Isolation and Characterization of Flavones from Artemisia monosperma

Lubna Abu-Niaaj, Ibrahim Katampe

ABSTRACT
Background: Artemisia monosperma (Delile) is a green aromatic shrub that grows widely in the deserts of Middle East, Africa and China. This plant is commonly used in folk medicine as a remedy of a wide range of illness including gastrointestinal disorders, inflammation, diabetes and microbial infection. The different categories of the secondary metabolites identified from Artemisia species are recognized for their biological activities as antioxidants, anti-inflammatory and antimicrobial compounds. Objective: This study aims to isolate new flavonoids from A. monosperma that might have potential biological activities thus be translated into pharmaceutical uses. Materials and Methods: Air-dried A. monosperma extraction was done using different organic solvents. The methanolic extract was selected for isolation of flavonoids using column chromatography and thin layer chromatography. The chemical structures of the isolated flavones determined based on spectroscopic analysis of ultraviolet, mass and nuclear magnetic resonance spectra. Results: Nine flavone aglycones were isolated and identified from the methanolic extract; four of which are reported for the first time from A. monosperma. These include: 5-Hydroxy-3',4', 6,7-tetramethoxyflavone; 3',5-dihydroxy-4',6,7-trimethoxyflavone (eupatorin); 5,6-dihydroxy-4',7-dimethoxyflavone(ladanein); and 2',4',5-trihydroxy-5', 6,7-trimethoxyflavone (arcapillin). The remaining five flavones were previously identified from this plant as : 4',5-Dihydroxy-3',6,7-trimethoxyflavone(cirsilineol); 5,7-dihydroxy-3',4',6-trimethoxyflavone (eupatilin); 4',5,7-trihydroxy-3',6-dimethoxyflavone(jaceosidin); 4',5-dihydroxy-6,7-dimethoxyflavone (circimaritin) and 4',5,7-trihydroxy-6-methoxyflavone (hispidulin). In addition, two acetophenone derivatives were isolated from fractions yielded selected flavones and these were identified as 4-hydroxyacetophenone and 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxy-ace-to-phenone. Conclusion: This successful isolation of these natural flavonoids from A. monosperma can contribute further to the evaluation of bioactive compounds against disorders including but not limited to inflammatory associated disorders and microbial infections.

Key words: Artemisia monosperma, Flavones, Antioxidants, Antimicrobial activity,
**Extraction of Plant**

Several organic solvents were used to extract flavonoids from the plant. The dried plant (7.0 Kg) was immersed in petroleum ether at room temperature for 10 days with frequent agitation to extract lipids. The petroleum ether was filtered, evaporated under reduced pressure to give a crude extract that was kept for later use. The plant material was further extracted repeatedly with multiple organic solvent systems as shown in Figure 1. All chemicals and organic solvents used were of analytical grade and purchased from Fisher Scientific.

**Fractionation of the Methanolic Crude Extract**

The methanolic crude extract (183 g) was subjected to fractionation using column chromatography and Thin Layer Chromatography (TLC). It was adsorbed on 150g silica gel, and loaded on a previously packed silica gel column with chloroform (8cm x 60cm, 1kg silica gel 60 PF254, 70-230 mesh). Elution of the column was initially with chloroform then the polarity was increased gradually by adding 95% ethanol and/or methanol. Eluents collected in 500 ml, analyzed by TLC, and combined into twelve fractions that were concentrated and subjected to further fractionation by column chromatography.

**Purification**

The identified flavones were isolated from eight fractions subjected to column chromatography. Each selected fraction was loaded on a silica column (0.35-0.50 Kg silica gel 60 PF254) packed in benzene: ethyl acetate (85%-75%). The loaded quantities were: fraction II (10.7g); fraction III (8.0g of 14.2g); fraction IV (11.7 g); fraction V (15g of 25 g), fraction VI (8.0g); fraction VII (7.5g); VIII (7.5g). The polarity of the eluted solvent was increased gradually until pure ethyl acetate was reached. Fractions were collected in 500ml and combined according to their TLC analysis. Further purification of fractions was achieved by either crystallization or by fractionation by TLC plates using suitable solvent systems. The structural elucidation of compounds was achieved by spectral analysis of 1H-NMR, UV, and mass data.

**Ultraviolet (UV) Analysis**

UV spectra were recorded on a Nikon 810 spectrophotometer. Identification of functional groups on the flavone nucleus was obtained by comparison of the spectrum of the compound in methanol to those obtained in the presence of acidic or basic shift reagents (sodium methoxide, aluminum chloride in presence or absence of hydrochloric acid).

**Mass Spectroscopy Analysis**

The mass spectral data obtained by the electron impact method (eim) at 70-90 eV on a kratos MS 9/50 double-focusing high resolution mass spectrophotometer.

**Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis**

The proton nuclear magnetic resonance (200 MHz 1H-NMR) spectra were recorded on a Brucker WP-200 spectrometer. Proton chemical shifts presented as parts per million (ppm) on a scale relative to tetramethylsilane using a deuterated chloroform peak at 7.26 ppm as an internal standard. Low-soluble compounds were dissolved in hexadeuteriodimethyl sulfoxide (DMSO-d6). The coupling constants are given in Hertz (Hz).

**RESULTS**

Four flavone aglycones are reported for the first time from A. monosperma. These are: 5-hydroxy-6,7,3',4'-tetramethoxyflavone, ladanein, eupatorin and arcapillin. Other five previously reported flavone aglycones are identified as: eupatilin, cirsimaritin, cirsilineol, hispidulin, and jaceosidin. Table 1 lists the obtained flavones, their chemical formula, common names, quantities, and molecular weights. The structural elucidation of compounds was achieved by spectral analysis of proton nuclear magnetic resonance, ultraviolet, and mass data. Table 2 lists the analysis of UV spectra, and Figure 2 shows the 1H-NMR spectra. The organic solvent mixtures used to obtain the flavones by crystallization or by fractionation using TLC plates as listed in Table 3.

Two acetophenone derivatives were also isolated and identified as 4-hydroxyacetophenone (Figure 3) and 3-(2-Hydroxymethyl-2-buten-4-yl)-4-Hydroxyacetophenone (Figure 4) from fractions previously yielded flavones. The TLC of fraction IV using chloroform: methanol: ammonia gas (8:2 v/v) produced 115 mg of 4-Hydroxyacetophenone which is known as piceol. 500 mg of 3-(2-Hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone was obtained by TLC of fractions V and VII using chloroform: methanol: ammonia gas (7:5: 2.5 v/v). The UV spectrum of this compound in methanol showed a band at 278 which was shifted to 354 upon addition of sodium methoxide indicating the presence of a phenolic group. The mass spectrum showed a molecular ion peak at m/e 202 due to the loss of a water molecule.

**DISCUSSION**

Flavones, a subgroup of flavonoids, have a basic skeleton that consists of three rings as shown below. The antioxidant activity of flavonoids is
related to their structure especially the hydroxy substitution of the aromatic A and B rings and the substitution pattern of the C-ring.²⁴

Table 1: Flavones isolated from *Artemisia monosperma* (Delile); chemical names and structures, common names, obtained quantities and molecular formula

<table>
<thead>
<tr>
<th>Chemical Name of Compounds</th>
<th>Chemical Structure</th>
<th>Common Name</th>
<th>Quantity (mg)</th>
<th>Mr (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  5-Hydroxy-3',4',6,7-tetramethoxyflavone</td>
<td><img src="image1.png" alt="Chemical Structure" /></td>
<td><em>N/A</em></td>
<td>175</td>
<td>358</td>
</tr>
<tr>
<td>2  3',5-Dihydroxy-4',6,7-trimethoxyflavone</td>
<td><img src="image2.png" alt="Chemical Structure" /></td>
<td><em>Eupatorin</em></td>
<td>70</td>
<td>344</td>
</tr>
<tr>
<td>3  5,6-Dihydroxy-4',7-dimethoxyflavone</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td><em>Ladanein</em></td>
<td>55</td>
<td>314</td>
</tr>
<tr>
<td>4  5,7-Dihydroxy-3',4',6-trimethoxyflavone</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
<td>Eupatilin</td>
<td>640</td>
<td>344</td>
</tr>
<tr>
<td>5  4',5-Dihydroxy-3',6,7-trimethoxyflavone</td>
<td><img src="image5.png" alt="Chemical Structure" /></td>
<td>Cirsilineol</td>
<td>280</td>
<td>344</td>
</tr>
<tr>
<td>6  4',5,7-Trihydroxy-3',6-dimethoxyflavone</td>
<td><img src="image6.png" alt="Chemical Structure" /></td>
<td>Jaceosidin</td>
<td>60</td>
<td>330</td>
</tr>
<tr>
<td>7  4',5-Dihydroxy-6,7-dimethoxyflavone</td>
<td><img src="image7.png" alt="Chemical Structure" /></td>
<td>Cirsinaritin</td>
<td>415</td>
<td>314</td>
</tr>
<tr>
<td>8  4',5,7-Trihydroxy-6-methoxyflavone</td>
<td><img src="image8.png" alt="Chemical Structure" /></td>
<td>Hispidulin</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>9  2',4',5-Trihydroxy-5',6,7-trimethoxyflavone</td>
<td><img src="image9.png" alt="Chemical Structure" /></td>
<td><em>Arcapillin</em></td>
<td>180</td>
<td>360</td>
</tr>
</tbody>
</table>

* Indicates flavones isolated for the first time from *A. monosperma*.

Table 2: UV spectra for the Isolated Flavones from *Artemisia monosperma*.

<table>
<thead>
<tr>
<th>Chemical Name of Compounds</th>
<th>Common Name</th>
<th>CH₃OH</th>
<th>NaOCH₃</th>
<th>AlCl₃</th>
<th>AlCl₃+HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  5-Hydroxy-3',4',6,7-tetramethoxyflavone</td>
<td>N/A</td>
<td>340, 278</td>
<td>340, 288</td>
<td>370, 286</td>
<td>360, 289</td>
</tr>
<tr>
<td>2  3',5-Dihydroxy-4',6,7-trimethoxyflavone</td>
<td>Eupatorin</td>
<td>338, 276</td>
<td>368, 310, 276</td>
<td>368, 284</td>
<td>354, 290</td>
</tr>
<tr>
<td>3  5,6-Dihydroxy-4',7-dimethoxyflavone</td>
<td>Ladanein</td>
<td>330, 274</td>
<td>368, 296sh, 274</td>
<td>354, 303sh, 280sh</td>
<td>350, 300</td>
</tr>
<tr>
<td>4  5,7-Dihydroxy-3',4,6-trimethoxyflavone</td>
<td>Eupatilin</td>
<td>342, 276</td>
<td>370, 310, 276</td>
<td>370, 282, 260</td>
<td>358, 290, 254</td>
</tr>
<tr>
<td>5  4',5-Dihydroxy-3',6,7-trimethoxyflavone</td>
<td>Cirsilineol</td>
<td>342, 276, 242</td>
<td>406, 304sh, 266</td>
<td>376, 286</td>
<td>364, 286</td>
</tr>
<tr>
<td>6  4',5,7-Trihydroxy-3',6-dimethoxyflavone</td>
<td>Jaceosidin</td>
<td>344, 274</td>
<td>408, 338, 258sh</td>
<td>378, 281sh, 261</td>
<td>364, 286</td>
</tr>
<tr>
<td>7  4',5-Dihydroxy-6,7-dimethoxyflavone</td>
<td>Cirsinaritin</td>
<td>332, 278</td>
<td>389, 296sh, 272</td>
<td>362, 302, 290sh</td>
<td>254, 300, 262sh</td>
</tr>
<tr>
<td>8  4',5,7-Trihydroxy-6-methoxyflavone</td>
<td>Hispidulin</td>
<td>334, 276</td>
<td>394, 326</td>
<td>362, 302</td>
<td>352, 300</td>
</tr>
<tr>
<td>9  2',4',5-Trihydroxy-5',6,7-trimethoxyflavone</td>
<td>Arcapillin</td>
<td>268, 288sh</td>
<td>422, 322sh, 288sh</td>
<td>406, 316, 276</td>
<td>398, 316sh, 278</td>
</tr>
</tbody>
</table>

We report the isolation and characterization of nine flavones from the methanolic extract of *A. monosperma*; four of which are reported for the first time from this plant. The five previously reported flavones from *Artemisia monosperma* are eupatilin,²⁹ cirsinaritin, cirsilineol, jaceosidin and hispidulin.²⁹ The data of ¹H-NMR, UV and mass obtained for the different flavones match those reported in the literature.²⁹,³⁰-³⁷ The two acetophenone derivatives are of importance because of the potential of their anti-oxidative and anti-inflammatory effects of acetophenones.²⁸

Figure 2: The 200 MHz ¹H-NMR spectra of isolated flavones from *A. monosperma*. 
Mass Spectra
The mass spectrum of each flavone displayed a molecular ion peak which matches its molecular formula. The fragmentation pattern was helpful in determining the substitution pattern because the resulting C-ring fragments as well as those of A and B rings are typical for each flavone. For example, the structure of arcapillin was confirmed by the mass spectrum which revealed the presence of the A and B rings which is indicative of the two methoxyl groups in ring A (Figure 6). All isolated flavones showed similar fragmentation patterns. The mass spectrum 4-hydroxyacetophenone showed a molecular ion peak at m/e 136 which corresponds to its molecular weight and the mass spectrum of 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone showed a molecular ion peak at m/e 202 due to the loss of a water molecule (Figure 5).

Ultraviolet Spectroscopy
A summary for the UV spectral peaks under different conditions are listed in Table 2. The existence of a flavone nucleus is indicated by the UV spectrum of a flavone in methanol based on the appearance of two major absorption peaks; band I and band II. The range of absorption for each band and subsequent bathochromic shift and change in the magnitude of peak intensity upon addition of different reagents determined the functional groups on the flavone nucleus. For example, the UV spectra for the two isolated acetophenone derivatives confirmed the presence of a phenolic group. The UV spectrum of 4-Hydroxyacetophenone in methanol showed a band at 277 which was shifted to 322 upon addition of sodium bicarbonate. This shift confirmed the presence of a phenolic hydroxyl group. The UV spectrum of 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone in methanol showed a band at 278 which was shifted to 354 upon addition of sodium methoxide indicating the presence of a phenolic group.
Proton Nuclear Magnetic Resonance (1H-NMR)

1H-NMR spectra of isolated flavones are shown in Figure 2. Analysis of spectra for all isolated compounds was similar to literature data. The two isolated acetophenone derivatives were confirmed by analysis of their 1H-NMR spectra.

The 4-hydroxyacetophenone showed a single peak at 2.6 ppm indicating the three protons of the acetyl group (Figure 3). The aromatic ring protons appeared as two doublets at 7.9 ppm and 6.9 ppm, a typical pattern for the para-substituted phenyl rings. Analysis of its 1H-NMR spectrum (Figure 4) of 3-(2-Hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone matches literature data.12

CONCLUSION

Artemisia monosperma is rich in different categories of secondary metabolites known for their diverse biological activities. Flavonoids subgroups are reputed for their potent antioxidant activity and for effectiveness against inflammation and aging. Thorough studies using Artemisia species are recommended to isolate new bioactive compounds in significant quantities that will allow further biological studies in order to identify drugs of natural origin against selective diseases including inflammatory associated diseases.

ACKNOWLEDGEMENT

The authors acknowledge Central State University for the provided technical support and University of Jordan for authentication of the plant.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ABBREVIATIONS

NMR: Nuclear Magnetic Resonance; UV: Ultraviolet; TLC: This Layer Chromatography; NaOCH: Sodium methoxide; AlCl3: Aluminum Chloride; HCl: Hydrochloric Acid; ppm: parts per million.

REFERENCES

SUMMARY

- Artemisia monosperma methanolic extract was fractionated to obtain nine flavone aglycones.
- This study reports four flavone aglycones for the first time from A. monosperma: 5-Hydroxy-3',4',6,7-tetramethoxyflavone; 3',5-dihydroxy-4',6,7-trimethoxyflavone (eupatorin); 5,6-dihydroxy-4',7-dimethoxyflavone (ladanein); and 2',4',5-trihydroxy-5',6,7-trimethoxyflavone (arcapillin).
- This study reports five flavone glycosides previously identified from A. monosperma and these are: 4',5-Dihydroxy-3',6,7-trimethoxyflavone (cirsilineol); 5,7-dihydroxy-3',4',8-trimethoxyflavone (eupatilin); 4',5,7-trihydroxy-3',6-dimethoxyflavone (jaceosidin); 5,5-dihydroxy-6,7-dimethoxyflavone (circimaritin); and 4',5,7-trihydroxy-6-methoxyflavone (hispidulin).
- Two acetophenone derivatives were isolated: 4-hydroxyacetophenone from the fraction yielded jaceosidin and 3-(2-hydroxymethyl-2-buten-4-yl)-4-hydroxyacetophenone from both fractions yielded circimaritin and arcapillin.

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