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Leaf essential oil composition and bioactivity of *Psidium guajava* from Kathmandu, Nepal

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Abstract

The essential oil from the leaves of *Psidium guajava*, collected from Kathmandu, Nepal, was obtained by hydrodistillation and analyzed by GC-MS. A total of 53 compounds were identified, accounting for 100% of the oil composition. The major components of the essential oil were (*E*)-nerolidol (35.6%) and (*E*)-caryophyllene (15.8%), with lower concentrations of (2*Z*,6*E*)-farnesol (6.7%), and ledol (5.5%). A cluster analysis of the major components of *P. guajava* leaf oils has revealed at least nine chemotypes; the sample from Nepal belongs to the nerolidol/caryophyllene chemotype. *P. guajava* leaf oil showed notable larvicidal activity against *Chaoborus plumicornis*, marginal nematocidal (*Caenorhabditis elegans*) and insecticidal (*Drosophila melanogaster*) activities, and showed no antimicrobial or cytotoxic activity.

Keywords: *Psidium guajava*, essential oil composition, (*E*)-nerolidol, (*E*)-caryophyllene, Nepal, chemotype, larvicidal.

1. Introduction

Psidium guajava L. (Myrtaceae) is native to Central and South America as well as the Caribbean [1]. *P. guajava* is known for its plethora of medicinal and therapeutic effects [1-3]. The leaves of this plant are used in traditional medicine for gastroenteritis, dysentery, and diarrhea, and leaf extracts have also been reported to show biological activities including antidiarrheal, antimicrobial, antioxidant, hepatoprotective, anti-allergy, antiplasmodial, antispasmodic, antidiabetic, anti-inflammatory, antinociceptive, and antitussive activities [1-3]. There have been several reports on the leaf oil compositions of *P. guajava* from various locations around the world, and there is wide variation in the compositions [4]. In this work, we present the leaf oil composition of *P. guajava* from Kathmandu, Nepal, and compare the composition with several previous analyses.

2. Materials and Methods

2.1 Plant Material

The leaves of *Psidium guajava* were collected from city of Kathmandu, (27° 42' 0" N, 85° 20' 0" E, 1305 m above sea level) in Kathmandu district in Bagmati Zone of Nepal on 16 May 2011. The plant was identified by Nawal Shrestha, and a voucher specimen has been deposited in the herbarium of the Tribhuvan University, Central Department of Botany, Kirtipur, Nepal. The air-dried sample (85 g) was crushed and hydrodistilled using a Clevenger type apparatus for 4 h to give a clear, colorless essential oil, which was stored at 4°C until analysis.

2.2 Gas Chromatographic – Mass Spectral Analysis

The essential oil of *Psidium guajava* was analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Injector temperature was 200 °C and detector temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2°/min to 220 °C. A 1% w/v solution of the sample in CH₂Cl₂ was prepared and 1 µL was injected using a splitless injection technique.

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [5] and stored on the MS library [NIST database (G1036A, revision D.01.00)/Chem Station data system (G1701CA, version C.00.01.080)]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The essential oil composition of *P. guajava* is summarized in Table 1.

2.3 Antimicrobial Screening

The leaf essential oil of *P. guajava* was screened for antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Aspergillus niger*; minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique as previously described [6,7].

2.4 Cytotoxicity Screening

The essential oil was tested for cytotoxicity against human MCF-7 breast adenocarcinoma cell (ATCC No. HTB-22) using the MTT assay for cell viability as previously described [6,7].

2.5 Invertebrate Toxicity Assays

The essential oil was tested for nematicidal activity against *Caenorhabditis elegans*, for larvicidal activity against *Chaoborus plumicornis* (glassworm), and insecticidal activity against *Drosophila melanogaster* (fruit fly) as described previously [8].

2.6 Hierarchical Cluster Analysis

A total of 18 *P. guajava* leaf oil compositions from the published literature as well as the composition from this study were treated as operational taxonomic units (OTUs). The percentage composition of 37 essential oil components was used to determine the chemical relationship between the various *P. guajava* essential oil samples by agglomerative hierarchical cluster (AHC) analysis using the XLSTAT software, version 2015.4.01. Pearson's correlation was selected as a measure of similarity, and the unweighted, pair-group method with arithmetic average (UPGMA) was used for cluster definition and to develop a dendrogram for the *P. guajava* samples. The resulting dendrogram is shown in Figure 1.

3. Results and Discussion

The essential oil of *Psidium guajava* was obtained in 0.5% yield and a total of 53 compounds were identified accounting for 100% of the composition (Table 1). The major components in the leaf essential oil were (*E*)-nerolidol (35.6%) and (*E*)-caryophyllene (15.8%), with lower concentrations of (*2Z,6E*)-farnesol (6.7%), and ledol (5.5%).

Antimicrobial screening of *P. guajava* leaf oil showed it to be ineffective with MIC of 625 µg/mL against *A. niger*, and > 1250 µg/mL against *P. aeruginosa*, *E. coli*, *S. aureus*, and *B. cereus*. The oil was also ineffective against MCF-7 cells as a cytotoxic agent with only 37.0±3.8% kill at a concentration of 100 µg/mL. The lack of cytotoxic activity in *P. guajava* leaf oil has been reported previously [9]. The oil was marginally nematocidal against *C. elegans* with LC₅₀ of 142 µg/mL and marginally insecticidal against *D. melanogaster* (LC₅₀ = 327 µg/mL). However, the essential oil did show larvicidal activity against *C. plumicornis* with LC₅₀ of 63.3 µg/mL. In comparison, *Cannabis sativa* leaf oil had lower nematicidal activity (LC₅₀ = 232 µg/mL), lower fruit fly insecticidal activity (LC₅₀ = 500 µg/mL), and much lower glassworm larvicidal activity (LC₅₀ = 227 µg/mL) [10].

A hierarchical cluster analysis was carried out using the essential oil compositions of 17 different analyses from Costa Rica [4], Arizona [11], Brazil [12-14], China [15], Nigeria [16], Ecuador [17], the Philippines [18], Cuba [19], Australia [21], Argentina [21], Taiwan [22], Egypt [23], Tahiti [24], and Tunisia [25], as well as this sample from Nepal. The cluster analysis (Figure 1) of the *P. guajava* leaf oils has revealed at least nine chemotypes: (1) a hexenal/benzaldehyde/cineole chemotype, represented by the sample from Costa Rica [4]; (2) a viridiflorol-rich chemotype represented by the sample from Tunisia [25]; (3) a bisabolene/sesquiphellandrene chemotype, represented by the sample from Arizona [8]; (4) a pinene/cineole chemotype (Brazil-1 [12], Brazil-2 [13], China [15]); (5) a limonene-rich chemotype (Nigeria [16], Ecuador [17], Philippines [18]); (6) a nerolidol/caryophyllene chemotype represented by a sample from Cuba [19] and this sample from Nepal; (7) a selinene/caryophyllene chemotype from Australia [20]; (8) a humulene/caryophyllene chemotype (Brazil-3 [14] and Argentina [21]); and (9) a caryophyllene/cineole chemotype (Taiwan [22], Egypt [23], Tahiti [24]). Thus, there is much variation in the chemical compositions of *P. guajava* leaf essential oils, and there does not seem to be a correlation with geographical location.

Table 1: Leaf essential oil composition of *Psidium guajava* from Kathmandu, Nepal.

| RI | Compound | % | RI | Compound | % |
|------|-----------------------------------------|------|------|-------------------------------------|-------|
| 1028 | Limonene | 0.17 | 1477 | α -Amorphene | 0.33 |
| 1031 | 1,8-Cineole | tr | 1481 | Germacrene D | tr |
| 1032 | Benzyl alcohol | 0.07 | 1492 | <i>trans</i> -Muurolo-4(14),5-diene | 0.21 |
| 1100 | Linalool | 0.27 | 1495 | <i>epi</i> -Cubebol | 1.01 |
| 1120 | <i>trans-p</i> -Mentha-2,8-dien-1-ol | tr | 1501 | α -Muurolole | 0.30 |
| 1124 | Chrysanthenone | tr | 1504 | (<i>Z</i>)- α -Bisabolene | 0.35 |
| 1134 | <i>cis-p</i> -Mentha-2,8-dien-1-ol | tr | 1510 | β -Bisabolene | 0.34 |
| 1139 | <i>trans-p</i> -Menth-2-en-1-ol | tr | 1516 | Cubebol | 1.30 |
| 1176 | Terpinen-4-ol | 0.11 | 1525 | δ -Cadinene | 4.03 |
| 1187 | (<i>3Z</i>)-Hexenyl butanoate | tr | 1533 | <i>trans</i> -Cadina-1,4-diene | 1.44 |
| 1189 | α -Terpineol | 0.90 | 1569 | (<i>E</i>)-Nerolidol | 35.59 |
| 1226 | Nerol | 0.64 | 1573 | (<i>3Z</i>)-Hexenyl benzoate | 0.28 |
| 1231 | (<i>3Z</i>)-Hexenyl 2-methylbutanoate | tr | 1584 | Caryophyllene oxide | 2.59 |
| 1252 | Geraniol | 1.48 | 1587 | Gleenol | 0.19 |
| 1324 | Methyl geranate | tr | 1601 | Ledol | 5.51 |
| 1346 | Benzyl butanoate | tr | 1616 | 1,10-di- <i>epi</i> -Cubenol | 0.27 |
| 1349 | α -Cubebene | 0.14 | 1628 | 1- <i>epi</i> -Cubenol | 2.42 |

| | | | | | |
|------|-------------------------------|-------|------|-----------------------------------|------|
| 1365 | Neryl acetate | 0.17 | 1636 | Caryophylla-4(12),8(13)-dien-5-ol | 0.41 |
| 1375 | α -Copaene | 2.82 | 1643 | Cubenol | 3.99 |
| 1382 | (3Z)-Hexenyl hexanoate | tr | 1646 | α -Muurolol | 3.05 |
| 1385 | Geranyl acetate | 0.50 | 1651 | β -Eudesmol | 0.34 |
| 1390 | β -Cubebene | tr | 1655 | α -Cadinol | 1.91 |
| 1419 | (E)-Caryophyllene | 15.80 | 1671 | <i>epi</i> - β -Bisabolol | 0.39 |
| 1451 | <i>cis</i> -Muurolo-3,5-diene | 0.22 | 1686 | α -Bisabolol | 0.12 |
| 1453 | α -Humulene | 2.53 | 1690 | Shyobunol | 0.46 |
| 1460 | <i>allo</i> -Aromadendrene | 0.46 | 1723 | (2Z,6E)-Farnesol | 6.70 |
| 1474 | Dauca-5,8-diene | 0.18 | | Total Identified | 100 |

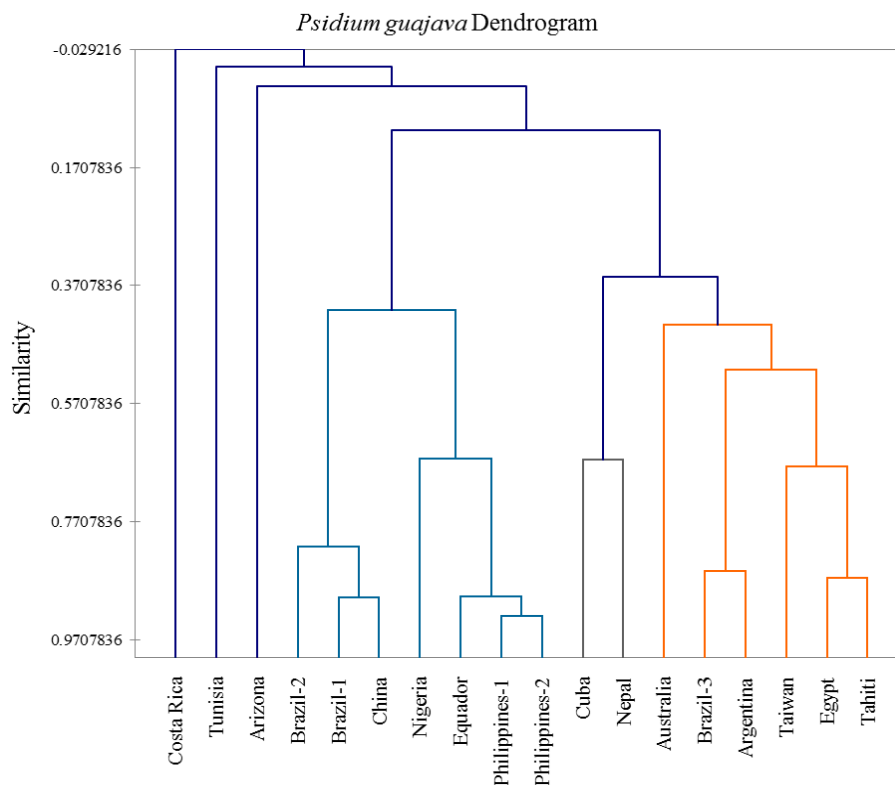


Fig 1: Dendrogram obtained from agglomerative hierarchical cluster of 18 *Psidium guajava* essential oil compositions.

4. Conclusions

Psidium guajava is an important herbal medicine in many cultures throughout the world. There is much variation in the leaf essential oil compositions, however, and which particular chemotype is available may have important implications for the traditional uses and biological activities of the plant. In this work, we have found nine different chemotypes of *P. guajava* based on essential oil composition. The sample of *Psidium guajava* from Nepal belongs to the nerolidol/caryophyllene chemotype.

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