ORIGINAL RESEARCH ARTICLE

HPTLC Fingerprinting Profile of Vacha (Acorus calamus Linn.)

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ABSTRACT

Introduction: Vacha (Acorus calamus Linn.) commonly called Sweet flag is a significant herb with well-recognized therapeutic potential. Choorna (powder) of its rhizome is the commonly used dosage form according to Ayurveda. High-performance thin-layer chromatography (HPTLC) fingerprinting of rhizome powder of Vacha was carried out to estimate the active constituents in the dosage form choorna. ß-asarone, which is the major phytoconstituent of rhizome, was used as standard.

Materials and Methods: HPTLC plate 5 × 10 cm, pre-coated with silica gel 60 F254 TLC plates, was used as stationary phase. Toluene: ethyl acetate: acetic acid (90:10:2) was used as mobile phase. Visualization was done under ultraviolet light 254 nm and 366 nm.

Results and Discussion: HPTLC chromatogram showed the presence of 3 comparable peaks at 254 nm and 2 comparable peaks at 366 nm, in methanolic extract of rhizome powder and standard.

Conclusion: This approach creates profile analysis of the plant rhizome and can be helpful for quality control and further researches.

1. INTRODUCTION

Vacha is a significant herb with well-recognized therapeutic potential in the purview of Ayurveda. It is botanically identified as Acorus calamus Linn., which is commonly called sweet flag. A. calamus Linn. is a semi aquatic, strongly aromatic, gregarious, and perennial tall herb belonging to the family Acoraceae.[1, 2] In Ayurveda, rhizome of the plant is utilized for therapeutic purposes. The Ayurvedic Pharmacopoeia of India recommends choorna (powder) of its rhizome in the dose of 60–120 mg for medicinal use.[12] Many references of Vacha are available in Ayurvedic classics in the context of navajata shishu poshana (confections to new born) and rasayana (rejuvenation therapy) with the ability to improve medha (grasping and retention of knowledge) and smriti (memory).[13, 14] It is frequently recommended as antiepileptic drug on account of its efficacy against apasmara (epilepsy).[15] It is an ingredient of formulations such as Pratham Brahma rasayana, Dvitiya Brahma rasayana, Saraswatarishta which confer medha and smriti.[6, 7] The drug is deepana (stimulate digestive fire) and amapacana (aid digestion of undigested food).[3, 9] The potential of Vacha to improve vak (eloquence) and swara (voice) is described in Ayurvedic classics.[10, 11] A vast variety of phytoconstituents is detected in different parts of A. calamus Linn. The main phytoconstituents in the rhizomes of the drug are ketones (α-asarone, β-asarone), alcohol group (sesquiterpene: farnesol, phenylpropene: methyleugenol), aldehydes (myrtenal, decadienal), terpenes ([Z]-ocimene, α and β-pinenes), polyphenolics (acoradin, galangin), glycosides (acorin), etc. The ability of drug to improve learning, memory, and other cognitive functions is supported by many studies.[12-14]

The immense therapeutic applications of Vacha demand better quality control and authentication. Chromatographic techniques help to isolate and identify individual components in the herbal drugs using stationary phase and mobile phase. Chromatographic fingerprint of genuine drug can represent active ingredients and prevent use of substandard and adulterated drugs. Thin-layer chromatography of alcoholic extract of Vacha on silicagel “G” plate using toluene: ethyl acetate (9:1) is described in the Ayurvedic Pharmacopoeia of India.[5] Currently, high-
performance thin-layer chromatography (HPTLC) fingerprinting is often used for estimation of active constituents of plant products due to its better resolution, reasonable accuracy, cost-effectiveness, and better results in shorter time. The present study was initiated to create detailed HPTLC fingerprinting of rhizome powder of Vacha for authentication. Comparison with this profiling can aid in sample recognition and confirmation in future researches. HPTLC fingerprinting of rhizome powder of Vacha was carried out to estimate the active constituents in the dosage form choorna. β-asarone, which is the major component of rhizome, was used as standard.[15]

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Drug

Dried rhizomes of Vacha were provided by Ambuja Institute of Ayurvedic Research and Documentation, Udayamperoor, Ernakulam district, Kerala. The drug authentication number was A-1/1020/IIB/2022. The rhizomes that were devoid of any contamination and infestation were selected. They were visually inspected for foreign matter and sorted.

2.2. Preparation of the Dosage form - choorna (Powder)

Rhizomes were chopped into small pieces. They were then powdered and sieved through mesh size 120. The powdered drug was stored in an air tight container Rhizome powder is shown in Figure 1.

2.3. Preparation of Test Solution

The powdered sample was soaked in methanol (4 × 20 mL each for 3 times, 3 days). The extracts were combined, filtered, and evaporated to dryness by rotary evaporator. Accurately weighed methanol extract (10 mg) was dissolved in methanol (1 mL) and thin-layer chromatography was carried out. 10 µL of the extract on was applied on HPTLC plate and developed to a distance of 8 cm using toluene: ethyl acetate: acetic acid (90:10:2). This solvent system was selected because it was used for thin-layer chromatography in the Ayurvedic Pharmacopoeia of India.[19] This method of development of HPTLC plate was also used by Ghosh et al., 2011.[16] After development, plate was allowed to dry in air and examined under ultraviolet light 254 nm and 366 nm.

2.4. Preparation of Standard

Standard (β-asarone) 233.4 ppm was dissolved in methanol and 10 µl of the standard was applied on HPTLC plate.

2.5. Visualization

The HPTLC plate was observed under UV light at 254 nm and 366 nm. Rf values and color of the resolved bands were recorded. After visualization and scanning, the plate was sprayed with anisaldehyde sulfuric acid reagent and heated at 105°C till the color of the bands appeared. Rf value and color of the bands were recorded.

2.6. HPTLC Conditions

HPTLC plate 5 × 10 cm, pre-coated with silica gel 60 F254 TLC plates (E. Merck) (0.2 mm thickness) with aluminum sheet support, was used. The spotting device was a CAMAG Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland); the syringe, 100 µL (from Hamilton); the developing chamber was a CAMAG glass twin trough chamber (5 × 10 cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software.

2.7. Procedure

HPTLC plate was developed using the solvent system in twin through chamber previously saturated with the solvent system for 30 min and washed the syringe twice with methanol. Plate was dried and placed in the scanner. All parameters of scanning, integration, and spectrum were entered in a file. For absorption reflection mode, plate was scanned in UV 254 nm and 366 nm using deuterium, tungsten, and mercury lamp, respectively. All the tracks were scanned and UV spectrum was scanned. Finger print of each track was taken. UV spectra spots could be compared in the spectrum display.

3. RESULTS

HPTLC fingerprinting profile of rhizome powder of A. calamus Linn. using beta asarone as standard:

3.1. Area and Peaks at 254 nm

3.1.1. Beta asarone standard

Total 4 peaks were obtained for beta asarone standard dissolved in methanol, at 254 nm. The peaks 1–4 were defined with max Rf value of −0.05 with area 219.1 AU, max Rf value of 0.05 with area 258.4 AU, max Rf value of 0.38 with area 1224.0 AU, and max Rf value of 0.75 with area 12014.9 AU, respectively, which are tabulated as follows: Table 1 shows area and peaks of beta asarone standard at 254 nm and Figures 2 and 6 shows overview graph of beta asarone standard at 254 nm.

3.1.2. Methanol extract of rhizome powder

Total 4 peaks were obtained for methanol extract of rhizome powder of A. calamus Linn. at 254 nm. The peaks 1–4 were defined with max Rf value of −0.04 with area 8611.6 AU, max Rf value of 0.36 with area 2513.8 AU, max Rf value of 0.75 with area 27626 AU, and max Rf value of 0.83 with area 997.3 AU respectively, which are shown in Table 2, Figure 3 and 6.

3.2. Area and Peaks at 366 nm

3.2.1. Beta asarone standard

Total 2 peaks were obtained for beta asarone standard dissolved in methanol, at 366 nm. The peaks 1 and 2 were defined with max Rf value of 0.38 with area 1070.7 AU and max Rf value of 0.74 with area 2385.4 AU, respectively, which are shown in Table 3, Figures 4 and 7.

3.2.2. Methanol extract of powder of dried rhizome

Total 7 peaks were obtained for methanol extract of dried rhizome powder of A. calamus Linn. at 366 nm. The peaks 1–7 were obtained with max Rf value of −0.04 with area 11457.2, max Rf value of 0.15 with area 274.1 AU, max Rf value of 0.33 with area 1616.9 AU, max Rf value of 0.47 with area 3983.8 AU, max Rf value of 0.47 with area 345.2 AU, max Rf value of 0.62 with area 1421.3 AU, and max Rf value 0.72 with area 3770.3 AU, respectively, which are shown in Table 4, Figure 5 and Figure 7.

3.3. Visualization under white light

TLC views of beta asarone standard and methanol extract of rhizome powder of Acorus calamus Linn. after derivatization in white light are shown in Figure 8.

4. DISCUSSION

Beta asarone is the phytoconstituent present in highest concentration in the volatile oil of A. calamus Linn. and is one of the main bioactive
10. CONFLICTS OF INTEREST

This study does not require ethical clearance as it is a laboratory study.

11. DATA AVAILABILITY
This is an original manuscript and all data are available for only review purposes from principal investigators.

12. PUBLISHERS NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES


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DOI link- https://doi.org/10.47223/IRJAY.2023.61207
Figure 1: Rhizome powder of *Acorus calamus* Linn

Figure 2: Overview graph of beta asarone standard at 254 nm

Figure 3: Overview graph of methanol extract of rhizome powder of *Acorus calamus* Linn. at 254 nm

Figure 4: Overview graph of beta asarone standard at 366 nm

Figure 5: Overview graph of methanol extract of dried rhizome powder of *Acorus calamus* Linn. at 366 nm

Figure 6: TLC views of beta asarone standard and methanol extract of rhizome powder of *Acorus calamus* Linn. at 254 nm
Table 1: Area and peaks of beta asarone standard at 254 nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Max Rf</th>
<th>End Rf</th>
<th>Area (AU)</th>
<th>Area %</th>
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<tbody>
<tr>
<td>1</td>
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<td>2</td>
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<td>3</td>
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<td>0.38</td>
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<td>1224.0</td>
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<tr>
<td>4</td>
<td>0.68</td>
<td>0.75</td>
<td>0.80</td>
<td>12014.9</td>
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Table 2: Area and peaks of methanol extract of rhizome powder at 254 nm

<table>
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<th>Max Rf</th>
<th>End Rf</th>
<th>Area (AU)</th>
<th>Area %</th>
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</table>

Table 3: Area and peaks of beta asarone standard at 366 nm

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<th>Max Rf</th>
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<th>Area %</th>
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<tr>
<td>1</td>
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<td>0.74</td>
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Table 4: Area and peaks of methanol extract of dried rhizome powder at 366 nm

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Rf</th>
<th>Max Rf</th>
<th>End Rf</th>
<th>Area</th>
<th>Area %</th>
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<td>0.72</td>
<td>0.81</td>
<td>3770.3</td>
<td>16.49</td>
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</tbody>
</table>

Figure 7: TLC views of beta asarone standard and methanol extract of rhizome powder of *Acorus calamus* Linn. at 366 nm

Figure 8: TLC views of beta asarone standard and methanol extract of rhizome powder of *Acorus calamus* Linn. after derivatization in white light