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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 nn 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.[2] 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).[3,4] It is frequently recommended as antiepileptic drug on account of its efficacy against *apasmara* (epilepsy).[5] It is an ingredient of formulations such as *Prathama Brahma rasayana, Dwitiya Brahma rasayana*, *Saraswatarishta* which confer *medha* and *smriti.*[6,7] The drug is *deepana* (stimulate digestive fire) and *amapacana* (aid digestion of undigested food).[8,9] 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.[2] Currently, high-

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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.* ß-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, Ernakulum 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 Pharmacopeia of India.[2] 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 finger printing 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 1616.9 AU, max Rf value of 0.37 with area 3983.8 AU, max Rf value of 0.47 with 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

constituents of the drug. HPTLC chromatogram of methanol extract of rhizome powder and standard beta asarone, at 254 nm and 366 nm, were recorded. Each peak in the chromatogram indicated presence of a specific chemical constituent. Peaks can be compared between test solution and standard for identification and quality control of *A. calamus* Linn.

At 254 nm, methanol extract of the rhizome powder and standard beta asarone showed presence of 4 peaks each. There was presence of 3 comparable maximum Rf values in both samples and peak intensities were found to be similar. The highest peak in both samples had Rf value of 0.75. Other 2 comparable Rf values in rhizome powder and standard were −0.04 and −0.05, 0.36 and 0.38, respectively. The peaks with comparable Rf values of −0.04 and −0.05 in powder and standard had areas of 8611.6AU and 219.1AU, respectively. The area of peaks with comparable Rf values of 0.36 and 0.38 in the powder and standard was 2513.8AU and 1224.0AU, respectively. The peaks with corresponding Rf value of 0.75 in both samples had area of 27626.3AU for rhizome powder and 12014.9AU for standard beta asarone.

At 366 nm, methanolic extract of the rhizome powder showed 7 peaks, while the standard beta asarone showed 2 peaks. There was presence of 2 comparable Rf values in both samples and peak intensities were found to be similar in both the samples. The highest peak in methanolic extract of rhizome had Rf value of −0.04 and area 11457.2 AU. The first comparable Rf values of rhizome powder and standard were 0.37 and 0.38 with areas of 3983.8 AU and 1070.7AU, respectively. The next comparable Rf values of rhizome powder and standard were 0.72 and 0.74 with area of 3770.3AU and 2385.4AU, respectively. The comparable peaks indicate the presence of beta asarone in the methanolic extract of rhizome powder.

5. CONCLUSION

HPTLC chromatogram showed the presence of 3 comparable peaks at 254 nm and at 2 comparable peaks at 366 nm, in methanolic extract of rhizome powder and standard. The procedure used in this research is repeatable and feasible. It offers scope for developing quality control and standardization of *Acorus calamus* Linn. Beta asarone has been identified in the plant's rhizome using HPTLC fingerprinting profile. This approach creates profile analysis of the plant and can be helpful for further researches.

6. ACKNOWLEDGMENT

Nil.

7. AUTHORS' CONTRIBUTIONS

All the authors contributed equally in design and execution of the article.

8. FUNDING

Nil.

9. ETHICAL APPROVALS

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

10. CONFLICTS OF INTEREST

Nil.

11. DATA AVAIBALITY

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

12. PUBLISHERS NOTE

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Figure 1: Rhizome powder of *Acorus calamus* Linn

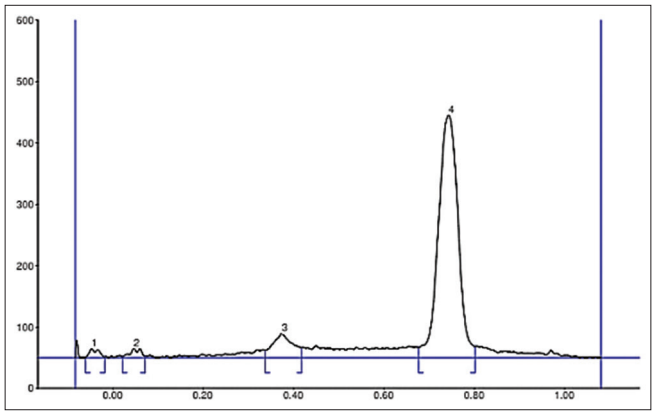

Figure 2: Overview graph of beta asarone standard at 254 nm

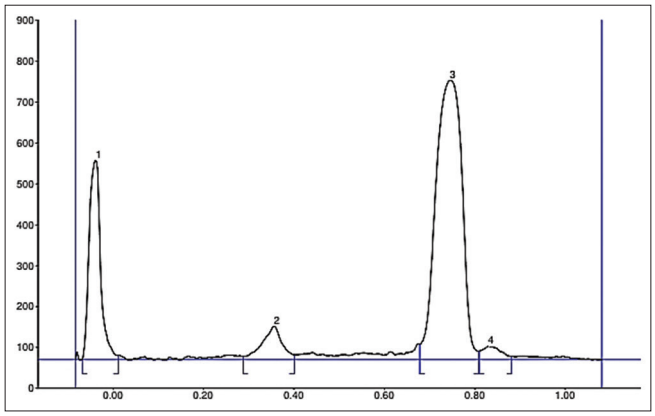

Figure 3: Overview graph of methanol extract of rhizome powder of *Acorus calamus* Linn. at 254 nm

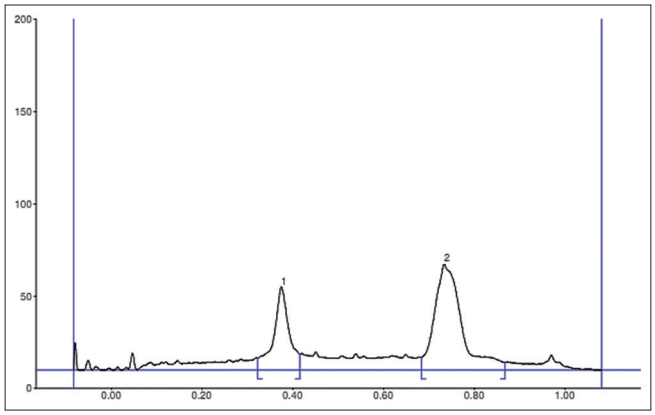

Figure 4: Overview graph of beta asarone standard at 366 nm

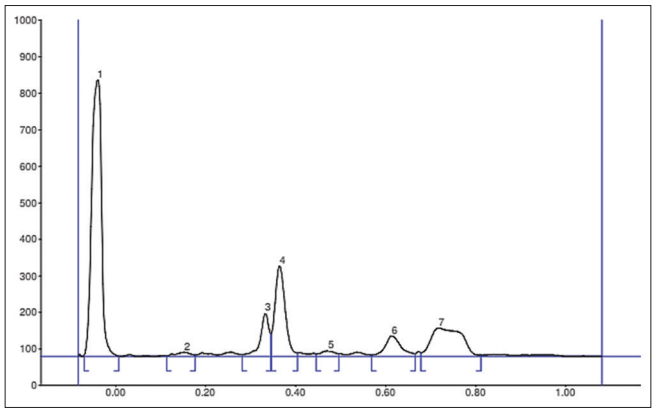

Figure 5: Overview graph of methanol extract of dried rhizome powder of *Acorus calamus* Linn. at 366 nm

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

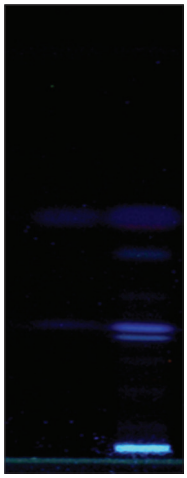

Figure 7: TLC views of beta asarone standard and methanol extract of rhizome powder of *Acorus calamus* Linn. at 366 nm

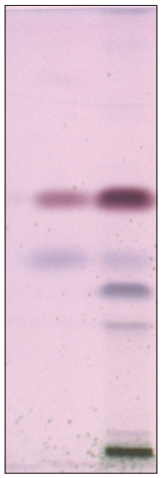

Figure 8: TLC views of beta asarone standard and methanol extract of rhizome powder of *Acorus calamus* Linn. after derivatization in white light

Table 1: Area and peaks of beta asarone standard at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	−0.06	−0.05	0.02	219.1	1.60
2	0.02	0.05	0.07	258.4	1.88
3	0.34	0.38	0.42	1224.0	8.92
4	0.68	0.75	0.80	12014.9	87.59

Table 2: Area and peaks of methanol extract of rhizome powder at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1	−0.07	−0.04	0.01	8611.6	21.66
2	0.29	0.36	0.40	2513.8	6.32
3	0.68	0.75	0.81	27626.3	69.50
4	0.81	0.83	0.88	997.3	2.51

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

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	0.32	0.38	0.42	1070.7	30.98
2	0.69	0.74	0.87	2385.4	69.02

Table 4: Area and peaks of methanol extract of dried rhizome powder at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1	−0.07	−0.04	0.01	11457.2	50.10
2	0.11	0.15	0.18	274.1	1.20
3	0.28	0.33	0.34	1616.9	7.07
4	0.35	0.37	0.41	3983.8	17.42
5	0.45	0.47	0.50	345.2	1.51
6	0.57	0.62	0.67	1421.3	6.21
7	0.68	0.72	0.81	3770.3	16.49