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آموزش مهارت نگارش کاربردی
در تدوین و چاپ مقاله
Detection of Sesquiterpene Lactones in Ten *Artemisia* Species Population of Khorasan Provinces

**Abstract**

**Objective**
During the last three decades sesquiterpenoid lactones have emerged as one of the largest groups of plant products with over 3000 naturally occurring substances known. The genus *Artemisia* characteristically contains this biologically active type of secondary metabolites. With respect to increasing interest in the cytotoxic, antibiotic, phototoxic and antineoplastic activities of sesquiterpene lactones, identification of these compounds in Khorasani *Artemisia* species, that some of them are endemic to Iran, can be worthwhile.

**Materials and Methods**
Ten species of *Artemisia* were collected from different parts of Khorasan Provinces of Iran. IR spectra recorded as KBr disks and in CH$_2$Cl$_2$ on a Unicam dp 110 spectrometer (UK). $^1$H-NMR (500 MHz) spectra were measured in CDCl$_3$ using a Bruker DRX 500 spectrometer. Number of scans was 32 for all of the samples. The Herz-Högenauer technique was applied for preparation of the terpenoid extracts.

**Results**
Our findings revealed that *A. absinthium*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghensis*, *A. santolina* and *A. sieberi* had a high amount of sesquiterpene lactones. A little or no sesquiterpene lactone had been detected in other species. Differentiation of saturated and unsaturated sesquiterpene lactones was one of the advantages of $^1$H-NMR application in this method.

**Conclusion**
Many of tested Khorasanian *Artemisia* species contained high amounts of sesquiterpene lactones.

**Keywords:** *Artemisia*, $^1$H-NMR, IR, Sesquiterpene lactone
Introduction

The genus *Artemisia* L. is one of the largest and most widely distributed of the Asteraceae (Compositae). This genus is a large and heterogenous genus, numbering over 400 species distributed mainly in the temperate zone of Europe, Asia and North America. These species are perennial, biennial and annual herbs or small shrubs. Leaves are alternate, capitula small, usually racemose, paniculate or capitate, inflorescence, rarely solitary. Involutural bracts in few rows, receptacle flat to hemispherical, without scales and sometimes hisrute. Florets all tubular, achenes obvoid, pappus absent or sometimes a small scarious ring (1-5). The genus in Iran has 30 species which 2 of them are endemic (5-7).

Some classes of the chemical composition of the genus include monoterpenes, sesquiterpenes, sesquiterpene lactones, flavonoids, coumarins, sterols, polyacetylenes etc (3, 8). Different species of *Artemisia* have a vast range of biological effects including antimalarial (8), cytotoxic (9), antibacterial, antifungal (8) and antioxidant (10) activity.

Sesquiterpene lactones form a group of important substances by its size (approximately 3000 known structures). This group of compounds has a rather scattered botanical distribution (11). They occur in the fungi and bryophytes, here and there in the angiosperms (Apiaceae, Lauraceae, Menispermaceae), and chiefly in the Asteraceae (12).

Sesquiterpene lactones have a broad range of biological activities. Many of them are antibacterial agents, especially against gram positive bacteria (13). Certain compounds in this series are also antifungal agents (8). Some of them are antiparasitics and antihelminthics (13-15). In addition, the alkylating potential of these compounds has led to the study of their cytotoxicity (9).

In the present work, the Herz-Högenauer procedure was used (11) for exclusive extraction of sesquiterpene lactones from the aerial parts of 10 *Artemisia* species. Subsequently, the IR and 1H-NMR experiments were performed on the mentioned extracts.

Materials and Methods

*Plant materials*

Ten species of *Artemisia* were collected from different parts of Khorasan Provinces (Table 1).

<table>
<thead>
<tr>
<th>Artemisia species</th>
<th>Location</th>
<th>Collection time</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>Islamabad near Maravehtapeh-Shahrabad road (height 940 m)</td>
<td>Sep. 15, 2003</td>
</tr>
<tr>
<td><em>A. annua</em></td>
<td>Islamabad near Maravehtapeh-Shahrabad road (height 940 m)</td>
<td>Sep. 15, 2003</td>
</tr>
<tr>
<td><em>A. biennis</em></td>
<td>Chovailly-Bajgiran road (height 1650 m)</td>
<td>Dec. 24, 2004</td>
</tr>
<tr>
<td><em>A. ciniformis</em></td>
<td>Maraveh tapeh-Shahrabad road (height 940 m)</td>
<td>Aug. 8, 2003</td>
</tr>
<tr>
<td><em>A. fragrans</em></td>
<td>Maravehtapeh-Shahrabad road (height 940 m)</td>
<td>Aug. 8, 2003</td>
</tr>
<tr>
<td><em>A. khorassanica</em></td>
<td>Chovailly-Bajgiran road (height 1650 m)</td>
<td>Dec. 23, 2003</td>
</tr>
<tr>
<td><em>A. kopetdaghensis</em></td>
<td>Bazangan lake (height 1030 m)</td>
<td>Aug. 8, 2003</td>
</tr>
<tr>
<td><em>A. santolina</em></td>
<td>Khosph-Birjand (height 1290 m)</td>
<td>Sep. 19, 2003</td>
</tr>
<tr>
<td><em>A. sieberi</em></td>
<td>Ghorogh Samieabad (height 909 m)</td>
<td>Sep. 15, 2003</td>
</tr>
<tr>
<td><em>A. turanica</em></td>
<td>Ghorogh Samieabad (height 909 m)</td>
<td>Sep. 15, 2003</td>
</tr>
</tbody>
</table>

Mozaffarian, Research Institute of Forest and Rangelands, Ministry of Jahad Keshavarzi, Iran, identified these plants. Voucher specimens of the species were deposited in the Herbarium of National Botanical Garden of Iran (TARI).
**Herz-Högenauer extraction procedure**

The Herz-Högenauer technique allows removal of chlorophyll and the very common plant phenolics by lead-(\(\Pi\))-acetate precipitation in the work-up procedure, thus providing a crude terpenoid sample for the subsequent chromatographic and/or spectral analysis. The procedure carried out was as follows:

Dried and ground plant material (20 g) was soaked overnight in dichloromethane (DCM; approx. 100 ml). The slurry product was filtered and the green filtrate evaporated in vacuo. The gummy residue dissolved in 96% ethanol (approx. 50 ml) and warmed to enhance solubilisation. An aqueous solution of lead-(\(\Pi\))-acetate 5%, was added dropwise for precipitation of the fatty acids, phenolics and chlorophyll. The precipitate removed using filtration by a pad of silica gel (230-400 mesh, Merck). The filtrate was concentrated on a water bath (40-50 ° C), until a viscous mass was obtained.

**General experimental procedures**

IR spectra were recorded as KBr disks and in CH\(_2\)Cl\(_2\) on a Unicam dp 110 spectrometer (UK). \(^1\)H-NMR (500 MHz) spectra were measured in CDCl\(_3\) using a Bruker DRX 500 spectrometer. Number of scans was 32 for all of the samples.

**Results**

Ten species of *Artemisia* (aerial parts) including *A. absinthium*, *A. annua*, *A. biennis*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghis*, *A. santolina*, *A. sieberi* and *A. turanica*, were extracted by the Herz-Högenauer technique.

In IR spectra, all the samples showed strong absorptions between \(\nu\) 1730 and 1780 cm\(^{-1}\), indicating a carbonyl function. Absorptions more than \(\nu\) 1760 cm\(^{-1}\) were considered as the \(\gamma\)-lactone moiety.

*A. absinthium*, *A. ciniformis*, *A. fragrans*, *A. khorassanica*, *A. kopetdaghis*, *A. santolina* and *A. sieberi* extracts displayed strong absorption peaks at \(\nu\) 1776, 1776, 1781, 1765, 1771, 1771 and 1786 cm\(^{-1}\), respectively, indicating a high content of sesquiterpene lactones in the terpenoid extract (Figure 1).

![Figure 1. IR spectrum of the terpenoid extract of *A. khorassanica*. The absorption of carbonyl function appeared at 1765 cm\(^{-1}\).](image)

Maximum absorption peaks of carbonyl function of *A. annua*, *A. biennis* and *A. turanica* appeared at 1729, 1709 and 1745 cm\(^{-1}\) (Figure 2). Therefore, the terpenoid extracts of the latter three samples probably contained a little sesquiterpene lactones.

![Figure 2. IR spectrum of the terpenoid extract of *A. biennis*. The absorption of carbonyl function appeared at 1709 cm\(^{-1}\).](image)

Diagnostic features in the \(^1\)H-NMR spectra of sesquiterpenoid \(\alpha\)-methylene \(\gamma\)-lactones are (Unsaturated sesquiterpene lactones) two doublets (\(J_B, c = 1-4\) Hz) which appear above and below 6 ppm (Proton B, Figure 3).
Sesquiterpene Lactones in *Artemisia*

![Diagram of lactone rings and artesimlin structure](image)

Figure 3. The protons A, B and C in saturated (1) and unsaturated γ-lactone rings (2) in the left and the structure of artemisinin with unusual lactone ring in the right.

On the other hand, a diagnostic feature in the $^1$H-NMR spectra of sesquiterpenoid α-methyl γ-lactones (Saturated sesquiterpene lactones) is a quintet between 2 and 3 ppm (Proton B). All of the diagnostic features in the $^1$H-NMR spectra are not usually obscured with other signals in the terpenoid extract. Therefore, the mentioned signals can be used for determination of the type of γ-lactone moiety. However, of the samples $^1$H-NMR spectra of the present studies, samples were also almost in agreement with the obtained results from IR experiments. As shown in table 2, the main type of sesquiterpene lactones in the terpenoid extract of *A. santolina*, *A. khorassanica*, *A. kopetdaghensis* and *A. sieberi* was saturated sesquiterpene lactones.

<table>
<thead>
<tr>
<th><em>Artemisia</em> species</th>
<th>Major carbonyl function in IR spectrum (cm$^{-1}$)</th>
<th>The result of $^1$H-NMR experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. absinthium</em></td>
<td>1776</td>
<td>Unusual SL*</td>
</tr>
<tr>
<td><em>A. annua</em></td>
<td>1729</td>
<td>Unsaturated SL</td>
</tr>
<tr>
<td><em>A. biennis</em></td>
<td>1709</td>
<td>A little or no SL</td>
</tr>
<tr>
<td><em>A. ciniformis</em></td>
<td>1776</td>
<td>Unsaturated SL</td>
</tr>
<tr>
<td><em>A. fragrans</em></td>
<td>1781</td>
<td>Unusual SL</td>
</tr>
<tr>
<td><em>A. khorassanica</em></td>
<td>1765</td>
<td>Saturated SL</td>
</tr>
<tr>
<td><em>A. kopetdaghensis</em></td>
<td>1771</td>
<td>Saturated SL</td>
</tr>
<tr>
<td><em>A. santolina</em></td>
<td>1777</td>
<td>Saturated SL</td>
</tr>
<tr>
<td><em>A. sieberi</em></td>
<td>1786</td>
<td>Saturated SL</td>
</tr>
<tr>
<td><em>A. turanica</em></td>
<td>1745</td>
<td>A little or no SL</td>
</tr>
</tbody>
</table>

* Sesquiterpene lactone

$^1$H-NMR spectra of these samples showed quintet signals (Proton A) between 2 and 3 ppm (Figure 4), whereas exocyclic methylene protons peaks (Proton B, Figure 3) at 5.5 and 6.2 ppm, indicating α-methylene γ-lactone, were not observed.

![Expanded 1H-NMR spectrum of A. santolina extract](image)

Figure 4. The expanded $^1$H-NMR spectrum of *A. santolina* extract with quintet signals of protons A of saturated γ-lactone ring.

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In contrast, \textit{A. annua}, and especially \textit{A. ciniformis}, contained more amounts of unsaturated than saturated sesquiterpene lactones. As mentioned above, the major carbonyl absorption was not observed at more than 1760 cm$^{-1}$ in IR spectrum of \textit{A. annua}, and similarly, only very small exocyclic protons signals were appeared in its $^1$H-NMR spectrum (Figure 5).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.png}
\caption{The expanded $^1$H-NMR spectrum of \textit{A. annua} extract with protons B (methylene protons) signals appeared at 5.46 and 6.18 ppm as doublets.}
\end{figure}

\section*{Discussion}
Regarding the terpenoid extracts of \textit{A. absinthium} and \textit{A. fragrans}, not only quintet signals (Proton A) but also exocyclic proton signals (Protons B) were not appeared. Since the IR spectra of these samples have clearly indicated the presence of the carbonyl function of $\gamma$-lactone, it may be concerned to the presence of unusual type of sesquiterpene lactones. As an example of unusual type of sesquiterpene lactones, artemisinin, the common well-known sesquiterpene lactone with antimalarial activity, had not $\gamma$-lactone ring in its structure (Figure 3). The absence of characteristic signals of saturated and unsaturated $\gamma$-lactone ring in $^1$H-NMR spectra of \textit{A. turanica} and \textit{A. biennis} was in agreement with the results of IR experiments, which did not show any absorption peaks for a $\gamma$-lactone ring carbonyl functional group.

It should be pointed out, however, the collection time of the plants could affect the content of sesquiterpene lactones. For two specimens of \textit{A. santolina}, collected in September and October, different results were observed. With respect to IR and $^1$H-NMR experiments, the latter specimen contained more amounts of sesquiterpene lactones than the former one.

\section*{Conclusion}
It is concluded that, if an exclusive procedure such as Herz-Högenauer or Bohlmann (11) is used for the extraction of terpenoid compounds, IR and $^1$H-NMR experiments can be fruitful methods for preliminary evaluation of sesquiterpene lactones in unknown plant extracts. To date, a few studies have been done on sesquiterpene lactones of \textit{A. fragrans} (16), \textit{A. absinthium} (17). This study revealed that \textit{A. absinthium}, \textit{A. ciniformis}, \textit{A. fragrans}, \textit{A. khorassanica}, \textit{A. kopetdaghensis}, \textit{A. santolina} and \textit{A. sieberi} had high amounts of sesquiterpene lactones and are worthwhile for the individual identification of their sesquiterpene lactones.

\section*{Acknowledgment}
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References

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