 m/z), caryophyllene oxide (6.16 min; M+ 135.2 m/z), α-terpineine (5.64 min; M+ 135.2 m/z), caryophyllene (6.72 min; M+ 243.4 m/z), nerylalcohol (7.28 min; M+ 149.0 m/z), benzylaldehyde (7.52 min; M+ 107.1 m/z), 2,4-ditertbutylphenol (1.52 min; M+ 107.1 m/z), benzylaldehyde (7.27 min; M+ 107.1 m/z), cinnamic acid (7.19 min; M+ 143.2 m/z) and thujone (1.67 min; M+ 241.4 m/z) standards (Sigma-Aldrich, St Louis, Mo) were injected separately for confirmation purposes. Tetradecanoic acid (8.16 min; M+ 226.4 m/z), hexadecanoic acid (9.72 min; M+ 243.4 m/z), 9Z-octadecenoic acid (10.78 min; M+ 268.4 m/z), 1110-3-octadecenoic acid (10.78 min; M+ 285.4 m/z), (Z,Z)-9,12-octadecadienoic acid (11.80 min; M+ 285.4 m/z) and negative controls, respectively. Additionally, some essential oils 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 film. The discs impregnated with water 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 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, terpineine, 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. friedrichstalianum 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 on 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, benzzenoids and phenylpropanes) 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 already been described as compounds of interest due to their potential as antimalarial drugs [26]. In the case of P. friedrichstalianum 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].

The major components (>3%) of dry season Costa Rican guava leaves essential oil were: 2,4-di-tert-buty phenol [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-tet-tert-buty phenol [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 the substance, in considerable percentages in other Myrtacea essential oils (Table 1). 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-dimethylenecyclo[7.2.0]undecan-5β-ol (8.4%), benzaldehyde (5.5%), caryophyllene oxide (8.1%), benzyl alcohol (4.5%).

Benzoc 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. friedrichstalianum 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-benzoxylbenzoic 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 when the chemical profiles are analyzed as four different groups of compounds, fruits of both species show rather similar compositions when 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

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### P. richardstalianum

| 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-pyryan-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-y) methyl-5-methoxy-1,4-benzooquinone (4.83) [6.8%] | | |
| globulol (4.07) [3.3%] | methyl formate (4.41) [4.7%] | ammonium acetate (2.50) [5.64%] |
| carpyrlifene oxide (3.81) [3.4%] | oleic acid (7.78) [4.4%] | octadecanoic acid (9.79) [5.3%] |
| 2,6-di-methylnaphthalene (5.20) [1.0%] | 1-nonadecene (3.89) [3.4%] | benzeneacetic acid (5.64) [6.2%] |
| (E)-hexen-3-ol (2.04) [0.9%] | 1-docosene (3.30) [2.9%] | N-phenylacetamide (5.64) [8.4%] |
| 2,4-di-tert-butylphenol (4.62) [2.9%] | 2-(hexyl ester) (4.26) [0.9%] | 1-butanol (1.40) [4.5%] |
| 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | 1,2-dimethyl-azetidine (4.29) | trans-cinnamic acid (7.22) [3.8%] |
| 2-hydroxy-3-(thiophen-2-yl)methyl-5-methoxy-1,4-benzooquinone (4.83) [6.8%] | | |
| dimethylenecyclodec-5-enol (4.26) [0.9%] | 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | 2-furancarboxylic acid (5.12) [2.6%] |
| (Z)-3-hexen-1-ol (1.99) [0.8%] | | |
| 3-methylpiridazine (3.76) | 2,4-di-tert-butyl-1,3-dioxol-2-one (1.73) | 3-methylpiridazine (3.76) |
| propylcyclopropane (1.89) | 1,2-ethanediol, monoformate (2.51) | 3-methylpiridazine (3.76) |
| 1-nonadecene (3.30) [0.4%] | 4-methyl-2-oxetanone (1.12) | 3-methylpiridazine (3.76) |
| methyl octadecyl ether (5.68) [2.2%] | | |
| 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | | |
| 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | | |
| 4-hidroxi-α,α,4-trimetylcyclohexane methanol (4.11) | | |
| 3-metil-1-butanol (1.55) [0.7%] | | |
| 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | | |
| methyl formate (2.51) | 2-methyl-aziridine (1.55) | 4-hidroxi-α,α,4-trimetylcyclohexane methanol (4.11) |
| 4-methyl-1-(1-methylethyl)-R-3-cyclohexen-1-ol (2.52) | | |
| N-propargyloxycarbonyl L-alanine | | |
| ammonium acetate (2.50) [5.64%] | | |
| 1,3-dioxol-2-one (1.73) | | |
| 4-hidroxi-α,α,4-trimetylcyclohexane methanol (4.11) | | |
| 2,4-di-tert-butylbenzoquinone (3.16) [1.3%] | | |
| 4-methyl-2-oxetanone (1.12) | | |
| cembratriene (2.66) [0.4%] | | |
| (E)-4-oxo-2-enal (3.17) [0.4%] | | |
| 1-nonadecene (3.30) [0.4%] | | |
| myrtenol (3.21) [0.3%] | | |
| octadecanoic acid (9.56) [0.3%] | | |
| 7-methoxycurcumarin (7.88) [0.3%] | | |

#### Trace compounds (i.e., <0.1%)

| Component | Leaf (dry season) | Leaves (rainy season) | Fruit |
|-----------|------------------|-----------------------|-------|
| isobutyl ether (2.13) | isovaleric acid (1.08) | | 4-methyl-2-oxetanone (1.12) |
| di-t-butylidicarbonate (2.37) | methyl cyclocetane (1.47) | | 3-penten-2-one (1.33) |
| trans-linalool oxide (6) acetate (2.71) | (2)-7-tetradecene (1.86) | | γ-terpinene (1.45) |
| 2,3-dimethyln-5-oxoanethestic acid, 5,5-dimethylcyclohexa-2,5-dien-1-one (2.78) | | | 2,4-di-tert-butyl-1,3-dioxol-2-one (1.73) |
| trans-pincaroceryl acetate (2.81) | methylcyt-hoxine oxime (3.02) | | 4-carene (1.63) |
| phosphonic acid, diethyl- methyl ester (3.47) | | | 1-methyl-3-(1-methylethyl) benzene (1.64) |
| 2,4,6-tris(1,1-dimethylethyl)4-methylcyclohexa-2,5-dien-1-one (3.50) | | | | |
| dimethyln sulfone (3.60) | | | 2,4-di-tert-butyl-1,3-thiaazinan-2-ylidenemethane (5.40) |
| trans-1-cadinol (4.32) | | | 1-tridecane (2.66) |
| (E)-2,4,6-tris(1,1-dimethylethyl)4-methylcyclohexa-2,5-dien-1-one (3.50) | | | (E)-4-oxo-2-enal (3.17) |
| 1,2-ethanediol, monoformate (2.51) | | | 1,1-dimethoxy-2-propanone (3.43) |
| trans-pincaroceryl acetate (2.81) | methylcyt-hoxine oxime (3.02) | | 4-carene (1.63) |
| 2,4,6-tris(1,1-dimethylethyl)4-methylcyclohexa-2,5-dien-1-one (3.50) | | | 2,4-di-tert-butyl-1,3-dioxol-2-one (1.73) |
| dimethyln sulfone (3.60) | | | 1,1-dimethoxy-2-propanone (3.43) |
| trans-1-cadinol (4.32) | | | (E)-4-oxo-2-enal (3.17) |
| (E)-2,4,6-tris(1,1-dimethylethyl)4-methylcyclohexa-2,5-dien-1-one (3.50) | | | 1,1-dimethoxy-2-propanone (3.43) |
| 1,2-ethanediol, monoformate (2.51) | | | 1,1-dimethoxy-2-propanone (3.43) |
| | | | 1,1-dimethoxy-2-propanone (3.43) |

#### Major compounds

- benzoyl benzyl disulfide (5.85) [1.0%]
- 2-nonadecene (4.48) [2.1%]
- 1H-pyrazolo[3,4-d]pyrimidin-4-amine (3.64) [3.6%]
- isoelemicin (4.96) [2.2%]
- 2H-pyran-2,6-(3H)-dione (3.86) [26.4%]
- 2-methyl-aziridine (1.55)
- 1,2,4,5-tetrazin-3-amine (3.17)
- 1,2-dimethyl-azetidine (4.29)
- Tetra decanoic acid (6.16) [9.5%]
- benzeneacetaldehyde (2.69)
- 3,5-di-tert-buty-4-hydrobenzaldehyde (5.34)
- diphenyl propanetrione (2.73)
- N-phenylacetamide (5.70)
- N-propargyloxycarbonyl L-alanine hexyl ester (2.83)

**Note:** The table lists major and trace compounds from the essential oils of *P. richardstalianum* leaves and fruits. The percentages indicate the relative abundance of each compound. Some compounds are trace compounds (i.e., <0.1%).
| Compound                                                      | Leaves (dry season) | Leaves (rainy season) | Fruit                  |
|---------------------------------------------------------------|---------------------|-----------------------|------------------------|
| 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione      | neointermedeol (4.66) [19.5%] | neointermedeol (4.52) [20.4%] | octadecanoic acid (9.74) [33.5%] |
| 2-oxopentanedioic acid (2.97)                                  | 2-hydroxy-2-methyl-butanolic acid methyl ester (1.77) [14.0%] | 2,4-di-tert-butylphenol (4.66) [12.7%] |
| 7-hexyl-2-oxepanone (6.81)                                    | neointermedeol (4.57) [9.0%] | 1-ethyl-2,4-dimethylbenzene (1.60) [8.6%] | neointermedeol (4.57) [9.0%] |
| 7-azabicyclo[4.2.0]octan-8-one (10.90)                         | benzaldehyde (2.43) [5.5%] | ura (2.00) [5.9%] | pentadecanoic acid (6.72) [3.8%] |
| 45 compounds                                                  | benzyl alcohol (3.46) [4.5%] | cryptomeridiol (6.45) [4.6%] | n-hexadecanoic acid (7.56) [2.4%] |
|                                                               | (Z)-2-hexenyl-2-ol (4.14) [2.2%] | (Z)-3-hexenyl-2-ol (4.14) [2.1%] | 1-octadecanol (3.33) [2.0%] |
|                                                               | D-limonene (1.41) [2.0%] | D-limonene (1.41) [2.0%] | D-limonene (1.41) [2.0%] |
|                                                               | octadecanoic acid (9.70) [3.9%] | (E)-3,7,11-trimethyl-1,6,10-dodecaadien-3-ol (3.88) [1.9%] | (E)-3,7,11-trimethyl-1,6,10-dodecaadien-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%] | eucalyptol (1.53) [1.5%] | eucalyptol (1.53) [1.5%] |
|                                                               | D-limonene (1.41) [2.0%] | D-limonene (1.41) [2.0%] | D-limonene (1.41) [2.0%] |
|                                                               | 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 octate (5.37) [1.5%] |
|                                                               | (Z)-3-hexen-1-ol, benzene (4.19) [1.1%] | benzyl benzoate (5.84) [1.2%] | heptadecanoic acid (8.35) [1.3%] |

**P. guajava**

| Major components | Leaves (dry season) | Leaves (rainy season) | Fruit |
|------------------|---------------------|-----------------------|-------|
| neointermedeol   | [19.5%]             | [20.4%]               | [33.5%] |
| 7-epi-α-selinene | [17.0%]             | [14.0%]               | [11.8%] |
| nerolidol        | [9.5%]              | [8.6%]                | [11.8%] |
| caryophyline     | [9.3%]              | [8.2%]                | [11.8%] |
| 7-epi-α-selinene | [2.1%]              | [4.1%]                | [2.0%]  |
| isoaaromadendrene| [1.1%]              | [1.5%]                | [1.9%]  |
| eucalyptol       | [2.1%]              | [1.1%]                | [1.8%]  |
| D-limonene       | [2.0%]              | [2.0%]                | [2.0%]  |
| muurola-4,10(14)-| [1.1%]              | [1.0%]                | [1.0%]  |
| α-terpineol      | [1.5%]              | [1.0%]                | [1.0%]  |
| (Z)-3-hexen-1-ol | [1.1%]              | [1.1%]                | [1.1%]  |
Table 1: Description and relative composition of fruit essential oils from two Psidium species and seasonal effect over the leaves' essential oils.

| Compound Description | Fruit Essential Oil Composition | Leaf Essential Oil Composition |
|----------------------|--------------------------------|-------------------------------|
| 2-cyclohexenes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 8.7% | 11% |
| 2-cyclohexenes-3-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 8.5% | 9% |
| 1,2-cyclohexadienes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 8.3% | 9% |
| 2-cyclohexenes-2-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 8.1% | 9% |
| 2-cyclohexenes-2,3-dione, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 7.9% | 8% |
| 2-cyclohexenes-2,3-dione, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 7.7% | 8% |
| 2-cyclohexenes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 7.5% | 8% |
| 2-cyclohexenes-3-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 7.3% | 8% |
| 1,2-cyclohexadienes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 7.1% | 8% |
| 2-cyclohexenes-2-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 6.9% | 7% |
| 2-cyclohexenes-2,3-dione, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 6.7% | 7% |
| 2-cyclohexenes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 6.5% | 7% |
| 2-cyclohexenes-3-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 6.3% | 7% |
| 1,2-cyclohexadienes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 6.1% | 7% |
| 2-cyclohexenes-2-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 5.9% | 6% |
| 2-cyclohexenes-2,3-dione, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 5.7% | 6% |
| 2-cyclohexenes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 5.5% | 6% |
| 2-cyclohexenes-3-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 5.3% | 6% |
| 1,2-cyclohexadienes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 5.1% | 6% |
| 2-cyclohexenes-2-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 4.9% | 5% |
| 2-cyclohexenes-2,3-dione, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 4.7% | 5% |
| 2-cyclohexenes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 4.5% | 5% |
| 2-cyclohexenes-3-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 4.3% | 5% |
| 1,2-cyclohexadienes-1-one, 3,4-dihydro-2H-furan-2-carboxarboxylic acid | 4.1% | 5% |

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)-germacrdenyl 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.
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 | *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 × 10¹⁰) | -                                           |                                           | 44.5 ± 1.9                     | -                             | 10.0 ± 0.5                   | -               | 29.0 ± 0.8                |
| **B. cereus** (2.70 × 10⁵)    | 7.7 ± 0.3                                   |                                           | 47.2 ± 2.2                     | 8.3 ± 0.4                     | 10.2± 1.4                    | -               | 33.7 ± 1.2                |
| **B. subtilis** (1.49 × 10⁶)   | -                                           |                                           | 37.3 ± 4.1                     | 10.3 ± 0.6                    | 10.0 ± 1.4                   | -               | 32.7 ± 2.1                |
| **P. aeruginosa** (1.07 × 10⁶) | -                                           |                                           | 4.2 ± 2.2                      | 10.0 ± 0.8                    | 9.7± 1.7                     | 9.7 ± 0.2       | 14.3 ± 0.5                |
| **P. mirabilis** (7.60 × 10⁵)  | -                                           |                                           | 36.8 ± 2.8                     | -                             | 10.7± 0.8                    | -               | 9.0 ± 1.6                 |
| **S. aureus** (7.00 × 10⁴)    | -                                           |                                           | 5.3 ± 1.5                      | 10.0 ± 0.5                    | 18.3± 2.4                    | 10.3 ± 1.5      | -                         |
| **B. cereus** (2.70 × 10⁵)    | -                                           |                                           | 22.3 ± 0.7                     | 8.2 ± 0.2                     | 14.2 ± 0.7                   | -               | -                         |
| **B. subtilis** (1.49 × 10⁵)   | 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) | -                                           |                                           | 38.3 ± 1.2                     | -                             | 14.0± 1.6                    | 12.7 ± 0.4      | -                         |
| **P. mirabilis** (7.60 × 10⁵)  | -                                           |                                           | 20.3 ± 0.6                     | 9.3 ± 0.6                     | 10.0 ± 1.2                   | 11.7 ± 0.8      | -                         |

*DMSO (used as a negative control), terpinene and cymene oils did not exhibit any inhibition zones (i.e. 0 mm). bInhibition zones reported as the median of three replicates.

Table 2: Antimicrobial activity for the recovered essential oils and some terpene standards.
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-dit tert-butylphenol [1] or α-terpinol [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 Myrtaceae species [43].

Finally, the rainy season P. guajava leaves’ oil is the most effective against the bacteria assayed exhibiting 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 leaves’ 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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**References**

1. Mc Vaugh R (1968) The genera of American Myrtaceae: an interim report. Taxon 17: 354-418.
2. Dahlgren R, Thorne RF (1984) The order Myrtales: circumscription, variation and relationships. Ann. Missouri Bot Gard 71: 633-696.
3. Govaerts R, Sobral N, Ashton P, Barrie F, Holst BK, et al. (2008) World Checklist of Myrtaceae. Kew Publishing, Royal Botanic Gardens, Surrey, UK.
4. Rojas-Rodriguez F, Torres-Córdoba G (2013) Trees Central Valley of Costa Rica Gen reproduction (Psidium friedrichsthalianum (Berg) Ndzu). Revista Forestal Mesoamericana Kourou 10: 30-31.
5. Segleaur Earle J (2008) Árboles medicinales: el guayabo. Kurú: Revista Forestal 5: 1-3.
6. Dlijas S, Čadanadov-Brunet J, Čekovčić G (2009) By-products of fruits processing as a source of phytochemicals. Chemical Industry & Chemical Engineering Quarterly 15: 191-202.
7. Shaabana HAE, El-Ghoraba AH, Shibamoto T (2012) Bioactivity of essential oils and their volatile aroma components. Review. J Essen Oil Res 24: 203-212.
