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COMPARATIVE PROFILING OF DIFFERENT PLANT SOURCES OF *VAASA* (ADHATODA SPECIES) USING HIGH PERFORMANCE THIN-LAYER CHROMATOGRAPHY

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ABSTRACT

Vaasa, an important medicinal plant extensively utilized in Ayurveda for its therapeutic benefits. There is an increasing demand of this plant, resulting to the availability of both wild and cultivated sources. Experts have recognized distinct morphotypes of Adhatoda species in Kerala, pointing to differences in active constituents and clinical effectiveness. This study aimed to conduct a comprehensive analysis of bioactive compounds in different plant sources of *Vaasa* (Adhatoda species) denoted as A1 (*Valiyaadalodakam*), A2 (*Cheriyaadalodakam*), A3 (Adhatoda species - *Vasika*), A4 (Adhatoda species - *Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke) using High-Performance Thin-Layer

Chromatography (HPTLC). The research involved the extraction of bioactive compounds from the leaves of these plant sources, utilizing optimized parameters for methanolic extraction. HPTLC analysis was conducted using the solvent system Toluene: Ethyl acetate: Formic acid, and the chromatograms were observed under ultraviolet light at 254nm and 366nm. After derivatization, Rf values and the color of the bands were recorded. The analysis of different *Vaasa* sources involved assessing chromatograms for the number of peaks, peak area, and Rf values. The chromatographic profiles revealed the presence of similar compounds in all samples, albeit with varying quantities. Additionally, the detection of extra compounds suggests variations in phytoconstituents among different plant sources of *Vaasa* (Adhatoda species). These findings not only contribute to the identification and quantification of bioactive compounds across diverse Adhatoda species but also illuminate potential variations in different plant sources, providing valuable insights into the botanical and chemical diversity of *Vaasa*.

Keywords: *Vaasa*, Adhatoda Species, High Performance Thin-Layer Chromatography

INTRODUCTION

Vaasa, also known as Malabar nut, has been extensively utilized in Ayurveda for various health conditions such as like *swaasa* (dyspnoea), *kaasa* (cough), *jwara* (fever), *chardhi* (vomit), and *raktapitta* (bleeding disorders).[1] The Ayurvedic Pharmacopeia of India designates *Adhatoda vasica Nees* as the official source of *Vaasa*,[2] yet some scholars propose *Adhatoda beddomei* C B Clarke as an alternative. In Kerala, locally referred to as *Aadalodakam*, two distinct varieties, *Valiyaadalodakam* and *Cheriyaadalodakam,* are identified based on their morphology.[3] *Cheriyaadalodakam,* considered smaller, is reputed to possess superior medicinal actions, particularly in Kerala.[1] The coexistence of widely occurring and cultivated varieties of *Vaasa* poses challenges in drug selection, leading to confusion among manufacturers, practitioners, and the laypeople who may randomly select plants for therapeutic formulations, disregarding official sources. Some scholars assert that the clinical efficacy is higher for the smaller variety of *Vaasa*.[1] Experts have identified

morphotypes of Adhatoda species in Kerala,[4] indicating variations in active constituents and clinical efficacy. The study underscores the imperative need for scientific validation in selecting *Vaasa* sources to ensure safety and efficacy in therapeutic formulations. There is an urgent need to scientifically validate the effectiveness of arbitrarily choosing *Vaasa* (Adhatoda species) sources for formulations, ensuring they do not lead to undesired outcomes. This approach would prevent potential adverse effects resulting from the random selection of different sources of *Vaasa* (Adhatoda species). This not only advances scientific understanding but also underscores the importance of careful plant selection, potentially guiding future clinical studies on the therapeutic potential of *Vaasa* for specific medical conditions.

Qualitative phytochemical studies identified the presence of alkaloids, flavonoids, saponins, sugars, tannins, and glycosides in different extracts of Adhatoda species.[5] High-Performance Thin-Layer Chromatography (HPTLC) is a contemporary and potent analytical technique with superior separation power, performance, and reproducibility compared to classic TLC.[6] A chromatographic fingerprint of a plant extract represents a pattern of common chemical constituents with pharmacologically active and/or chemical characteristics.[7] The present study aimed to conduct a comprehensive analysis of bioactive compounds in different plant sources of *Vaasa* (Adhatoda species) denoted as A1 (*Valiyaadalodakam*), A2 (*Cheriyaadalodakam*), A3 (Adhatoda species - *Vasika*), A4 (Adhatoda species - *Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke) using High-Performance Thin-Layer Chromatography (HPTLC).

MATERIALS AND METHODS

Collection of plant sources of *Vaasa* (Adhatoda species)

Saplings of Valiyaadalodakam and Cheriyaadalodakam were acquired from the premises of Government Ayurveda College in Tripunithura. Kerala Agricultural University (KAU) introduced two high-yielding Vaasa varieties, Vasika and Ajagandhi, characterized by a vasicine content of 2.5% following comparative yield trials.[8] The saplings of these varieties were procured from the Sales Centre of the Department of Plantation Crops and

Spices at Kerala Agricultural University (KAU) in Mannuthy, Thrissur. Additionally, the Centre for Medicinal Plants Research Institute (CMPR) in Kottakkal housed a mother plant of Adhatoda beddomei C B Clarke.[4] Saplings of Adhatoda beddomei C B Clarke were sourced from the Herbal Garden at the Centre for Medicinal Plants Research Institute (CMPR) in Kottakkal. These samples were denoted as A1, A2, A3, A4, and A5, respectively for descriptive purpose.

Table No. 1 Sources of *Vaasa* (Adhatoda species) collected and their collection sites with respective sample codes

Sources of *Vaasa* (Adhatoda species)	Sample code	Collection site
Valiyaadalodakam	A1	Campus of Government Ayurveda college,Tripunithura
Cheriyaadalodakam	A2	Campus of Government Ayurveda college, Tripunithura
Adhatoda species - *Vasika*	A3	Sales centre of Department of Plantation Crops and Spices, Kerala Agricultural University, Mannuthy
Adhatoda species- *Ajagandhi*	A4	Sales centre of Department of Plantation Crops and Spices, Kerala Agricultural University, Mannuthy
Adhatoda beddomei C B Clarke	A5	Herbal garden, Centre for Medicinal Plants Research, Institute (CMPR), Kottakkal

HPTLC analysis

a. Preparation of test solution

Methanolic extract was prepared with 0.5g sample in 10 ml methanol.

b. HPTLC conditions

HPTLC plate consists of 5 × 10cm, precoated with silica gel 60 F254 TLC plates (E. Merck) (0.2mm thickness) with aluminium sheet support. The spotting device was a CAMAG

Linomat V Automatic Sample Spotter (CamagMuttenz, Switzerland); the syringe, 100μL (from Hamilton); the developing chamber was a CAMAG glass twin trough chamber (5 × 10cm); the densitometer consisted of a CAMAG TLC scanner 3 linked to WINCATS software.

c. Procedure

The HPTLC plate was developed to a distance of 8 cm using the solvent system Toluene: Ethyl acetate: Formic acid (5:4:0.5) by applying 2µl of the methanolic extract. Then the plate was dried and observed under ultraviolet light 254nm&366nm. After visualization and scanning, the plate was sprayed with anisaldehyde sulphuric acid reagent and heated at 105 degrees Celsius till the colour of the bands appear. The Rf value and colour of the bands were recorded (CKL/ANL/HPTLC 001).

RESULTS

HPTLC finger printing profile

a. HPTLC finger printing profile of methanol extract of sample A1 (*Valiyaadalodakam*)

Area and peaks of methanol extract at 254nm

Total 5 peaks were obtained for methanol extract of dried leaf powder of sample A1 (*Valiyaadalodakam*) at 254 nm. These 5 peaks were defined with max Rf value of -0.01 with area 1576.2AU, max Rf value of 0.08 with area 200.5AU, max Rf value of 0.73 with area 528.5AU, max Rf value of 1.00 with area 180.7AU, max Rf value of 1.21 with area 456.4AU, respectively, which are tabulated as follows.

Table No. 2 Area and peaks of methanol extract of sample A1 (*Valiyaadalodakam*) at 254nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.05	-0.01	0.02	1576.2	53.57
2.	0.07	0.08	0.11	200.5	6.81
3.	0.69	0.73	0.76	528.5	17.96
4.	0.98	1.00	1.03	180.7	6.14
5.	1.17	1.21	1.24	456.4	15.51

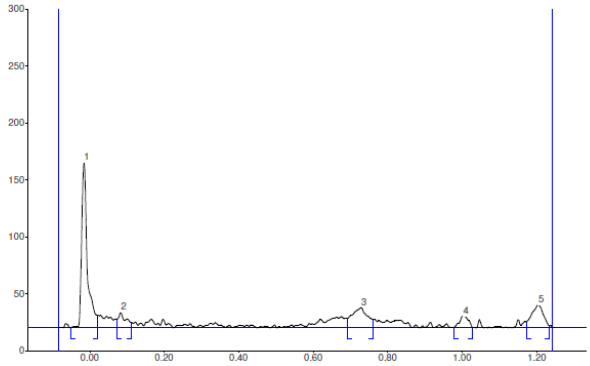


Diagram 1: Overview graph of methanol extract of sample A1 at 254nm

Area and peaks of methanol extract at 366 nm

Total 11 peaks were obtained for methanol extract of dried leaf powder of sample A1 (*Valiyaadalodakam*). The peaks were obtained with max Rf value of -0.01 with area 1544.7 AU, max Rf value of 0.03 with area 924.9AU, max Rf value of 0.62 with 230.9 AU, max Rf value of 0.68 with area 302.8AU, max Rf value of 0.71 with 380.9AU, max Rf value of 0.75 with area 262.0AU, max Rf value 0.83 with area 411.4 AU, max Rf value of 0.95 with area 148.2AU, max Rf value 1.01with area 1040.9 AU, max Rf value of 1.12 with area 3017.1, max Rf value 1.21with area 13446.8AU respectively, which were tabulated as follows.

Table No.3 Area and peaks of methanol extract of sample A1 (*Valiyaadalodakam*) at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area	Area %
1.	-0.04	-0.01	0.01	1544.7	7.11
2.	0.01	0.03	0.07	924.9	4.26
3.	0.60	0.62	0.64	230.9	1.06
4.	0.66	0.68	0.69	302.8	1.39
5.	0.69	0.71	0.73	380.9	1.75
6.	0.73	0.75	0.78	262.0	1.21
7.	0.81	0.83	0.88	411.4	1.90
8.	0.93	0.95	0.97	148.2	0.68
9.	0.98	1.01	1.04	1040.9	4.79
10.	1.05	1.12	1.15	3017.1	13.90
11.	1.15	1.21	1.24	13446.8	61.94

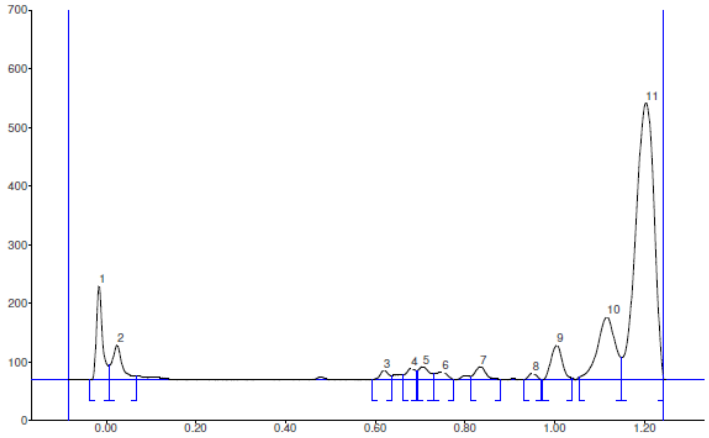


Diagram 2: Overview graph of methanol extract of sample A1 at 366nm

b. HPTLC finger printing profile of methanol extract of sample A2 (*Cheriyaadalodakam*)

Area and peaks of methanol extract at 254nm

Total 4 peaks were obtained for methanol extract of dried leaf powder of sample A2 (*Cheriyaadalodakam*) at 254 nm. These 4 peaks were defined with max Rf value of -0.02 with area 5223.5AU, max Rf value of 0.73 with area 788.7AU, max Rf value of 0.83 with area 397.9AU, max Rf value of 1.21 with area 742.8AU respectively, which are tabulated as follows.

Table No.4 Area and peaks of methanol extract of sample A2 (*Cheriyaadalodakam*) at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.03	-0.02	0.07	5223.5	73.03
2.	0.70	0.73	0.78	788.7	11.03
3.	0.82	0.83	0.85	397.9	5.56
4.	1.17	1.21	1.24	742.8	10.38

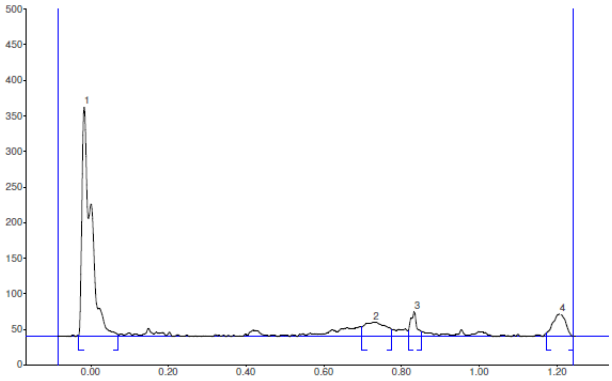


Diagram 3: Overview graph of methanol extract of sample A2 at 254nm

Area and peaks of Methanol extract at 366nm

Total 10 peaks were obtained for methanol extract of dried leaf powder of sample A2 (*Cheriyaadalodakam*) The peaks were obtained with max Rf value of -0.02 with area 2759.1AU, max Rf value of 0.02 with area 6014.6AU, max Rf value of 0.20 with 429.5 AU, max Rf value of 0.62 with area 383.0 AU, max Rf value of 0.69 with 325.7AU, max Rf value of 0.71 with area 530.6AU, max Rf value 0.75 with area 746.9 AU, max Rf value of 0.82 with area 1157.5AU, max Rf value 1.00 with area 633.9 AU, max Rf value of 1.12 with area 1247.5 AU respectively, which were tabulated as follows.

Table No. 5 Area and peaks of methanol extract of sample A2 (*Cheriyaadalodakam*) at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.02	0.00	2759.1	19.39
2.	0.00	0.02	0.10	6014.6	42.27
3.	0.19	0.20	0.22	429.5	3.02
4.	0.59	0.62	0.64	383.0	2.69
5.	0.66	0.69	0.69	325.7	2.29
6.	0.69	0.71	0.73	530.6	3.73
7.	0.73	0.75	0.79	746.9	5.25
8.	0.79	0.82	0.89	1157.5	8.13
9.	0.98	1.00	1.03	633.9	4.46
10.	1.04	1.12	1.15	1247.5	8.77

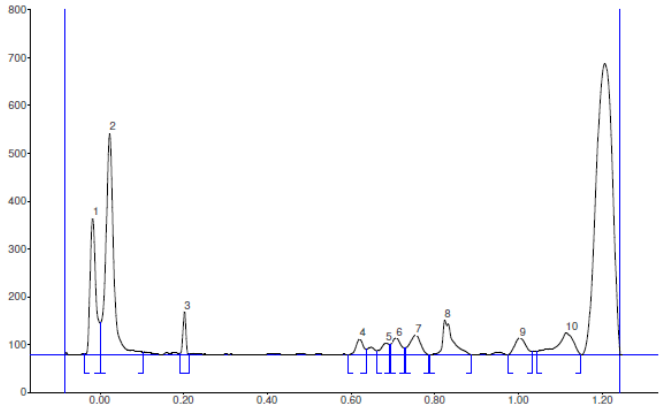


Diagram 4: Overview graph of methanol extract of sample A2 at 366nm

c. HPTLC finger printing profile of methanol extract of sample A3 (Adhatoda species-*Vasika*)

Area and peaks of Methanol extract at 254nm

Total 4 peaks were obtained for methanol extract of dried leaf powder of sample A3 (Adhatoda species-*Vasika*) at 254 nm. These 4 peaks were defined with max Rf value of -0.01 with area 3822.5AU, max Rf value of 0.67 with area 351.1AU, max Rf value of 0.74 with area 596.2AU, max Rf value of 1.21 with area 389.2AU, respectively, which are tabulated as follows.

Table No.6 Area and peaks of methanol extract of sample A3 (Adhatoda species-*Vasika*) at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.01	0.05	3822.5	74.09
2.	0.63	0.67	0.68	351.1	6.80
3.	0.70	0.74	0.77	596.2	11.56
4.	1.18	1.21	1.23	389.2	7.54

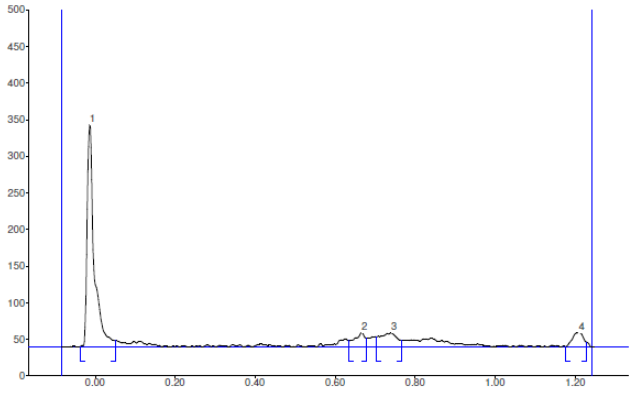


Diagram 5: Overview graph of methanol extract of sample A3 at 254nm

Area and peaks of Methanol extract at 366nm

Total 11 peaks were obtained for methanol extract of dried leaf powder of sample A3 (Adhatoda species-*Vasika*). The peaks were obtained with max Rf value of -0.01 with area 2060.4, max Rf value of 0.03 with area 1363.0AU, max Rf value of 0.07 with 320.3 AU, max Rf value of 0.18 with area 278.6 AU, max Rf value of 0.28 with 409.9 AU, max Rf value of 0.63 with area 571.8AU, max Rf value 0.65 with area 257.9 AU, max Rf value of 0.71 with area 691.6AU, max Rf value 0.87 with area 1271.7 AU, max Rf value of 1.01 with area 217.7, max Rf value 1.11 with area 338.7 AU respectively, which were tabulated as follows

Table No.7 Area and peaks of methanol extract of sample A3 (Adhatoda species-*Vasika*) at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.01	0.01	2060.4	26.48
2.	0.01	0.03	0.06	1363.0	17.52
3.	0.06	0.07	0.14	320.3	4.12
4.	0.14	0.18	0.21	278.6	3.58
5.	0.27	0.28	0.31	409.9	5.27
6.	0.60	0.63	0.64	571.8	7.35
7.	0.64	0.65	0.67	257.9	3.31
8.	0.68	0.71	0.78	691.6	8.89
9.	0.79	0.87	0.91	1271.7	16.34
10.	0.98	1.01	1.03	217.7	2.80
11.	1.10	1.11	1.15	338.7	4.35

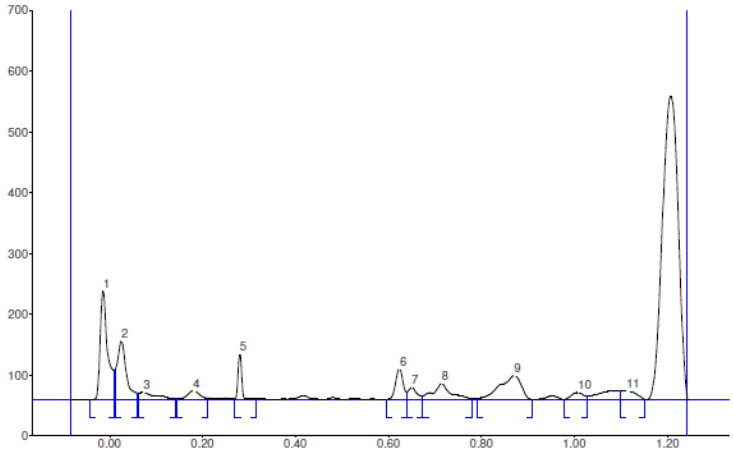


Diagram 6: Overview graph of methanol extract of sample A3 at 366nm

d. HPTLC finger printing profile of methanol extract of sample A4 (Adhatoda species-*Ajagandhi*)

Area and peaks of Methanol extract at 254nm

Total 3 peaks were obtained for methanol extract of dried leaf powder of sample A4 (Adhatoda species-*Ajagandhi*) at 254 nm. These 3 peaks were defined with max Rf value of -0.01 with area 3646.7AU, max Rf value of 0.75 with area 476.9AU, max Rf value of 1.21 with area 385.0AU, respectively, which are tabulated as follows.

Table No.8 Area and peaks of methanol extract of sample A4 (Adhatoda species-*Ajagandhi*) at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.01	0.05	3646.7	80.88
2.	0.73	0.75	0.78	476.9	10.58
3.	1.18	1.21	1.24	385.0	8.54

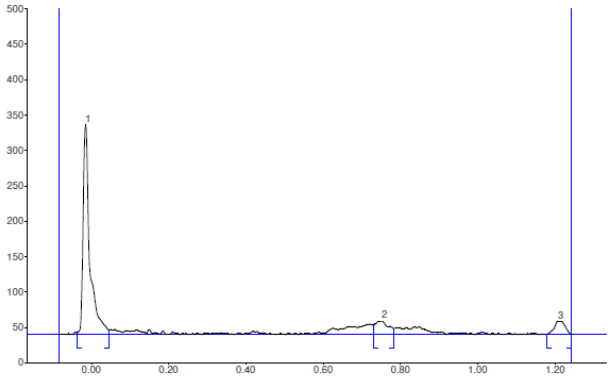


Diagram 7: Overview graph of methanol extract of sample A4 at 254nm

Area and peaks of Methanol extract at 366nm

Total 10 peaks were obtained for methanol extract of dried leaf powder of sample A4 (Adhatoda species-*Ajagandhi*). The peaks were obtained with max Rf value of -0.01 with area 2320.1, max Rf value of 0.02 with area 2514.7AU, max Rf value of 0.21 with 75.1 AU, max Rf value of 0.63 with area 403.0 AU, max Rf value of 0.72 with 753.2 AU, max Rf value of 0.76 with area 315.7AU, max Rf value 0.85 with area 574.7 AU, max Rf value of 0.88 with area 431.7AU, max Rf value 1.01 with area 358.7 AU, max Rf value of 1.13 with area 964.0 respectively, which were tabulated as follows

Table No.9 Area and peaks of methanol extract of sample A4 (Adhatoda species-*Ajagandhi*) at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.05	-0.01	0.01	2320.1	26.63
2.	0.01	0.02	0.09	2514.7	28.87
3.	0.20	0.21	0.24	75.1	0.86
4.	0.61	0.63	0.64	403.0	4.63
5.	0.67	0.72	0.75	753.2	8.65
6.	0.75	0.76	0.80	315.7	3.62
7.	0.80	0.85	0.87	574.7	6.60

8.	0.87	0.88	0.91	431.7	4.96
9.	0.99	1.01	1.04	358.7	4.12
10.	1.04	1.13	1.16	964.0	11.07

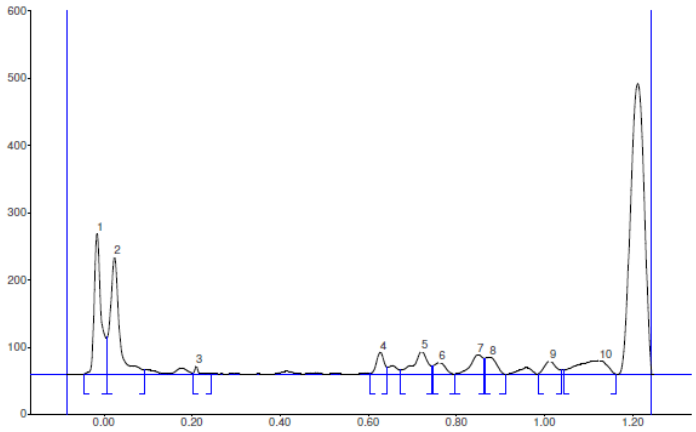


Diagram 8: Overview graph of methanol extract of sample A4 at 366nm

e. HPTLC finger printing profile of methanol extract of sample A5 (*Adhatoda beddomei* C B Clarke)

Area and peaks of Methanol extract at 254nm

Total 3 peaks were obtained for methanol extract of dried leaf powder of sample A5 (*Adhatoda beddomei* C B Clarke) at 254 nm. These 3 peaks were defined with max Rf value of -0.01 with area 3548.8AU, max Rf value of 0.43 with area 218.2AU, max Rf value of 1.15 with area 62.6AU respectively, which are tabulated as follows.

Table No.10 Area and peaks of methanol extract of sample A5 (*Adhatoda beddomei* C B Clarke) at 254 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.01	0.04	3548.8	92.67
2.	0.40	0.43	0.47	218.2	5.70
3.	1.12	1.15	1.16	62.6	1.64

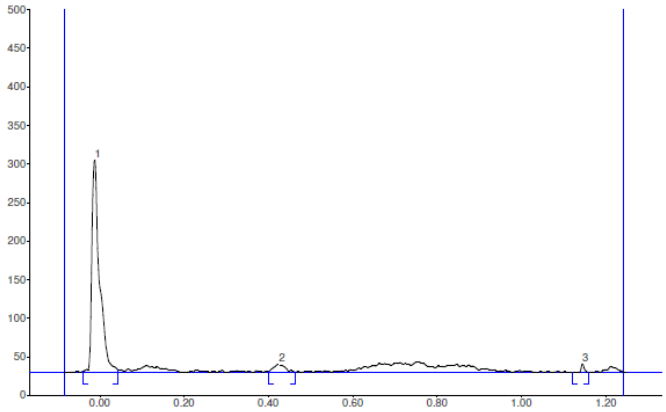


Diagram 9: Overview graph of methanol extract of sample A5 at 254nm

Area and peaks of Methanol extract at 366 nm

Total 7 peaks were obtained for methanol extract of dried leaf powder of sample A5 (Adhatoda beddomei C B Clarke). The peaks were obtained with max Rf value of -0.01 with area 2540.2, max Rf value of 0.03 with area 926.3AU, max Rf value of 0.08 with 396.0 AU, max Rf value of 0.18 with area 397.1 AU, max Rf value of 0.63 with 205.5 AU, max Rf value of 0.88 with area 1654.1AU, max Rf value 1.14 with area 595.1 AU, respectively, which were tabulated as follows

Table No. 11 Area and peaks of methanol extract of sample A5 (*Adhatoda beddomei* C B Clarke) at 366 nm

Peak	Start Rf	Max Rf	End Rf	Area (AU)	Area %
1.	-0.04	-0.01	0.02	2540.2	37.83
2.	0.02	0.03	0.06	926.3	13.80
3.	0.06	0.08	0.14	396.0	5.90
4.	0.15	0.18	0.22	397.1	5.91
5.	0.59	0.63	0.65	205.5	3.06
6.	0.79	0.88	0.92	1654.1	24.64
7.	1.05	1.14	1.17	595.1	8.86

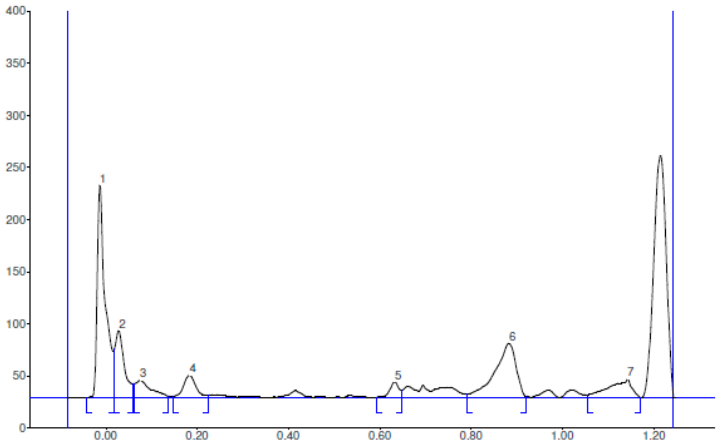
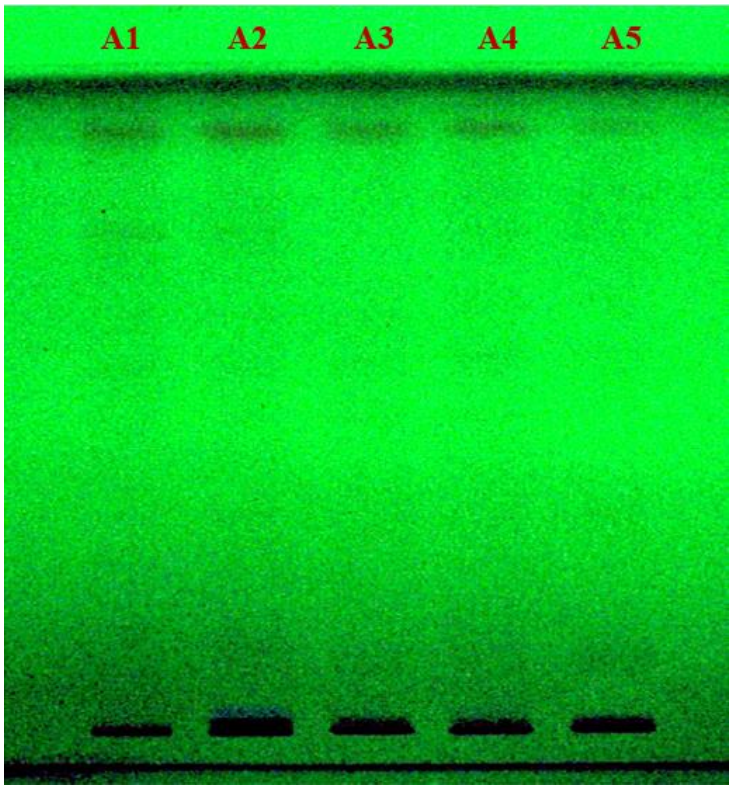
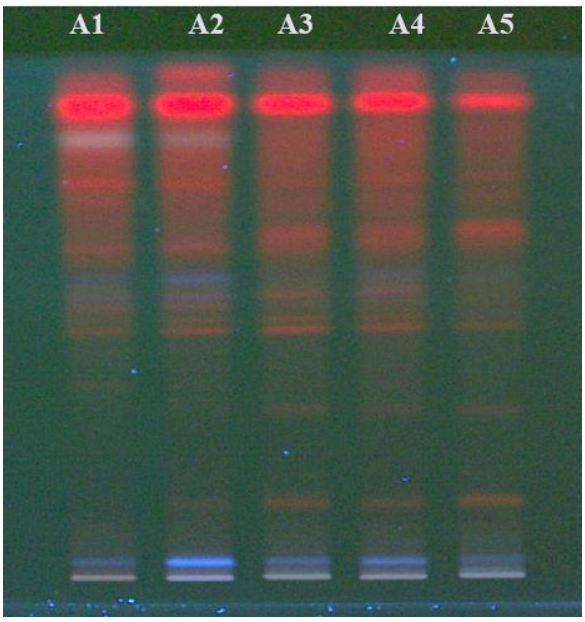


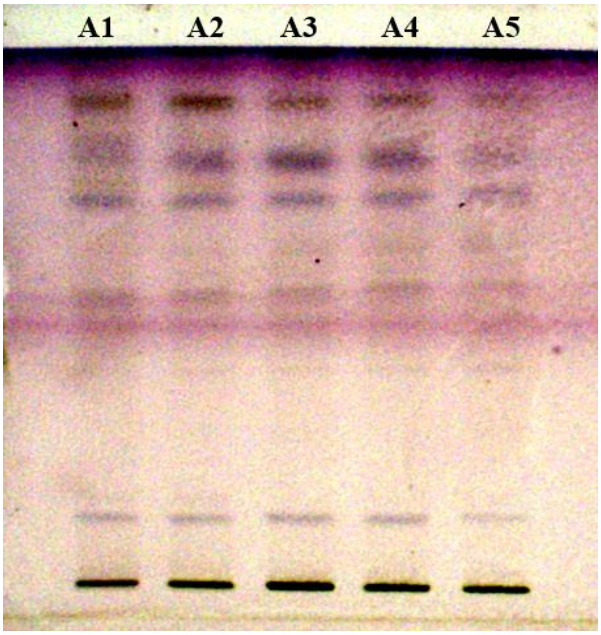
Diagram 10: Overview graph of methanol extract of sample A5 at 366nm



Picture No: 1 HPTLC views of methanol extract of sources of *Vaasa* (Adhatoda species) at 254 nm: A1 (*Valiyaadalodakam*); A2 (*Cheriyaadalodakam*); A3 (Adhatoda species - *Vasika*); A4 (Adhatoda species - *Ajagandhi*); and A5 (*Adhatoda beddomei* C B Clarke)



Picture No: 2 HPTLC views of methanol extract of sources of *Vaasa* (Adhatoda species) at 366 nm: A1 (*Valiyaadalodakam*); A2 (*Cheriyaadalodakam*); A3 (Adhatoda species - *Vasika*); A4 (Adhatoda species - *Ajagandhi*); and A5 (*Adhatoda beddomei* C B Clarke)



Picture No: 3 HPTLC views of methanol extract of sources of *Vaasa* (Adhatoda species) at white light (after derivatization): A1 (*Valiyaadalodakam*); A2 (*Cheriyaadalodakam*); A3 (Adhatoda species - *Vasika*); A4 (Adhatoda species - *Ajagandhi*); and A5 (*Adhatoda beddomei* C B Clarke)

DISCUSSION

The current study involved conducting High Performance Thin Layer Chromatography (HPTLC) fingerprinting analysis on powdered leaves of five samples of *Vaasa* (Adhatoda species). The analysis utilized a solvent system composed of Toluene: Ethyl acetate: Formic acid (5:4:0.5),[2] and the chromatograms are observed under ultraviolet light at 254nm and 366nm wavelengths. The result obtained are compared with findings from a related study (Sini et al.)[9] that investigated in crude methanolic extract of *Adhatoda vasica* Nees and *Adhatoda beddomei* C B Clarke with a solvent system of Toluene: Ethyl acetate: Methanol. The HPTLC analysis of different sources of *Vaasa* (Adhatoda species) are performed by assessing the obtained chromatograms with respect to the number of peaks, peak area, and Rf values.

Under 254nm wavelength, the chromatographic profiles of the samples showed 5 peaks for sample A1 with a total peak area of 2942.3; 4 peaks for sample A2 with a total peak area of 7152.9; 4 peaks for sample A3 with a total peak area of 5159; 3 peaks for sample A4 with a total peak area of 4,508.6; and 3 peaks for sample A5 with a total peak area of 3829.6. Sample A1 exhibited the highest number of peaks, but its total peak area is comparatively lower than that of the other samples. On the other hand, sample A2 demonstrated the greatest peak area among all the samples. On comparing the peak area with Rf values, the maximum peak areas of sample A1is 1576.2 with Rf value -0.01; maximum peak area of sample A2 is 5223.5 with Rf value -0.02; maximum peak area of sample A3 is 3822.5 Rf value -0.01; maximum peak areas of sample A4 is 3646.7 with Rf value -0.01; maximum peak areas of sample A5 is 3548.8 with Rf value -0.01. So, sample A1, sample A3, sample A4 and sample A5 exhibited similar Rf values (-0.01) which indicates the presence of similar phytoconstituent in these samples. But the peak area in sample A1 is lesser than other samples. The maximum peak area of sample A3, sample A4, and sample A5 are comparable. Sample A2 shows a comparable Rf value (-0.02) with the other samples. Similarly, another compound with maximum Rf value of 1.21 is present in sample A1, sample A2, sample A3, and sample A4. At this maximum Rf value the maximum peak area is for sample A2 (742.8) and then for sample A1 (456.4). Sample A3 and A4 exhibited comparable peak areas at this

maximum Rf value (1.21). This compound is absent in sample A5. In sample A1 and sample A2 another compound is present with maximum Rf value of 0.73. The maximum peak area for this compound is greater in sample A2 (788.7) when compared with sample A1 (528.5). A comparable maximum Rf values is present in sample A3 (0.74) and sample A4 (0.75).

Under 366nm wavelength, the chromatographic profiles of samples showed 11 peaks for sample A1 with a total peak area of 21710.6; 10 peaks for sample A2 with a total peak area of 14228.3; 11 peaks for sample A3 with a total peak area of 7529.8; 10 peaks for sample A4 with a total peak area of 8387.9; and 7 peaks for sample A5 with a total peak area of 6714.3. Sample A5 is with least peak number and total peak area where as sample A1 is with highest peak area among all samples. On comparing peak area with maximum Rf values, the maximum peak area of sample A1 is 13446.8 at maximum Rf value 1.21. This compound is absent in other samples. The maximum peak area for sample A2 was 6014.6 at maximum Rf value of 0.02. Similar compound is present in sample A4 with maximum peak area of 2514.7. Sample A1, sample A3 and sample A5 showed a comparable compound with maximum Rf value 0.03. The maximum peak area of sample A3 is 2060.4 with a maximum Rf value -0.01. Sample A1, sample A4, and sample A5 is with same Rf value with a peak area of 1544.7, 2320.1, and 2540.2 respectively. At this Rf value sample A5 exhibits maximum peak area. Sample A2 is with comparable maximum Rf value as -0.02 with a peak area of 2759.1. In sample A1 and sample A2 similar compound is present with maximum Rf value of 0.62 with a peak area of 230.9 and 383.0 respectively. Sample A4 and sample A5 exhibited with a comparable compound with maximum Rf value 0.63. In sample A3 there are two comparable compounds present with maximum Rf value at 0.63 and 0.65. In sample A1 a compound with maximum Rf value at 0.68 is present. In sample A2 a comparable compound is present with maximum Rf value at 0.69. In sample A1, sample A2, and sample A3 a similar compound is present at a maximum Rf value of 0.71 with peak area of 380.9, 530.6, 691.6 respectively. In sample A4 a comparable compound is present at maximum Rf value of 0.72 with a peak area of 753.2. In sample A5 this compound is absent. A comparable compound is present in sample A1 at maximum Rf value of 0.83 with a peak area of 411.4; sample A2 at maximum Rf value of 0.82 with a peak area of 530.6; sample A3 at maximum Rf value 0.87 with a peak

area of 1271.7; sample A5 at maximum Rf value 0.88 with a peak area of 1654.1. Sample A4 is with two such comparable compounds at maximum Rf value of 0.88 and 0.85. Sample A1and sample A2 is with same compound at maximum Rf value of 1.12 with peak area of 3017.1 and 1247.5 respectively. Similar compound is present in sample A3 at maximum Rf value of 1.11 with a peak area of 338.7 and in sample A4 at maximum Rf value of 1.13 with a peak area of 964.0. Also is present such compound in sample A5 at maximum Rf value 1.14 with a peak area of 595.1.

When analysing the chromatographic profiles of various sources of *Vaasa* (Adhatoda species), it becomes evident that similar compounds are present in each sample. However, the quantities of these compounds differ among the plants. Moreover, there are additional compounds present, that may indicate variations in phytoconstituents among the different samples of Adhatoda species.

CONCLUSION

A study involving HPTLC fingerprinting was conducted on the powdered leaves of these samples. The chromatographic profiles indicated the presence of similar compounds in all samples, though with differing quantities. Sample A1 exhibits more peaks compared to other samples with a total peak area of 21710.6 under 366nm. Sample A5 is with least peak number and total peak area of 6714.3 under 366nm. Sample A2 with highest total peak area of 7152.9 and sample A1 with least total peak area of 2942.3 under 254nm. Remarkably, additional compounds were detected, suggesting variations in phytoconstituents among the different Adhatoda species samples. The chromatographic profiles revealed the presence of similar compounds in all samples, albeit with varying quantities. Additionally, the detection of extra compounds suggests variations in phytoconstituents among different plant sources of *Vaasa* (Adhatoda species). These findings not only contribute to the identification and quantification of bioactive compounds across diverse Adhatoda species but also illuminate potential variations in different plant sources, providing valuable insights into the botanical and chemical diversity of *Vaasa*.

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CONFLICT OF INTEREST : NIL

FINANCIAL ASSISTANT : NIL

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