CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF FATTY ACIDS FROM PICRAMNIA POLYANTHA FRUITS

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ABSTRACT

Picramnia species have been estimated in traditional medicine for its healing properties, usually used in the form of infusion. Picramnia polyantha is an unusual species to be found in various regions of Mesoamerica. In this work the biological activity and the lipidic composition of the fruits of P. polyantha was preliminarily studied, in addition we report the toxicity of the diethyl ether extract of the fruit and the principal isolated compounds using the Artemia salina L. assay. The results showed the toxicity of the crude diethyl ether extract of the fruits of P. polyantha (LD$_{50} = 0.51$ µg/mL) where the fatty acids were the predominant petroselinic and tariric acids and to a lesser extent myristic, palmitic, stearic and palmitoleic acids; the mixture of all these acids showing the highest toxicity (LD$_{50} = 0.19$ µg/mL) than the original extract. Aloe-emodin and the β-sitosterol were also isolated. The structural elucidation of metabolites isolated was by GC-MS, $^1$H-NMR and $^{13}$C-NMR.

Keywords: Picramnia polyantha; fatty acids; Artemia salina L.; toxic activity.

RESUMEN

Especies del género Picramnia han sido apreciadas en Medicina Tradicional por sus propiedades curativas, que habitualmente se utilizan en forma de infusión. Picramnia polyantha es una especie inusual que se puede encontrar en diversas regiones de Mesoamérica. En este trabajo se estudió preliminarmente la actividad biológica y la composición lipídica de los frutos de P. polyantha, además se reporta la toxicidad del extracto de éter etílico de los frutos y los compuestos principales aislados usando el ensayo de Artemia salina L.

Los resultados mostraron la toxicidad del extracto crudo (DL$_{50} = 0.51$ µg/mL) donde los ácidos grasos predominantes fueron los ácidos petroselinico y tarírico, y en menor medida los ácidos mirístico, palmitico, esteárico y palmitoleico; la mezcla de todos ellos presentó mayor toxicidad (DL$_{50} = 0.19$ µg/mL) que el extracto original. También fueron aislados aloe-emodina y β-sitosterol. La dilucidación estructural de los metabolitos obtenidos se realizó por GC-MS, RMN-$^1$H y RMN-$^{13}$C.

Palabras clave: Picramnia polyantha; ácidos grasos; Artemia salina L.; toxicidad.
INTRODUCTION

The Picramnia genus had been considered within the family Simaroubaceae; nevertheless, due to the morphologic and chemotaxonomic differences occurring in the species that constitute this genus, it has recently been proposed that Picramnia has to be to a new category, the Picramniaceae family (Fernando and Quinn, 1995; Jacobs, 2003).

The genus Picramnia was described by Swartz in 1788, based on P. antidesma from Jamaica. Since that time, over 100 names have been published for the estimated 45 (Thomas, 1988) or 89 (Jacobs, 2003) described species. Because México and Central America were among the first areas of the New World to be explored botanically, the study of genera such as Picramnia suffers from a paradox: while there remain undiscovered taxa and areas to explore, much of the superfluity of names in Picramnia centers upon several variable taxa common in the region which were collected and described independently on numerous occasions (Thomas, 1988). Diverse species of this genus have been appreciated in traditional medicine for their curative properties, the bark and leaves have been used in infusions to treat some intestinal complaints, erysipelas, and venereal diseases (Standley and Steyermark, 1946; Morton, 1981; Martínez, 1989; Heywood, 1993). So, Thomas (1988) mentions that P. polyantha is a synonymy species of P. quaternaria; this last taxa is used in Costa Rica, as a tonic to stimulate the appetite and digestion and as febrifuge (Morton, 1981). On the other hand, there are few reports about the biological activity and composition of the fruit of this genus of plants; however some fruits from Picramnia species are edible as P. gracilis and P. latifolia (Morton, 1981).

Picramnia polyantha is an uncommon species found from Honduras to Guerrero, Mexico, including Puebla and Oaxaca states. Seemingly typical of Villa Alta and of the mountains of Chinantla, in Oaxaca, it is a shrub approximately 3 m tall with slender branches, terminal paniculate inflorescence, reddish petals and leaflets ranging from ovate to oblong-lanceolate; this species is apparently restricted to limestone deposits (Standley and Steyermark, 1946; Thomas, 1988; Heywood, 1993).

The precedents of Picramnia in Traditional Medicine, the type of anthraquinonic derivatives that have been isolated from this species (Solis et al., 1995; Hernández-Medel et al., 1996; Hernández-Medel et al., 1999; Rodríguez-Gamboa et al., 2000; Hernández-Medel and Pereda-Miranda, 2002; Jacobs, 2003; Diaz et al., 2004) and the scant knowledge that has been generated regarding the possible biological activity of its fruits, are the basis of our interest in discovering the secondary metabolites of P. polyantha fruits. Research on the fatty acid composition of the fruits oils of three species of Picramnia, P. latifolia, P. bonplandiana, and P. sellowii showed the presence of tariric and petroselinic acid, the more abundant being the former (62.9-85.3 %), with only a small quantity of petroselinic acid (0.8-2.9 %) (Spencer et al., 1970; Stuhlfauth et al., 1985). Thus in this work, the biological activity and the preliminary lipidic composition of P. polyantha fruits were studied.

MATERIALS AND METHODS

Solvent purification

The solvents used (hexane, benzene, ethyl ether, methylene chloride, ethyl acetate and methanol) were purchased from Técnicas Químicas (México) and were purified through distillation with rectification columns.

Thin layer chromatography (TLC)

Merck 60 GF<sub>254</sub> aluminum sheets 0.2 mm in thickness were used for thin layer chromatography (TLC) with several solvents (hexane, methylene chloride, ethyl acetate)
as eluents. The chromogens used were ultraviolet light (365 and 254 nm) and 2 % CoCl$_2$ in 10 % H$_2$SO$_4$.

**Column chromatography (CC)**
The separation and purification of the extract and compounds were performed with column chromatography (CC) with silica gel Merck, Kieselgel 60 with particle size 0.063-0.200 mm and 0.040-0.063 mm, as well as glass columns having several diameters and sizes (1.0 x 60 cm; 1.5 x 60 cm; 0.7 x 40 cm) according to the quantity of samples treated.

**Gas Chromatography-Mass Spectrometry (GC-MS)**
An Agilent 6890N GC- 5975 inert MS (Shanghai, China), operating in the electron impact (EI) mode, and a 60 m DB-23 column (50% cyanopropyl-methylpolysiloxane), internal diameter of 0.25 mm and a film thickness of 0.25 µm were used. The injector port was held at 250°C, and the oven was programmed from 100 to 250°C. The quantification of the FA was performed by GC.

**NMR spectroscopy**
The $^1$H NMR and $^{13}$C NMR data for some of the individual compounds were obtained using a Varian Mercury 300 (Palo Alto, CA, USA) spectrometer, and CDCl$_3$ and TMS as internal reference.

**Plant material**
The *Picramnia polyantha* plant was collected from Vega del Sol (Asuzul), municipality of Santa Maria Jacatepec, Oaxaca, in Mexico. The vegetable material was separated into stem, leaves and fruits, and dried at room temperature. A voucher specimen was deposited at the Herbarium of the Centro de Investigaciones Biológicas (XALU), of the Universidad Veracruzana, Mexico.

**Ethyl ether extract of *P. polyantha* fruits**
The process of extraction consisted of an exhaustive maceration of the fruits. Dried and finely chopped fruits (39.3 g) were macerated in ethyl ether (600 mL x 3) at room temperature during approximately 2 weeks. The extracts were concentrated under reduced pressure to provide the crude ethyl ether extract (16.0 g).

**Ether extract purification**
Phase separation and the purification of extract components included steps of precipitation, re-crystallization and the use of several chromatography columns. The raw ethyl ether extract was treated with acetone (500 mL), yielding a precipitate (ppt). Both the soluble fraction (ASF) and the ppt were analyzed.

The ppt was re-crystallized with a methylene chloride-acetone (2:3 v/v) system at room temperature and needle shaped crystals of white color were produced. The TLC of this precipitate showed only two spots with rf-values 0.42 and 0.37, respectively, in a hexane-ethyl acetate (9:1 v/v) system with CoCl$_2$ as the chromogenic agent. Its polarity, its coloration with CoCl$_2$ and the fact that the hydrolysis with KOH did not change the rf-values, indicated the possibility of a fatty acids mixture (FA). The separated crystals had a melting point (mp) between 48 and 51°C. The melting points were measured with a Fisher-Johns apparatus (Pittsburgh, PA, USA) and were uncorrected.

The acetone-soluble fraction (ASF) was concentrated to dryness and submitted to diverse chromatographic methods to isolate two known compounds, which were characterized by spectroscopic methods.

**Fatty acids methylation and separation**
The methyl ester derivatives of FA components were obtained following the Anu and Rao methodology (Anu and Rao, 2002). The crude oil of reaction was separated through CC with silica gel (0.040-0.063 mm) and the elution was performed successively with hexane, and mixtures of hexane-benzene (9:1, 8:2, 7:3, 6:4, 5:5 v/v and 100% benzene). Two fractions were obtained with
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**Biological tests of extract activity**

Activity tests were performed on the raw extract and the isolated lipidic fraction. A toxicity bioassay was carried out using brine shrimp eggs (*Artemia salina* L.), in accordance with the procedure described by (McLaughlin, 1991). Briefly, in this method, *Artemia salina* eggs were incubated at 27°C in artificial sea water (3.7% solution of iodine free salt in distilled water). After 48 h, the obtained nauplii were considered to be ready for the evaluations.

The testing material consisted of 4 mg of *Picramnia polyantha* ethyl ether extract and 4 mg of lipidic fraction, which were dissolved in 4 mL of methylene chloride. Then, aliquots of 1000, 100, 10 and 1 µL were placed in vials and evaporated to dryness. To facilitate the dissolution of the samples, 50 µL of methyl sulfoxide (DMSO, J.T. Baker, México) were added to each vial. Saline solution was used for dilutions and as control.

To each vial, 1 mL of saline solution and 10 shrimp were added; the volume was adjusted to 5 mL with saline solution and was allowed to incubate for 24 h. After this time period, the survivors were counted, and the percentages of deaths at each dose were recorded according to Abbot’s formula (McLaughlin, 1991):

\[
\%M = \frac{m_e - m_b}{10 - m_b} \times 100
\]

where \(m_e\) = dead shrimp in the sample and \(m_b\) = shrimp dead in the blank.

This evaluation was performed in triplicate.

**Experimental data processing for LD\textsubscript{50} estimate and statistical analyses**

Lethal dose 50 (LD\textsubscript{50}) was calculated from experimental data by regression analyses, with the TableCurve Windows software (TableCurve Windows); the goodness of fit was judged by R\textsuperscript{2} value (R>0.90) and ANOVA test for regression.

**RESULTS AND DISCUSSION**

The bioactivity of *P. polyantha* fruit ethyl ether extract, using *Artemia salina*, exhibited its toxicity as LD\textsubscript{50} = 0.51 µg/mL, indicating the presence of compounds with possible biological activity. The separation of the extract (16.0 g) from non-polar components was realized using acetone, and the resulting precipitate was re-crystallized from methylene chloride-acetone, this produced 7.9 g of white needles whose mp = 48-51°C and whose weight represented 49 % of the original extract. The toxicity evaluation of this precipitate was positive too, with LD\textsubscript{50} = 0.19 µg/mL. The TLC analysis of the ppt (FA) indicated the presence of two main spots with rf = 0.42 and 0.37 (hexane-ethyl acetate 9:1 v/v) respectively. The methyl ester derivatives of the ppt were obtained by the Anu and Rao methodology (2002) which produced two main spots by means of TLC (rf = 0.77 and 0.63 in hexane-ethyl acetate 9:1 v/v), which were separated by means of CC.

The GC-MS analysis of the mixture with an rf = 0.77 showed five peaks corresponding to five methyl ester derivatives of FA.

The mass spectra of peaks 1, 3, and 5 were similar, i.e., a base peak at m/z 74 (McLafferty rearrangement), fragments separated by 14 units of atomic mass (uma), besides at M-31 (M-CH\textsubscript{3}O\textsuperscript{+}) peak. In addition, they showed molecular ions with m/z 242, 270 and 298 peaks respectively, which correspond with the molecular formula for saturated FA myristic C14:0 (C\textsubscript{15}H\textsubscript{30}O\textsubscript{2}), palmitic C16:0 (C\textsubscript{17}H\textsubscript{34}O\textsubscript{2}) and stearic C18:0 (C\textsubscript{19}H\textsubscript{38}O\textsubscript{2}) acids, in agreement with information from pertinent literature (Pretsch et al., 1989; Silverstein et al., 1991; McLafferty and Turecek, 1993).

The complexity of the mass spectrum
of peak 2 suggested the presence of a double bond, in addition to the base peak at m/z 74 (McLafferty rearrangement), the M⁺-31 (M⁺-CH₃O) peak, and the molecular ion at m/z 268, which corresponded to the molecular formula of palmitoleic acid C₁₆:1 (C₁₇H₃₆O₂) (Pretsch et al., 1989; Silverstein et al., 1991; McLafferty and Turecek, 1993).

The mass spectrum of peak 4 exhibited a molecular ion at m/z 296 indicating, as in the case of peak 2, the presence of a double bond. Also, it presented fragments at m/z 59 and 74, characteristic of the loss of CO₂, CH₃, and CH₂COCH₃, respectively, as well as m/z 222 according to M⁺-74, and m/z 265 due to M⁺-31. These spectral data were in accordance with the structure of the methyl ester of petroselinic acid C₁₈:1 (C₁₉H₃₄O₂) (Guiet et al., 2003, and computer database). To support these interpretations, we obtained the ¹H NMR spectrum, which showed the following signals δ 5.38 (dd, J = 7.1, 8.3 Hz, H-6 and H-7), 3.66 (s, OCH₃), 2.32 (t, J = 7.2 Hz, H-2), 1.96 (m, H-5 and H-8), 1.62 (m, H-3), 1.25 (s) and 0.87 (t, J = 7.2 Hz, H-18, CH₃). The coupling constant (J= 8.3 Hz) was in agreement with the cis double bond geometry. In addition the vinyl proton signal was compared with cis and trans fatty acids spectra, making evident the similarity to cis compounds, which is due to smaller coupling constants for cis (6-15 Hz) than for trans (11-18 Hz). (Christie, 2009)

The GC-MS analysis of the mixture with rf value of 0.63 showed a major component whose mass spectrum exhibited a molecular ion m/z of 294 with formula C₁₈H₃₄O₂. In addition to the fragments m/z 220 (M⁺-74) and m/z 263 (M⁺-31), it showed a very intense fragment at m/z 154 indicating the presence of a triple bond in the position C-6 (¹³C NMR δ 80.73 (C-7), 79.35 (C-6)), which agreed with the structure of the methyl derivative of tariric acid (Li et al., 2003). Table 1 summarizes the GC-MS information about the identified fatty acids.

<table>
<thead>
<tr>
<th>FA-methyl ester</th>
<th>M⁺ [m/z]</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirystic acid</td>
<td>C₁₅H₂₉O₂</td>
<td>242</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>C₁₇H₃₄O₂</td>
<td>270</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>C₁₇H₃₆O₂</td>
<td>268</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>C₁₇H₃₆O₂</td>
<td>298</td>
</tr>
<tr>
<td>Petroselinic acid</td>
<td>C₁₉H₃₄O₂</td>
<td>296</td>
</tr>
<tr>
<td>Tariric acid</td>
<td>C₁₉H₃₄O₂</td>
<td>294</td>
</tr>
</tbody>
</table>

* Traces, < 1% of total FA

The remaining ASF extract (51 %) presented a very complex miscellany of compounds; nevertheless, the repeated use of CC on silica gel yielded two known compounds, which were identified as aloe-emodin and β-sitosterol (Hernández-Medel et al., 1999) through comparison with authentic samples and spectral data.

The isolated FA blend presented a higher toxicity (LD₅₀ = 0.19 µg/mL) than the original extract (LD₅₀ = 0.51 µg/mL), which made it clear that the toxicity stemmed precisely from the miscellany of FA.

Tariric acid was the most abundant in the lipid fraction (81.40 %), which means that P. polyantha fruit can be a potential source of this acid. It has been found that tariric acid has a diversity of uses as a pesticide (Fatope et al., 2000), antibiotic (Li et al., 2003), etc.

Petroselinic acid, whose presence was believed to be limited to members of the families Umbelliferae and Apiaceae (Guiet et al., 2003), has already been found in other families such as Geraniaceae (Tsevegsuren et al., 2004), Cornaceae and Sterculiaceae (Jacobs, 2003). The petroselinic acid structure presents a double bond at the C-6 position, which gives this acid an important industrial potential. Through the breakage of this double bond, petroselinic acid may be the precursor lauric acid, which is a component of detergents and surfactants, in addition to adipic acid (6:0 dicarboxylic), which is the precursor of nylon 6,6. On the other hand, when petroselinic acid forms
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Triacylglycerids, it makes itself resistant to hydrolysis by pancreatic lipase; then the oils with petroselinic triacylglycerids can acquire value as low-calory fats (Ohlrogge et al., 1995). This acid has been used in cosmetology (Barrett et al., 2002) and recently it has been found to be a powerful inhibitor of topoisomerase (Suzuki et al., 2000).

These rare FA, especially tariric acid, have been a motive for study at the genetic level (Cahoon et al., 2005) to characterize the metabolism that produces them, since the position of the double and triple bond that they possess in C-6 makes them special because animal tissues are deficient in introducing double bounds in positions previous to C-9, counting from the terminal methyl. This knowledge should be useful in the production of new fatty acids in the oils of seeds from transgenic plants.

### CONCLUSIONS

The presence of tariric and petroselinic acid in the oils of seeds from other species of *Picramnia* has been established (Stuhlfauth et al., 1985), the most abundant being the former (57.6-85.3 %) with only a small quantity of petroselinic acid (3.31-7.67 %). Nevertheless, this is the first time that they have been isolated, together with miristic, palmitic, stearic and palmitoleic acids from *P. polyantha* fruit, a species that should be considered as a potential source of tariric acid.

### ACKNOWLEDGMENT

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### REFERENCES


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Table 2. Results from the bioassay of the extract and FA from *P. polyantha*.

<table>
<thead>
<tr>
<th>C*</th>
<th>Ethyl-Ether extract</th>
<th>FA fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dead shrimp*</td>
<td>SD %M</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Control</td>
<td>3.33</td>
<td>0.57</td>
</tr>
<tr>
<td>0.2</td>
<td>6</td>
<td>1.41</td>
</tr>
<tr>
<td>2.0</td>
<td>7</td>
<td>2.82</td>
</tr>
<tr>
<td>20.0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>200.0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>LD₅₀</td>
<td>0.51 *</td>
<td>0.19 **</td>
</tr>
</tbody>
</table>

*Dead shrimp average of 10 in triplicate assays

*Concentration in µg of sample/mL of saline solution

**With R = 0.9486

§ With R = 0.9949


TableCurve Windows, v1.0, Jandel Scientific, San Rafael CA.
