Comparative Study of HPTLC Fingerprint of β-Asarone Content between Leaves and Rhizome of *Acorus calamus* L.

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Abstract: β-asarone as a major compound of Sweet Flag (*Acorus calamus* L.) family acoraceae. β-asarone accumulate on rhizome and distribute generally on the other part of plant including on leaves. The aim of this study is determine of β-asarone contents of Sweet Flag (*A. Calamus* L.) between rhizome and leaves by HPTLC method. Rhizome and leaves of Sweet Flag (*A. Calamus* L.) extracted by Stahl steam distillation. Extract rendemen is 2.4% mL/g from rhizome and 4.5% mL/g from leaves. Extract and β-asarone eluted by n-hexane:ethyl acetat (9:1). TLC plate scanned at maximum wavelength 298 nm. The result of β-asarone contents between rhizome and leave are 5.1386 µg/mL equivalent 0.23246 µg/g and 4.21866 µg/mL equivalent 0.22499 µg/g respectively.

Key word: β-asarone, *Acorus calamus* L., HPTLC.

Introduction

*Acorus calamus* L. (AC) is a perennial plant with flavoring scent that grow in aquatic environments. It has a long history of medical, cultural, and ritual use and hence was spread outside its indigenous areas in Asia and is now found across Australia, Europe, and North America¹ In India, Traditional use of AC in Ayurvedic medicine is documented for treatment of insomnia, neurosis, and remittent fever.²³

AC has been widely used alone or combined with other herbs in traditional Chinese medicine over centuries. Recent studies have suggested that β-asarone is one of the main bioactive constituents of its essential oil. Growing evidence has demonstrated that β-asarone has the properties of antifungal⁴. β-asarone is affect to central nervous system (CNS)⁵, induces apoptosis at colon cancer cell⁶, hallucinogenic⁷. The rhizome and essential oil preparations thereof contain high concentrations of α- and β-asarone, which are believed to be pharmacologically active components.⁸ The aromatic constituents namely asarylaldehyde in roots asarone in leaves are responsible for the smell of volatile oil⁹.
Currently HPTLC is often used as an alternative to HPLC for the quantification of plant products because of its simplicity, accuracy, cost-effectiveness and rapidity\textsuperscript{10}. HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time\textsuperscript{11}.

Material and Method

Twenty five gram of the air dried leaves and rhizome powder was subjected to hot extraction using Sthal steam distillation for 5 hours. The distillation of sample was repeated three times until came by oily substance. The oily substance from distillation was collected and measured the volume. HPTLC process base on Gunalan, G., et al method with any modification; the oil resulting from both of samples were applied to a commercial 10 cm × 10 cm precoated HPTLC silica gel 60-plate (Merck) on various concentration, β-asarone used as a standard.

Fifteen milliliters of mobile phase consisting of n-hexane and ethyl acetate in the ratio of 9:1 v/v was added into a single-trough chamber, to saturate it for 15 min. The plate in the chamber was developed upward over a path of upper mark. The fluorescent image was examined under UV 254 nm by using a UV viewer cabinet (CAMAG). They were captured with a WinCATS Planar Chromatography Manager documentation system (CAMAG). The excitation wavelength was 298 nm in reflection mode and the exposure time was 3 secon.\textsuperscript{12}

Result and Discussion

Sthal steam distillation results shown on table 1. Rendemen of 2.4% mL/g from rhizome and 4.5% mL/g from leaves.

Table 1. Distillation Result

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Weight (g)</th>
<th>Volume of Water (mL)</th>
<th>Oily Substance (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>75</td>
<td>750</td>
<td>1.8</td>
</tr>
<tr>
<td>Rhizomes</td>
<td>75</td>
<td>750</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Most of the phenylpropanoids were isolated by steam distillation. An oily substance namely calamol was extracted which was found to be an allyl trimethoxy benzene derivative. It is isomeric with asarone\textsuperscript{13}.

Total β-asarone content from samples shown on table 2. β-asarone content on rhizome is 5.10795 µg/mL and β-asarone content on rhizome is 4.21866 µg/mL.

Table 2. Total β-asarone Content from Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repetitions</th>
<th>Area</th>
<th>β-asarone Content (µg/µl)</th>
<th>Average Content (µg/µl)</th>
<th>Average Content (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaves</td>
<td>I</td>
<td>12498.88</td>
<td>4105.62</td>
<td>4218.66</td>
<td>4.21866</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>14850.07</td>
<td>4937.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>11105.85</td>
<td>3612.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizomes</td>
<td>I</td>
<td>18111.98</td>
<td>6092.01</td>
<td>5107.95</td>
<td>5.10795</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>19077.00</td>
<td>6433.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>8804.65</td>
<td>2798.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HPTLC chromatogram shown on figure 1 and β-asarone fingerprint from samples showed 10 tracks on figure 2.

Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and TCHM (Chinese traditional herbal medicine). The optimized chromatographic fingerprint is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs. HPTLC fingerprint analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug. 

Figure 1. HPTLC chromatogram (1-4 β-asarone standard, 5-7 leaves and 8-10 rhizome)

Figure 2. Fingerprint of β-asarone (4 tracks in front are standard, 3 tracks in middle are leaves and 3 tracks rhizomes)
Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

Standard curve on figure 3 was used for determine of β-asarone content on both of sample. On this research we use serial standard from 100 ppm, 200 ppm, 300 ppm and 400 ppm.

![Figure 3. Standard curve for samples determination](image)

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ppm</td>
<td>6335.49</td>
</tr>
<tr>
<td>200 ppm</td>
<td>19612.35</td>
</tr>
<tr>
<td>300 ppm</td>
<td>23449.10</td>
</tr>
<tr>
<td>400 ppm</td>
<td>30488.07</td>
</tr>
</tbody>
</table>

\[
y = 897.1 + 76.3x \\
r = 0.97089
\]

The maximum wave length showed on figure 4, this figure base on measurement of β-asarone standard at 298 nm.

![Figure 4. Spectrum of maximum wave length from samples](image)
Calculation result based on the regression standard curve obtained that \( \beta \)-asarone content on rhizome and leaves are 5.10795 µg/mL and 4.2186 µg/mL.

Conclusion

The existence of \( \beta \)-asarone on leaves can be used to determine the concentration. By comparing the concentration on rhizomes can give a data for identification and quality control on AC plant.

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References