Leaf and flower essential oil compositions of *Gliricidia sepium* (Fabaceae) from Costa Rica

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Abstract

*Gliricidia* is a small genus of flowering plants in the Fabaceae family. *Gliricidia sepium* is a multipurpose tree native to Mesoamerica and possibly northern South America. The chemical composition of the hydrodistilled essential oils of *G. sepium*, growing wild in the Central Pacific coast of Costa Rica, was analyzed by capillary gas chromatography-flame ionization detector (GC-FID) and capillary gas chromatography-mass spectrometry (GC-MS) using the retention indices on DB-5 type capillary column. A total of 96 and 109 compounds were identified in the leaf and flower oils, respectively, corresponding to 87.9% and 89.2% of the total amount of the oils. The leaf oil consisted mainly of aliphatics (54.9%) and terpenoids (28.1%). The major compounds from the leaf oil were pentadecanal (18.7%), (Z)-phytol (7.8%), methyl linolenate (6.0%) and nonanal (5.1%). The flower oil consisted mainly of aliphatics (58.9%) and terpenoids (25.8%). The major components of the flower oil were hexadecanoic acid (19.7%), myrtanol (7.7%) and (E)-nerolidol (5.9%).

Keywords: *Gliricidia sepium*, Fabaceae, essential oil composition, hexadecanoic acid, pentadecanal, Costa Rica.

1. Introduction

The Fabaceae (Leguminosae) is a large family of flowering plants with about 727 genera and over 19300 species distributed throughout the world [1]. Around the world, the family has great economic value because some species are important agricultural plants utilized for food (*Phaseolus* L. spp., beans, *Glycine max* (L.) Merr., soya, *Pisum sativum* L., peas, *Arachis hipogaea* L., peanut, etc.) Also, some species are farmed as a source of timber for construction and furniture. *Gliricidia* Kunth comprises a small number of taxa. It is constituted of low-branching flowering trees distributed mainly in tropical America. The most prominent species in this plant genus is *G. sepium* known for presenting large numbers of pink flowers which attract carpenter bees, mainly *Xylocopa fimbriata* [2] and by its dark hardwood with good potential for making pulp and paper [3].

*Gliricidia sepium* (Jacq.) Kunth ex Walp, is commonly known as “Madero negro” in Costa Rica. It is a fast growing tree which is used in the tropics for firewood, lumber, fodder and soil improvement. The tree is used as living fence posts and to provide shade in coffee and cacao plantations. It is native to Central America ranging from southern Mexico to northern South America where it occurs in a number of ecological zones and at altitudes ranging from sea level to 1200 m [4, 5]. The prevalence of natural stands in the tropical dry forest of the Pacific slope might indicate that this environment represents the true natural habitat of the species in Costa Rica [6].

*Gliricidia sepium* is a small to medium-size deciduous tree (usually about 2-15 m tall). The plant presents alternate, pinnately compound leaves, of 15 to 35 cm long, the leaflets are elliptical or lanceolate, 3 to 6 cm long and 1.5 to 3 cm wide. There are 6-24 opposed leaflet pairs and a terminal leaflet. They are dull green and pale grayish beneath, sometimes tinted bronze or purple. The inflorescences are racemes (showy clusters) and have 5 to 15 cm long, borne at the base of leaves. The flowers are pink, pink-lavender with splash of yellow or white with a conspicuous red-brown calyx [4, 7, 8]. Seed pod (legume) is dark brown, oblong, flat, from 10 to 12 cm long and 1.5 to 2 cm wide.

The leaf decoction of *G. sepium* is used in several countries as an expectorant and as a febrifuge and cold remedy [7]. Also, the leaves are used in baths for malaria, and poultice on
skin erysipelas, bruises and sores [7]. In Guatemala and Costa Rica, the bark decoction is used against protozoal diseases and for the treatment of impetigo and other skin diseases [7]. Leaves, roots and seeds are toxic to mice and giant pocket gophers (“taltuzas”) [9]. Some traditional uses and biological activities of several extracts of *G. sepium* were listed and reviewed recently by Lim [10].

The phytochemistry of *G. sepium* has been extensively investigated. This plant contains mainly flavonoids and allied phenolics [11-16], lignans [17], other aromatics [18-20] and triterpenic saponins [21, 22]. Previously, the composition of the essential oils from leaves, flowers [23] and bark [24] of *G. sepium* cultivated in Kerala, India, was published. The aim of this work was to examine the chemical composition of the oils obtained from the leaves and flowers of *G. sepium* growing wild in the Central Pacific coast of Costa Rica.

2. Materials and methods

2.1 Plant Materials

Leaves and flowers of *G. sepium* (Fabaceae), growing wild in Costa Rica, were collected during the flowering period in March-April, 2009, near Judas de Chomes (10° 06’ 27.58” N, 84° 54’ 46.94” W, 38 m elevation) in the Pacific coast, Province Puntarenas, Costa Rica. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 93891).

2.2 Isolation of the essential oils

The fresh leaflets and flowers were subjected to hydrodistillation at atmospheric pressure, for 3 h using a modified Clevenger-type apparatus. The distilled oils were collected and dried over anhydrous sodium sulfate, filtered and stored at 0-10 °C in the dark, for further analysis. The yield of the pale yellowish oil from the leaflets was 0.012 % (v/w) and from the flowers was 0.005% (v/w).

2.3 Gas chromatography (GC-FID)

The oils of *Gliricidia sepium* were analyzed by GC-FID (gas chromatography with flame ionization detector) using a Shimadzu GC-2014 gas chromatograph. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μm), (MDN-5S, Supelco), with a LabSolutions, Shimadzu GC Solution, Chromatography Data System, software version 2.3. Operating conditions were: carrier gas N₂, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min, 280 °C (2 min); sample injection port temperature 250 °C; detector temperature 280 °C; split 1:60.

2.4 Gas chromatography-mass spectrometry (GC-MS)

The analyses by gas chromatography coupled to mass selective detector were performed using a Shimadzu GC-17A gas chromatograph coupled with a GCMS-QP5000 apparatus and CLASS 5000 software with Wiley 139 and NIST computer databases. The data were obtained on a poly (5% phenyl-95% methylsiloxane) fused silica capillary column (30 m x 0.25 mm; film thickness 0.25 μm), (MDN-5S). Operating conditions were: carrier gas He, flow 1.0 mL/min; oven temperature program: 60-280 °C at 3 °C/min; sample injection port temperature 250 °C; detector temperature 260 °C; ionization voltage: 70 eV; ionization current 60 μA; scanning speed 0.5 s over 38-400 amu range; split 1:70.

2.5 Identification of the constituents

The oil components were identified using the retention indices (RI) on DB-5 type column [25] and by comparison of their mass spectra with those published in the literature [26] or those of the author’s database. To obtain the retention indices for each peak, 0.1μL of n-alkane mixture (Sigma, USA, standard mixture C₆-C₃₂, R 8769) was injected under the same experimental conditions reported above. Integration of the total chromatogram (GC-FID), expressed as area percent, has been used to obtain quantitative compositional data.

3. Results and Discussion

The composition of the hydrodistilled essential oils from leaves and flowers of *Gliricidia sepium* are presented in Table 1. The components are listed in order of elution on a MDN-5S column. Table 1 gives the relative percentages of single components, their experimental retention indices (RI) with reference to a homologous series of linear alkanes (C₆-C₃₂) and, for purposes of comparison, literature values (Lit. RI) are given.

The chemical analysis of the leaf oil showed that the major classes of constituents were aliphatics (54.9%) and terpenoids (28.1%) (See Table 2, where the percentages of the various classes of constituents of the oils are indicated). Among the 58 aliphatic compounds identified, the major constituents were aldehydes (ca. 30%). Pentadecanal (18.7%) and nonanal (5.1%) were de main ones. Of the twenty-six terpenoids identified, none was found in high concentration. The major ones were pinocarvone (1.5%), myrtenol (1.3%), β-cyclocitrinal (1.1%), α-pinene (1.0%) and β-caryophyllene (1.0%). These results differ markedly from those reported for the chemical composition of *G. sepium* leaf oil obtained from plants growing at Kerala, southwestern India on the Malabar Coast [23]. Kaniampady *et al.* reported that the leaf essential oil was characterized by large amounts of propylene glycol (25.1%), (the authors claimed that it was the first report of propylene glycol obtained from a natural source), coumarin (18.2%), (Z)-3-hexen-1-ol (17.7%) and β-farnesene (14.2%) [23]. The data of the oil samples from Costa Rican leaves do not appear to confirm the presence of that main constituent and afforded only minute amounts of (Z)-3-hexen-1-ol (0.2%) and coumarin (0.8%).

The flower oil consists mainly of aliphatics (58.9%) with 67 compounds identified (Table 2) and terpenoids (25.8%) with 35 compounds identified. The major components of the flower oil (Table 1) were hexadecanoic acid (19.7%), myrtenol (7.7%), (E)-nerolidol (5.9%), linoelene acid (3.4%), and heptacosane (3.1%). The flower oil of India [23] contains coumarin (43.1%), hydroquinone (21.6%) and myrtenol (12.7%). Of the 26 compounds reported in the Indian sample [23] only five compounds were present in Costa Rican samples. The flower oil from Costa Rica lacks coumarin and hydroquinone, the major compounds from Indian oil sample. Also, the chemical composition of the flower oil differs markedly from that of India. The flower oil composition was characterized by unbranched homologous series of hydrocarbons (C₁₅-C₂₉), alcohols (C₆-C₁₂), aldehydes (C₆-C₁₀), ketones, fatty acids, esters and many unsaturated aliphatic
compounds. Some of these compounds are probably biosynthesized from fatty acids. Among the group of the monoterpenoids, several compounds were identified with a typical pinane carbon skeleton: α-pinene, trans-pinocarveol, pinocarvone (1.8%), pinocamphol, myrtenol (7.7%), cis- and trans-myrtyrnils and myrtenyl acetate.

Table 1: Chemical and percentage composition of the essential oils from leaves and flowers of *Gliricidia sepium*.

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI</th>
<th>Lit. RI</th>
<th>Class</th>
<th>Leaf %</th>
<th>Flower %</th>
<th>Identification method</th>
</tr>
</thead>
<tbody>
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<td>Methylbenzene</td>
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<td>773</td>
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<td>839</td>
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<td>844</td>
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<td>859</td>
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<td>p-Cymene</td>
<td>1021</td>
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<td>1024</td>
<td>M</td>
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<td>(E)-3-Octen-2-one</td>
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<td>M</td>
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<td>Thuj-3-en-10-ol</td>
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<td>Structure</td>
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Table 2: The chemical class distribution in the essential oils of *Gliricidia sepium*.

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Total identified: 87.9% 89.2%
Number of compounds: 96 109

4. Conclusions
The leaf and flower essential oils of *Gliricidia sepium* were dominated by aliphatic compounds (54.9-58.9%). Hexadecanoic acid (19.7%), pentadecanal (18.7%) and nonanal (5.1%) were found to be the main constituents. Among terpenoids, the flower oil yields myrtenol (7.7%) and (E)-nerolidol (5.9%). The composition of leaf and flower essential oils of *Gliricidia sepium* from Chomes, Pacific coast of Costa Rica, appear to be markedly different to the composition of the oils from samples of the same morphological parts from cultivated material at Kerala, India.

5. Acknowledgments
The authors are grateful to Vicerrectoría de Investigación, Universidad de Costa Rica (UCR) for financial support and to C. O. Morales (Escuela de Biología, UCR) for the botanical identification.

6. References


