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CHEMICAL CONSTITUENTS OF *Tamarindus indica* L. LEAVES

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● Resumen

El tamarindo (*Tamarindus indica* L.) es un árbol tropical con múltiples usos, empleado principalmente por las propiedades nutritivas de sus hojas y frutos. Ambas partes presentan también propiedades medicinales reportadas, pero para el caso de las hojas, la información disponible sobre su composición química es limitada. En este trabajo, se determina la composición química de dos extractos de las hojas empleando para ellos técnicas de cromatografía gaseosa/espectrometría de masas (GC/MS), cromatografía en capa delgada de alta resolución/espectroscopía UV (HTLC-UV) y espectrometría por plasma inductivamente acoplado (ICP-AES). Los resultados confirman la presencia de aceites esenciales, ácidos grasos libres y esterificados, flavonoides y compuestos relacionados, pero también describen la presencia de ocho nuevos compuestos e importantes niveles de selenio y otros micro-elementos nunca antes reportados para la planta. En su conjunto, muchos de los compuestos determinados pueden ser relacionados con las propiedades atribuidas por la población: la antibacteriana y la antioxidante, en especial la relacionada con desórdenes hepáticos.

Palabras clave: *tamarindus indica*, ácidos grasos, aceites esenciales, flavonoides, microelementos.

● Abstract

Tamarind (*Tamarindus indica* L.) is a multipurpose tropical tree used primarily because its fruits and leaves have eatable properties. Both parts of the plant have also medicinal uses, but in the specific case of the leaves, little information about their chemical composition is available. In this paper, we explore the tamarind leaves’ composition of two extracts employing Gas Chromatography/Mass Spectrometry (GC/MS), High Performance Thin Layer Chromatography-Ultraviolet spectroscopy (HTLC-UV) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) techniques. Results confirm the production of essential oils, free and conjugated fatty acids, flavonoids, and other compounds, but also describe the presence of eight new compounds for this part of the plant and important levels of Selenium and other micro-elements not previously reported. As a whole, many of the detected compounds can be related to the traditional folk use: Antibacterial and antioxidant, particularly in liver diseases.

Keywords: *tamarindus indica*, fatty acids, essential oil, flavonoids, micro-elements.

● Introduction

*Tamarindus indica* L. or tamarind, as it is commonly known, is a medium-sized tree belonging to the *Caesalpinaceae* family. It’s a multipurpose tropical tree used primarily for its fruits, which are eaten fresh or processed, used as a seasoning or spice; but also as medicine beverages. In fact, their fruits are the most studied and valuable medicinal part of the plant, which has often been reported as curative in several pharmacopoeias. This millenary tree has been taken into consideration from ancient times;
documented evidences about its cultivation appear in Egypt around 400BC, and it is mentioned in the Indian Brahmasamhita Scriptures between 1200-200 BC. Also about 370-287 BC Theophrastus wrote on plants and two descriptions refer to tamarind, even though not named as such /1/.

Thai traditional medicine recognizes *Tamarindus indica* fruit as digestive, carminative, laxative, expectorant and blood tonic /2/. Many other properties have been also reported like Hypolipemic and antioxidant /3/, anti-inflammatory /4/, antimicrobial /5/, cytotoxic /6/, against gastrointestinal spasms /7/, and modifying the complement system /8/.

These extraordinary properties of tamarind fruits along with the interest on the polysaccharide and antioxidant seed compounds, keep the attention of the scientific community on tamarind tree, leaving relegated to a secondary level the other parts of the plant. On the other hand, tamarind leaves retain a more empiric use. As the fruits, tamarind leaves are also edible and are used to make curries, salads, stews and soups in many countries, but especially in times of scarcity, even when their protein ratios (4.0-5.8%) /9/ are not too far from those reported for fruits (2.0-7.1%) /10/. In fact, most of the chemical studies of tamarind leaves are focused in their eatable properties, with flavonoid compounds as the only exception, which have been reported with certain frequency /11,12/. Other classes of compounds reported are the triterpenoids lupanone and lupeol /13/, and some essential oil with benzyl benzoate and limonene as major compounds /14/.

During the latest years, the descriptions of the pharmacological potential of tamarind leaves are coming out. The hepatoprotective and the antimicrobial activity look like the most prominent activities, both associated to flavonoid and/or phenol presence. Our research group was investigating the real influence of these compounds on the antimicrobial activity, but no quantitative relationship was possible to establish /15/. Due to the relative poor knowledge about the chemical composition of tamarind leaves, no specific association compound/activity is suggested; giving rise to this investigation in which an exploration of the phytochemical constituents is proposed.

### Material and methods

#### Plant material

Tamarind leaves were collected in November 2008 from a tamarind population in Santiago de Cuba, eastern part of Cuba. A voucher specimen registered as 052216 was deposited at the herbarium of the biology department, University of Oriente, Cuba.

#### Plant extraction

The collected leaves were sun dried and then powdered with the help of a blender. Two kinds of extracts were prepared.

**Extract 1:** Twenty-five grams of the leaf powder was placed in the thimble and extracted with 150 mL of Chloroform, using a Soxhlet apparatus for 12 h. The extract was concentrated using rotary flash evaporator under reduced pressure at temperatures below 35 °C.

**Extract 2:** Fifty grams of the powered leaves were prepared as fluid extract by percolation method with four-day extractions, using ethanol 70 % as solvent.

**Fractions from extract 2:** Fluid extract was fractioned by a successive liquid-liquid separation method described for flavonoid and other related compounds /16/. Three extractions with 50 mL of n-hexane were used to generate the first fraction in this liquid-liquid separation. Successive extractions with chloroform, ethyl acetate and n-butanol were employed to obtain the other three fractions. All four fractions were dried by reduced pressure at temperatures below 50 °C and re-dissolved in absolute ethanol.

#### Chemical analysis

**Gas Chromatography-Mass spectrometry (GC-MS) analysis**

The extract 1 and the two first fractions of the extract 2 were analyzed by a Gas Chromatography-Mass spectrometry (GC-MS) analysis on a FISONS
Trio 1000 system. The experimental conditions were an SPB-1 fused silica column of 30 m · 0.32 mm, 0.25 μm film thickness, with a temperature program from 30 °C (3min), 4 °C/min till 250 °C (10 min.). Carrier gas (helium) flow rate was 1 ml/min and mass spectra were measured by electron ionization (EI) at 70 eV. Identification was made comparing mass spectra and GC retention indices (RI) with those of our IDENT data bank included in the system. Some mass spectra are also compared with literature data /17/.

**High Performance Thin Layer Chromatography-Ultraviolet spectroscopy (HTLC-UV)**

The last two fractions of the second extract were analyzed by High Performance Thin Layer Chromatography (HPTLC LINOMAT IV) coupled with a UV-visible densitometer (SCANNER II) both CAMAG from Switzerland. A bi-dimensional method employing butanol-acetic acid-water (65:15:25 v/v/v) and acetic acid 5 % as mobile phases was used, meanwhile Silica gel 60F 254 plates 10x10 with internal fluorescent indicator (Merck EMD, Germany), were employed as static phase /18/. For all compounds detected, three UV-spectrums were determined controlled by the Peak Purity option included in CATS software (Planar Chromatography Manager) version 3.20. Compound assignment was developed comparing the data obtained with literature /19-22/ and considering the previous tamarind leaves flavonoids reports.

**Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES)**

**Elemental assay:** A Nitric Acid - Perchloric Acid digestion of one gram of dried leaves, and both extracts were investigated for elemental analysis by ICP-AES, in a SPECTRO ARCOS spectrometer from Germany. Appropriate working standard solution was prepared for each element. The calibration curves and the data were statistically analyzed by using fitting of straight line by the least square method. As a way to evaluate the total inorganic substance, Total Ash determination was developed as it is described in literature /23/.

**Results and discussion**

**Gas Chromatography-Mass spectrometry (GC-MS) analysis**

The Gas Chromatography-Mass spectrometry (GC-MS) analysis on the first extract reveals 18 peaks, yielding an acceptable 90.83 % (table 1). Taking into consideration the chemical nature of the compounds extracted; great varieties of chemical compounds (essential oils, fatty acids, polyphenols and others) yield the extract 1. Previous papers signed the essential oils production in tamarind leaves /15,16/. Limonene, linalool antranilate, and p-cymene are some of those before mentioned. However, the compounds, Diphenyl-ether, Longifolene, Caryophyllene and 6,10,14-trimethylpentadeca-5,9,13-trien-2-one are also essential oils but no previous reports appear in literature. The amino substituent of the volatile linalool antranilate compound can be the cause of why tamarind leaves give an intermittent and weak evidence of alkaloid /24/.

The Diphenyl-ether is a compound with a limited distribution on plants. Nevertheless some reports in green tea, and others species as *Isatis tinctoria*, *Capparis spinosa* L., *Rosa damascena* Mill, y *Mangifera indica* L. has been found /25/. Compounds 6 and 7 have a phenolic nature. Compound 6 (2,6-di-tert-butyl-4-methylphenol) is a well recognized natural antioxidant widely distributed on plants, and compound 7 is a chemical derivate from them, but not commonly reported in the plant kingdom. These two compounds have not been previously reported for tamarind plant before.
Polyacetylenes are plant metabolites derivates from the latest steps of acetate pathway, being expressed in the final stages of this metabolic via and common in high-development plants. The compound 3-eicosine belongs to this class of metabolites that has been cited as potent antimicrobial and citotoxic substance. Compound Cryptopinone have a carbonyl function, and has been referred in several species on plant Kingdom, but neither 3-eicosine nor Cryptopinone were previously reported for *Tamarindus indica* specie.

Free and conjugated fatty acids and β-Sitosterol are also detected in the extract 1. These kinds of compounds with a high value not only as nutrient but also as antioxidant and antimicrobial /26,27/ were reported in previous papers in India and Pakistan /28,29/, but never in tamarind trees growing at the American continent. As well as these previous studies, C_{16:0} and C_{18:1} appear as the main fatty acids in tamarind leaves, but also the long chain fatty acid Methyl 15-tricosenoate is detected.

This diversity of compounds provides new elements to justify the two more reported pharmacological properties for tamarind leaves: Antioxidant and antimicrobial activity. This study also affords eight compounds not previously reported for *Tamarindus indica* leaves.

In the GC-MS analysis of extract 2, n-hexane and chloroform fractions results only in six and seven identified peaks as is shown in table 2. The relative high solvent polarity employed in this second extract (ethanol 70%) should be the cause of these few peaks found.
In n-hexane fraction, all extracted compounds result already identified in the extract 1. Even when chloroform and n-hexane belong to the same category of non-polar solvents, their diverse polarities allow chloroform fraction to extract different kinds of compounds, mainly those with acidic characteristic. Tartaric acid appears in higher concentration. This compound is reported as the responsible of the most outstanding characteristic of tamarind: its sweet acidic taste. Many other acidic compounds’-types are also extracted in this fraction. Hexadecanoic and 10-Octadecenoic acids identification occurs in both fractions, confirming that the most common fatty acid chains in tamarind leaves are C_{16} and C_{18}.

**High Performance Thin Layer Chromatography-Ultraviolet Spectroscopy (HTLC-UV)**

The latest ethyl acetate and n-butanol fractions from extract 2 were analyzed by High Performance Thin Layer Chromatography-Ultraviolet spectroscopy (HTLC-UV).

Because of the methodology employed, these fractions should be rich in flavonoids and related compounds. Those compounds have a strong absorption in UV region and this fact has been used in their chemical detection, quantification and characterization. Our studies reveal a total of 8 and 9 spots in ethyl-acetate and n-butanol fractions respectively, but only 3 and 4 spots were detected in enough concentration to be considered as main compounds and with conditions to be analyzed by the technique employed. These results are showed in table 3.

From table 3 it is inferred that spots from 1 to 6 are flavonoid derivates, and more specific flavone type, because the two characteristic band of absorption in the range 260-270 μm (band II) and 330-365 μm (band I), while spot 7 should be a polyphenol, kind of compound usually extracted together with flavonoids. Another important information from the flavonoid UV spectrum is the arithmetic difference between the both absorbance peaks to define the pattern of substitution in B ring. Values under 83 mean a single OH substitution (Spots 3 and 6), whereas over 83 mean double OH substitution (spots 1, 2, 4 and 5).

Spot 3, gives a perfect chromatography and spectroscopic match with apigenin, while spots 1 and 2 with almost identical spectroscopy features but absorption in UV region and this fact has been used in their chemical detection, quantification and characterization. Our studies reveal a total of 8 and 9 spots in ethyl-acetate and n-butanol fractions respectively, but only 3 and 4 spots were detected in enough concentration to be considered as main compounds and with conditions to be analyzed by the technique employed. These results are showed in table 3.

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Spots 1, 2, 4 and 5 are a set of flavonoids that have been identified as apigenin, luteolin, quercetin, and myricetin, respectively. The identification of these flavonoids is based on their characteristic UV absorption maxima and the comparison of their UV spectra with those of reference compounds. The retention times of these flavonoids are consistent with their reported values in the literature.
different chromatography behavior, were matched with luteolin 7-o-glucoside and luteolin respectively. The same analysis was developed for the n-butanol fraction, matching the experimental data with the data bank and literature. All the identified compounds were previously reported for tamarind leaves by Dehesa et al. in 2006 /12/.

**TABLE 3. IDENTIFIED COMPOUNDS IN THE EXTRACT 2 BY HPTLC-UV (ETHYL ACETATE AND N-BUTANOL FRACTIONS)**

<table>
<thead>
<tr>
<th>Spot</th>
<th>λ₁</th>
<th>λ₂</th>
<th>λ₃</th>
<th>Assigned comp.</th>
<th>Spot</th>
<th>λ₁</th>
<th>λ₂</th>
<th>λ₃</th>
<th>Assigned comp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.31</td>
<td>212</td>
<td>267</td>
<td>358</td>
<td>luteolin 7-o-glucoside</td>
<td>4</td>
<td>0.26</td>
<td>205</td>
<td>260</td>
</tr>
<tr>
<td>2</td>
<td>0.45</td>
<td>212</td>
<td>267</td>
<td>357</td>
<td>luteolin</td>
<td>5</td>
<td>0.44</td>
<td>214</td>
<td>266</td>
</tr>
<tr>
<td>3</td>
<td>0.62</td>
<td>203</td>
<td>267</td>
<td>336</td>
<td>Apigenin</td>
<td>6</td>
<td>0.62</td>
<td>213</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>0.63</td>
<td></td>
<td>322</td>
</tr>
</tbody>
</table>

**Inductively coupled plasma atomic emission spectrometry (ICP-AES)**

Dried leaves and both extract were investigated for elemental analysis. Traditionally, this kind of study determines common elements as Sodium, Phosphorus, Magnesium, Calcium, Potassium and others. In fact, the few reports found refer these elements /1, 29/. But none of these elements is associated to the antioxidant or antimicrobial activity, for this reason we focused our study on multi-valence cations in which an electron transfer may happen and indeed be involved in the antioxidative process, or elements which form parts of antioxidant metallo-proteins. By this way, common elements as aluminium, iron, copper, lead, selenium and zinc were considered, as well as others less common as cadmium, chromium, cobalt, manganese, nickel, strontium, molybdenum and vanadium.

The results of these cation determinations in dried leaves and both prepared extracts are showed in table 4. Metals in plants normally appear forming salts or complexes, but usually retain their hydrophilic nature, for this reason the low results obtained for extract 1 are not a surprise, due to the non-polar nature of chloroform solvent. Even when ethanol 70 % is much polar than chloroform, it cannot extract high quantities of the total metals present in leaves, meaning a maximum of 30 % for Manganese and minimum of 1,13 % for Nickel. The values of total ashes determined in leaves and both extracts confirms these observations. These differences between one metal to other must depend on the substrate nature.

**TABLE 4. ELEMENTS CONCENTRATIONS AND TOTAL ASHES DETECTED IN TAMARINDUS INDICA L. LEAVES BY ICP-AES**

<table>
<thead>
<tr>
<th>Element</th>
<th>Leaves (µg/g)</th>
<th>Extract 1 (µg/g)</th>
<th>Extract 2 (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>5,37</td>
<td>0,013</td>
<td>1,181</td>
</tr>
<tr>
<td>Cd</td>
<td>0,019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0,830</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0,250</td>
<td>-</td>
<td>0,079</td>
</tr>
<tr>
<td>Cu</td>
<td>7,900</td>
<td>0,196</td>
<td>0,857</td>
</tr>
<tr>
<td>Fe</td>
<td>16,160</td>
<td>0,241</td>
<td>1,107</td>
</tr>
<tr>
<td>Mn</td>
<td>2,500</td>
<td>0,027</td>
<td>0,750</td>
</tr>
<tr>
<td>Ni</td>
<td>0,461</td>
<td>-</td>
<td>0,052</td>
</tr>
<tr>
<td>Pb</td>
<td>0,730</td>
<td>-</td>
<td>0,056</td>
</tr>
<tr>
<td>Sr</td>
<td>0,325</td>
<td>-</td>
<td>0,051</td>
</tr>
<tr>
<td>Zn</td>
<td>7,990</td>
<td>0,031</td>
<td>0,292</td>
</tr>
<tr>
<td>Mo</td>
<td>0,250</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>4,723</td>
<td>0,083</td>
<td>1,341</td>
</tr>
<tr>
<td>Totalashes</td>
<td>3,600 %</td>
<td>0,093 %</td>
<td>0,550 %</td>
</tr>
</tbody>
</table>
Copper and Iron appears in upper concentrations than previous studies /1, 29/, also Lead concentrations are superior, but not in toxic levels. On the other hand, cadmium, zinc and specially manganese are detected in lower concentrations.

In spite of all, tamarind leaves have good quantities of the most prominent pro-oxidant/antioxidant cations: iron, cupper and selenium, therefore this property should be expressed in polar tamarind formulations.

**Conclusion**

Tamarind leaves are worldwide reported for their antioxidant and antimicrobial activity, but it hasn’t been possible to establish a relationship with the chemical composition due to the scanty information availed. In this study, we detected eight components not previously reported, and confirmed the high fatty acid and polyphenol production in Tamarindus indica L leaves. In addition, high concentration of the most prominent pro-oxidant/antioxidant cations is described. All this information give light to the pretended intention to find chemical proof that support the pharmacological activities previously described for tamarind leaves.

**Bibliography**