

## ORIGINAL RESEARCH ARTICLE

# Phytochemical Evaluation of Different Plant Sources of *Vaasa* (*Adhatoda* Species)

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### ABSTRACT

**Introduction:** *Vaasa*, a valuable medicinal plant widely used in Ayurvedic medicine, has experienced growing demand, resulting in the availability of both wild and cultivated sources. Experts have identified morphotypes of *Adhatoda* species in Kerala, indicating variations in active constituents and clinical efficacy. This study specifically focused on assessing physicochemical parameters and phytochemical constituents of different sources of *Vaasa*.

**Materials and Methods:** The parameters such as foreign matter, total ash, acid insoluble ash, water insoluble ash, moisture content, volatile oil content, fiber content, tannin content, sugar content, phenol content, pH, qualitative analysis of ash, extractive values, alkaloids, saponins, flavonoids, tannins, steroids, phenols, carbohydrates, and proteins were estimated in different sources of *Vaasa* (*Adhatoda* species): A1 (*Valiya adalodakam*), A2 (*Cheriyā adalodakam*), A3 (*Adhatoda* species - *Vasika*), A4 (*Adhatoda* species - *Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke).

**Results and Discussion:** The results revealed comparable physicochemical parameters between sample A1 and *Adhatoda vasica* Nees, as well as between sample A5 and *Adhatoda beddomei* C B Clarke, as documented in authentic texts and research articles. In qualitative analysis, no differences were found between samples A2, A3, A4, and A5 compared to sample A1. The study revealed quantitative differences in physicochemical and phytochemical evaluation among the powdered leaf samples.

**Conclusion:** Qualitative analysis did not reveal significant differences among samples A2, A3, A4, and A5 compared to sample A1. However, quantitative variations were observed in both physicochemical and preliminary phytochemical evaluation of the powdered leaf sample. The findings in this study contribute to a better understanding and identification of various *Vaasa* sources in Ayurvedic medicine, essential for quality control and further research.

## 1. INTRODUCTION

*Vaasa*, also known as Malabar nut, holds significant medicinal importance in Ayurveda, traditionally used for centuries to address conditions such as *swaasa* (dyspnea), *kaasa* (cough), *jwara* (fever), *chardi* (vomit), and *raktapitta* (bleeding disorders).<sup>[1]</sup> The official source of *Vaasa*, according to the Ayurvedic Pharmacopeia of India, is *Adhatoda vasica* Nees.<sup>[2]</sup> However, some scholars suggest *Adhatoda beddomei* C B Clarke as an alternative source. In Kerala, locally known as *Aadalodakam*, there are two distinct varieties – *Valiya*

*adalodakam* and *Cheriyā adalodakam*, identified based on their morphology. *Cheriyā adalodakam* is considered smaller and reputed to have better medicinal actions, particularly in Kerala.<sup>[1]</sup> Challenges arise in drug selection due to the coexistence of both widely occurring and cultivated varieties of *Vaasa*. This complexity leads to confusion among medicinal drug manufacturers, practitioners, and common people who may randomly choose different plants for therapeutic formulations, ignoring official sources. Additionally, some scholars claim that the clinical efficacy is higher for the smaller variety of *Vaasa*.<sup>[1]</sup> Experts have identified morphotypes of *Adhatoda* species in Kerala, indicating variations in active constituents and clinical efficacy. The study emphasizes the need for scientific validation in selecting *Vaasa* sources to ensure safety and efficacy in therapeutic

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formulations. The phytochemical composition of different *Adhatoda* species can vary due to factors such as geographical location, climate, and soil conditions. The study on the phytochemical composition of different *Vaasa* sources aims to provide empirical evidence supporting or refining traditional knowledge. The study investigated to ensure the safe and effective selection of specific *Vaasa* plants for diverse medicinal purposes. There is a need of hour to scientifically validate the effectiveness of arbitrarily choosing *Vaasa* (*Adhatoda* species) sources for formulations, ensuring they do not lead to undesired outcomes. This would prevent potential adverse effects caused by the random selection of different sources of *Vaasa* (*Adhatoda* species). This study specifically focused on assessing physicochemical parameters, such as foreign matter, total ash, acid insoluble ash, water insoluble ash, moisture content, volatile oil content, fiber content, tannin content, sugar content, phenol content, pH, qualitative analysis of ash, extractive values, and phytochemical constituents (alkaloids, saponins, flavonoids, tannins, steroids, phenols, carbohydrates, and proteins) of different sources of *Vaasa* (*Adhatoda* species).

## 2. MATERIALS AND METHODS

### 2.1. Collection of Plant Sources of *Vaasa* (*Adhatoda* Species)

Saplings of *Valiya adalodakam* and *Cheriyadaalodakam* were gathered from the Government Ayurveda College campus in Tripunithura. Kerala Agricultural University (KAU) introduced two high-yielding varieties of *Vaasa*, namely *Vasika* and *Ajagandhi*, with a vasicine content of 2.5% following comparative yield trials.<sup>[3]</sup> Saplings of both varieties were obtained from the Sales Centre of the Department of Plantation Crops and Spices at KAU in Mannuthy, Thrissur. The Centre for Medicinal Plants Research Institute (CMPR) in Kottakkal hosted a mother plant of *Adhatoda beddomei* C B Clarke.<sup>[4]</sup> Saplings of *Adhatoda beddomei* C B Clarke were collected from the Herbal Garden at the CMPR in Kottakkal. These samples were denoted as A1, A2, A3, A4, respectively, for descriptive purposes.

### 2.2. Preparation of *Patra Churna* (Powdered Leaves)

The fresh leaves of different sources of *Vaasa* (*Adhatoda* species) were inspected visually for any foreign matter and washed thoroughly with water separately to eliminate actual contaminations such as soil and mud. They were then dried in the shade separately. Once dried well, they were separately ground into a fine powder using a motor grinder and filtered through a mesh size of 120. The powdered leaves were stored individually in clean and airtight bottles for further analysis (Figures 1-5).

### 2.3. Reagents and Apparatus Required

Concentrated and dilute hydrochloric acid, xylene, concentrated and dilute sulfuric acid, concentrated and dilute nitric acid, sodium hydroxide solution, lead acetate solution, sodium oxalate, potassium permanganate, anhydrous sodium carbonate, petroleum ether, cyclohexane, acetone, alcohol, Fehling's solution A&B, chloroform water, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, neutral ferric chloride, magnesium ribbon, methylene blue reagent, sodium bicarbonate solution and copper sulfate, catechol, Folin Ciocalteu phenol reagent. Silica crucible, round bottom flask, conical flask, standard flask, Dean and stark's apparatus, Clevenger's apparatus, Soxhlet apparatus, Bunsen burner, water condensers, hot air oven, muffle furnace, heating mantle, glass beakers, glass beads, petri dishes, test tubes, glass lids, measuring jars, funnel, glass rods, watch glass, burettes, pipettes, Whatman filter paper.

## 2.4. Procedure

### 2.4.1. Determination of the physicochemical parameters

The physicochemical properties such as determination of foreign matter, total ash, acid insoluble ash, water insoluble ash, moisture content, volatile oil content, fiber content, tannin content, sugar content, phenol content, pH, and qualitative analysis of ash were separately evaluated in the powdered leaves of samples of *Vaasa* (*Adhatoda* species)-A1 (*Valiya adalodakam*), A2 (*Cheriyadaalodakam*), A3 (*Adhatoda* species-*Vasika*), A4 (*Adhatoda* species-*Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke). The ash obtained from each plant was subjected to qualitative analysis to confirm the presence or absence of acid radicals such as carbonate, phosphate, sulfate and chloride, and basic radical of potassium.

### 2.4.2. Determination of extractive values

The cold and hot water-soluble extractive values, cold and hot alcohol soluble extractive values of test drugs of *Adhatoda* species)-A1 (*Valiya adalodakam*), A2 (*Cheriyadaalodakam*), A3 (*Adhatoda* species-*Vasika*), A4 (*Adhatoda* species-*Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke), were estimated in the study using petroleum ether, cyclohexane, acetone, and alcohol which were the solvents used for successive solvent extraction.

### 2.4.3. Phytochemical parameters

Phytochemical constituents such as alkaloids, saponins, flavonoids, tannins, steroids, phenols, carbohydrates, and proteins were screened to detect the presence or absence of samples of *Vaasa* (*Adhatoda* species)-A1 (*Valiya adalodakam*), A2 (*Cheriyadaalodakam*), A3 (*Adhatoda* species-*Vasika*), A4 (*Adhatoda* species-*Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke). Petroleum ether, cyclohexane, acetone, and alcohol extracts of the above test drugs of *Adhatoda* species were subjected to qualitative analysis for detecting the presence of steroids, alkaloids, flavonoids, and phenols.

## 3. RESULTS

### 3.1. Physicochemical and Preliminary Phytochemical Evaluation

The physicochemical and preliminary phytochemical evaluation of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)-A1 (*Valiya adalodakam*), A2 (*Cheriyadaalodakam*), A3 (*Adhatoda* species - *Vasika*), A4 (*Adhatoda* species - *Ajagandhi*), and A5 (*Adhatoda beddomei* C B Clarke) was assessed separately.

### 3.2. Determination of Physicochemical Parameters

Physicochemical parameters were estimated for powdered leaves of different sources of *Vaasa* (*Adhatoda* species) separately. The observations are listed in Table 1.

### 3.3. Qualitative Analysis of Ash

Qualitative analysis of ash of powdered leaves of different sources of *Vaasa* (*Adhatoda* species) is assessed separately, as shown in Table 2.

### 3.4. Determination of Extractive Values (Water Soluble and Alcohol Soluble)

Hot water-soluble and alcohol-soluble extractive values of powdered leaves of different sources of *Vaasa* (*Adhatoda* species) are assessed separately and are listed in Table 3.

### 3.5. Determination of Successive Solvent Extractive Values

The successive solvent extractive values of powdered leaves of different sources of *Vaasa* (*Adhatoda* species) were assessed separately in solvents such as petroleum ether, cyclohexane, acetone, and alcohol (Table 4).

### 3.6. Determination of the Phytochemical Constituents

#### 3.6.1. Qualitative analysis

The results obtained in qualitative analysis of powdered leaves of different sources of *Vaasa* (*Adhatoda* species) were assessed separately (Table 5).

#### 3.6.2. Qualitative analysis of successive solvent extractives

Results obtained from the qualitative analysis of successive solvent extractives in petroleum ether, cyclohexane, acetone, and alcohol of powdered leaves of different sources of *Vaasa* (*Adhatoda* species) are shown in Table 6.

## 4. DISCUSSION

### 4.1. Determination of Physicochemical Parameters

In the present study, foreign matter was absent in all the samples of the *Vaasa* (*Adhatoda* species), which indicates the purity of the samples. The total ash value indicates the quantity of residue left after ignition. Acid-insoluble ash quantifies the silica content in the substance, particularly as sand and siliceous earth, which contributes to the overall total ash. Water-insoluble ash accounts for the portion of the total ash that remains insoluble in water. The physicochemical parameters such as foreign matter, total ash, acid insoluble ash of *churna* (powder) of sample A1 are comparable with *Adhatoda vasica* Nees in Ayurvedic Pharmacopoeia of India,<sup>[2]</sup> Quality Standards of Indian Medicinal Plants by ICMR,<sup>[5]</sup> and research articles (Nandhini and Ilango., Unnati *et al.*).<sup>[6,7]</sup> Also, these parameters of sample A5 are comparable with *Adhatoda beddomei* C B Clarke explained in Quality Standards of Indian Medicinal Plants by ICMR<sup>[8]</sup> and research article (Nandhini and Ilango).<sup>[6]</sup> Water insoluble ash was additionally determined in this study and the values obtained for sample A1, sample A2, sample A3, sample A4, and sample A5 are 18.15%, 16.8%, 14.6%, 14.91%, and 13.88%, respectively. The moisture content of sample A1 is comparable with the article published by Chauhan *et al.*<sup>[9]</sup> For the five samples examined in this study, no volatile oil was extracted. However, according to the findings of Sarker *et al.*, the hydrodistillation of *Adhatoda vasica* (Nees) leaves yielded 0.096% (v/w) of essential oil.<sup>[10]</sup> The fiber content obtained for the five samples is comparatively greater than while comparing with the research work by Singh *et al.*<sup>[11]</sup> This may be due to geographical variation during the collection of the leaves. There are no references available for *Adhatoda beddomei* C B Clarke in determining the fiber content and volatile oil. Hence, the present study can be taken into account as a reference for the future. The tannin and phenol content values obtained in this study are comparable with the values to previous research work (Kumar *et al.*, Nandhini and Ilango).<sup>[6,12]</sup> The tannin content is fairly consistent across all five samples. However, the phenol content is comparatively less in sample A1 (1.39%) when compared with other samples of *Vaasa* (*Adhatoda* species). In the current study, sample A1 lacks total sugar whereas, sample A2, sample A3, sample A4, and sample A5 had total sugar content of 1.34%, 2.68%, 1.59%, and 2.28%, respectively. The results of total sugar in *Adhatoda* species in the present study were lesser while compared with the previous research work (Singh *et al.*).<sup>[11]</sup> This may be due to geographical variation during the collection of the leaves. The reducing sugar was absent in sample A1, sample A2, sample

A3, and Sample A4. However, in sample A5, 2.21% of reducing sugar was present. For pH analysis, litmus paper tests demonstrated that all five samples turned blue, indicating an alkaline nature. The quantitative pH measurements for each sample are quite similar and fall within the acceptable range according to the Ayurvedic Pharmacopoeia of India.<sup>[2]</sup> The findings from the experiments conducted on sample A2, sample A3, and sample A4 could be considered significant reference points for future research, especially since these experiments are carried out for the first time on these plants. The qualitative analysis of ash for each sample revealed the presence of acid radicals such as carbonate, phosphate, chloride, and sulfate, as well as basic radicals like potassium.

### 4.2. Determination of Extractive Values

The extractive values of powdered leaves of samples of *Vaasa* (*Adhatoda* species) are assessed. The extractive values of *Adhatoda vasica* Nees are available in Ayurvedic Pharmacopoeia of India and reported by Unnati *et al.*<sup>[7]</sup> The extractive values of *Adhatoda beddomei* C B Clarke are suggested by Nandhini and Ilango.<sup>[6]</sup> In the present study, cold water-soluble extractive values and cold alcohol-soluble extractive values of five samples are comparatively similar. In addition to this, hot water soluble and hot alcohol soluble extractive values are also determined. Sample A4 exhibits notably a higher hot water-soluble extractive value (8.08%) in comparison to other samples. Conversely, sample A2 exhibited the lowest quantity of hot water-soluble extractive value (5.30%). Sample A1, sample A3, and sample A5 showed comparable values in this regard. The hot alcohol soluble extractive values obtained for sample A1, sample A2, sample A3, sample A4, and sample A5 are 2.93%, 1.7%, 1.91%, 2.72%, and 2.32%. The highest hot soluble extractives are obtained from sample A1 and the least from sample A2. The successive solvent extraction was carried out individually on five samples using petroleum ether, cyclohexane, acetone, and alcohol as solvents. The extractive values of petroleum ether and alcohol extracts are compared to a previous study on *Adhatoda vasica* Nees conducted by Kumar *et al.*<sup>[13]</sup> Notably, the extractive values obtained from this study differed from the values reported in the literature. This variation could be attributed to inconsistencies arising from geographical differences during the leaf collection process. There are no relevant references available for *Adhatoda beddomei* C B Clarke. So, these values can be considered for future reference. Among the solvents used, the highest extractive value is with petroleum ether, while the lowest extractive value is from cyclohexane. There are no established references accessible for the cyclohexane and acetone extracts. The findings from this study can be considered as a potential point of reference for future research.

### 4.3. Determination of the Phytochemical Constituents

In earlier studies, qualitative analysis was carried out on powdered leaves, revealing the presence of chemical constituents such as alkaloids, flavonoids, saponins, carbohydrates, proteins, phenols, steroids, and tannins across different extract types (Nandhini and Ilango).<sup>[6]</sup> In the current study, both alcoholic and aqueous extracts of the powdered drug are individually employed to detect the presence of alkaloids, flavonoids, phenols, and steroids. Meanwhile, the aqueous extract was utilized to identify saponins, carbohydrates, proteins, and tannins in powdered leaves in each sample. Alkaloids, flavonoids, and phenols are present in both alcoholic and aqueous extract of each sample. Carbohydrates and tannin are detected in the aqueous extract of each sample. Steroids are absent in aqueous and alcoholic extract of each sample. Proteins were not detected in the aqueous extract of individual sample. In the current study, a qualitative analysis was conducted on the successive solvent extracts of powdered leaves from

five samples of *Vaasa* (*Adhatoda* species), using petroleum ether, cyclohexane, acetone, and alcohol. The findings are consistent across all samples. Alkaloids are identified in all samples except for the acetone extract. Alcoholic extracts exhibited the presence of steroids, alkaloids, flavonoids, and phenols. Moreover, flavonoids are also detected in the cyclohexane extract alongside the alcoholic extract. Steroids are only found in the alcoholic extracts of each sample, while phenols are exclusively present in the alcoholic extracts across all samples.

## 5. CONCLUSION

The physicochemical characteristics of sample A1 closely align with those reported for *Adhatoda vasica* Nees in authoritative texts and research publications. Similarly, the physicochemical parameters of sample A5 exhibit similarities with *Adhatoda beddomei* C B Clarke, as documented in authentic sources. Physicochemical and preliminary phytochemical screenings were performed on each powdered leaves sample highlighting quantitative differences among the samples. The experiments conducted on samples A2, A3, and A4 are particularly noteworthy as they represent the 1<sup>st</sup>-time investigations on these plants, providing valuable reference points for future research. Qualitative analysis did not reveal significant differences among samples A2, A3, A4, and A5 compared to sample A1. However, quantitative variations were observed in both physicochemical and preliminary phytochemical screenings of the powdered leaf sample. The study underscores substantial phytochemical diversity among different *Adhatoda* species, emphasizing the variability within the *Adhatoda* genus. These findings contribute to an enhanced understanding and identification of diverse sources of *Vaasa* in Ayurvedic medicine, crucial for quality control and guiding future research endeavors.

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## 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 AVAILABILITY

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

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**Table 1:** Physicochemical parameters of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

S. No.	Parameters	Sample A1	Sample A2	Sample A3	Sample A4	Sample A5
1	Foreign matter	Nil	Nil	Nil	Nil	Nil
2	Total ash	18%	16.5%	14.01%	14.5%	13.95%
3	Acid insoluble Ash	0.83%	0.76%	0.9%	1.03%	0.79%
4	Water-insoluble ash	18.15%	16.8%	14.6%	14.91%	13.88%
5	Moisture content	13%	10%	10%	11%	15%
6	Volatile oil	Nil	Nil	Nil	Nil	Nil
7	Fiber	18.87%	14.53%	11.96%	11.21%	17.11%
8	Tannin content	6.756%	6.456%	6.375%	6.389%	6.501%
9	Total sugar	Nil	1.34%	2.68%	1.59%	2.28%
10	Reducing sugar	Nil	Nil	Nil	Nil	2.21%
11	Phenol	1.39%	2.56%	2.78%	2.93%	2.68%
12	Ph	8.70	8.26	8.80	8.89	9.07

**Table 2:** Qualitative analysis of ash of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

S. No.	Experiment	Sources of <i>Vaasa</i> ( <i>Adhatoda</i> species)				
		Sample A1	Sample A2	Sample A3	Sample A4	Sample A5
1	Carbonate	+	+	+	+	+
2	Phosphate	+	+	+	+	+
3	Chloride	+	+	+	+	+
4	Sulfate	+	+	-	+	-
5	Potassium	+	+	+	+	+

**Table 3:** Extractive values (water soluble and alcohol soluble) of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

S. No	Type of extractives	Sources of <i>Vaasa</i> ( <i>Adhatoda</i> species)				
		Sample A1 (%)	Sample A2 (%)	Sample A3 (%)	Sample A4 (%)	Sample A5 (%)
1	Cold water soluble	26.8	24.3	25.8	26.3	25.39
2	Hot water soluble	7.8	5.30	6.99	8.08	7.08
3	Cold alcohol soluble	5.9	4.5	5.6	5.7	5.16
4	Hot alcohol soluble	2.93	1.7	1.91	2.72	2.32

**Table 4:** Extractive values (in different solvents) of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

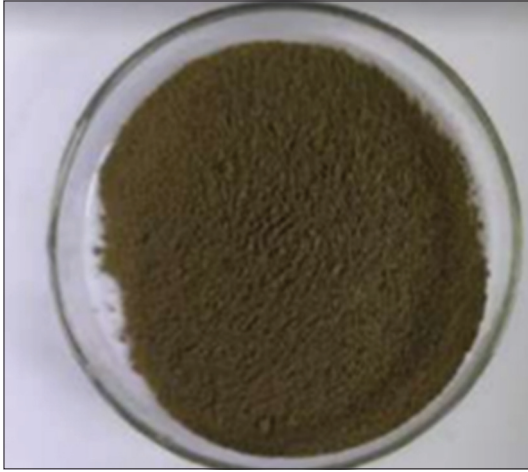
S. No.	Solvents	Sources of <i>Vaasa</i> ( <i>Adhatoda</i> species)				
		Sample A1 (%)	Sample A2 (%)	Sample A3 (%)	Sample A4 (%)	Sample A5 (%)
1	Petroleum ether	5.99	4.98	5.01	5.18	5.06
2	Cyclohexane	0.25	0.2	0.22	0.2	0.3
3	Acetone	1.62	2.46	1.55	1.70	1.26
4	Alcohol	1.97	0.88	1.28	0.99	2.13

**Table 5:** Qualitative phytochemical analysis of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

S. No.	Experiment	Sources of <i>Vaasa</i> ( <i>Adhatoda</i> species)				
		Sample A1	Sample A2	Sample A3	Sample A4	Sample A5
1	Alkaloids	+	+	+	+	+
	a. Dragendroff's test					
	b. Meyer's test	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Saponins	+	+	+	+	+
4	Carbohydrates	-	-	-	-	-
	c. Fehling's test					
	d. Benedict's test	+	+	+	+	+
5	Proteins	-	-	-	-	-
6	Phenols	-	-	+	+	+
	e. Ferric chloride test					
	f. Lead acetate test	+	+	+	+	+
7	Steroids	-	-	-	-	-
8	Tannins	-	-	-	-	-
	g. Ferric chloride test					
	h. Lead acetate test	-	-	-	-	-

**Table 6:** Qualitative phytochemical analysis of solvent extracts of powdered leaves of different sources of *Vaasa* (*Adhatoda* species)

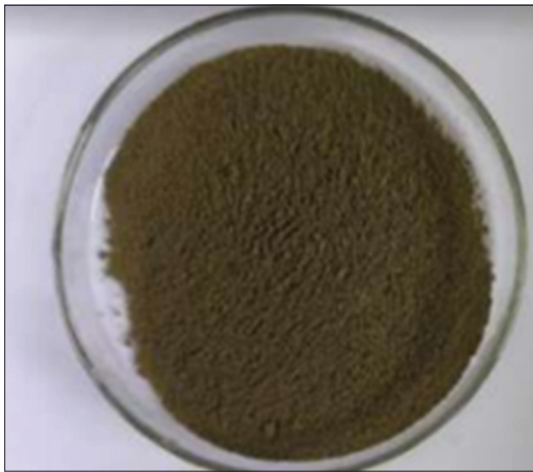
Samples of <i>Vaasa</i> ( <i>Adhatoda</i> species)	Extract	Sources of <i>Vaasa</i> ( <i>Adhatoda</i> species)			
		Steroids	Alkaloids	Flavonoids	Phenols
Sample A1	i. Petroleum ether	-	+	-	-
	ii. Cyclohexane	-	+	+	-
	iii. Acetone	-	-	-	-
	iv. Alcohol	+	+	+	+
Sample A2	i. Petroleum ether	-	+	-	-
	ii. Cyclohexane	-	+	+	-
	iii. Acetone	-	-	-	-
	iv. Alcohol	+	+	-	+
Sample A3	i. Petroleum ether	-	+	-	-
	ii. Cyclohexane	-	+	+	-
	iii. Acetone	-	-	-	-
	iv. Alcohol	+	+	-	+
Sample A4	i. Petroleum ether	-	+	-	+
	ii. Cyclohexane	-	+	+	-
	iii. Acetone	-	-	-	-
	iv. Alcohol	+	+	-	+
Sample A5	i. Petroleum ether	-	+	-	-
	ii. Cyclohexane	-	+	+	-
	iii. Acetone	+	-	-	-
	iv. Alcohol	+	+	-	+



**Figure 1:** Powder of sample A1 (*Valiya adalodakam*)



**Figure 3:** Powder of sample A3 (*Adhatoda* species-Vasika)



**Figure 2:** Powder of sample A2 (*Cheriyi adalodakam*)



**Figure 4:** Powder of sample A4 (*Adhatoda* species-Ajagandhi)



**Figure 5:** Powder of sample A5 (*Adhatoda beddomei* C B Clarke)