# PHYTOCHEMICAL SCREENING OF *Vernonia cinerea* Linn. WHOLE PLANT USING HPTLC

**Seema R. Sapale[1], Vikas V. Vaidya[2], Mr. Siddhesh Babaji Mangaonkar[3], Willy J. Shah[4]**

[1] *Department of Chemistry, Kirti M Doongursee College, Mumbai, India*

[2] *Department of Chemistry, Royal College of Arts, Science and Commerce, Mumbai India*

[3] *Department of Chemistry, Sonopant Dandekar, Palghar, India*

[4] *Department of Chemistry, Annasaheb Vartak College of Arts, Commerce, Science, Vasai, India*

**ABSTRACT**

*The present study aims to investigate different phytochemicals present in whole plant of Vernonia cinerea Linn. The plant were collected and authenticated from Blatter Herbarium, St. Xavier's college, Mumbai, India. The sample was washed, dried in hot air oven and were grinded to form fine powder. The powder were subjected to different method for phytochemical screening. The phytochemical constituents from Vernonia cinerea Linn. whole plant powder were extracted as per Harborne method and separated using HPTLC and it was found that the whole plant contained maximum amount of Polar extract (Quaternary alkaloids and N- oxides). The method can be used as a reference to check the quality of the plants during bulk collection.*

**Keyword:-** *Phytochemical screening, Vernonia cinerea Linn, HPTLC*

## INTRODUCTION

Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. In all these operations, methods are needed for separation, purification, identification of the many different constituents present in plants. Thus, advances in our understanding of Phytochemistry are directly related to successful exploitation of known techniques, and the continuing development of new techniques to solve outstanding problems as they appear. Phytochemistry progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals and its chromatographic techniques. Evaluation of plant materials and their derived products has always been an important part of the analyst. However, over the year the nature and the degree of this evaluation have changed. Initially it was considered sufficient to authenticate the plant material by comparing with standard botanical description or monograph. Later, it was realized that for determination of adulterants, the above data must be supplemented with both, microscopical analysis and confirmatory chemical tests for the constituents present.

This development slowly until the middle of the century when rapid advances in the knowledge of chemistry of plant drugs were made and new, improved methods for analysis, identification, and estimation of the active ingredients were developed. This led to the requirement that drugs should confirm to a phytochemical as well as morphological monograph. Plant constituents of medicinal importance form an extensively diverse group of chemical compounds showing greater variation in solubility and stability. They can be broadly classified as follows [1]: Alkaloids, Carbohydrates, Glycosides, Phenols, Proteins, Resins, Tannins, Essential oils, Fats and Waxes. The present study aims to determine the phytochemical profile of the plant.

## MATERIALS AND METHOD

### 1. Collection and Storage

The plant material of *Vernonia cinerea* Linn. were collected from Mumbai, India and authenticated from Blatter Herbarium, St. Xavier's college, Mumbai, India and were used as reference sample for further analysis. *Vernonia cineria* Linn. whole plant were collected, washed thoroughly, shade dried for a week, packed in absorbant paper and kept in incubator (Temp-40ºC) for 2-3 days. The plants were labelled with details including name of the plant (Genus, Species, and Family), common name, date of collection and region of collection. The dried raw materials were removed from the incubator and then powdered using a mixer-grinder and sieved through BSS mesh number 85. This powder was stored in commercially available airtight Pearlpet bottles.

### 2. Phytochemical screening

Following procedure was employed for determination of phytochemical profile of *Azadirachta indica* A.Juss leaves and *Vernonia cinerea* Linn. whole plant powder.

Five gram of dried powder of *Azadirachta indica* A.Juss leaves and *Vernonia cinerea* Linn. whole plant was Soxhlet extracted with a mixture of Methanol and Distilled Water (150 ml) in the ratio 4: 1. The extract was cooled and filtered through Whatmann filter paper No. 41 into a dry and pre weighed beaker.

A. The residue was extracted with 125 ml of Ethyl Acetate and filtered into a dry, pre- weighed beaker. The residue obtained after filtration comprised of plant fibers. Weight of the extract was noted down, the plant fibers were dried in an oven and percent *Crude Fiber* was calculated.

B. The filtrate obtained from step 2 was evaporated to dryness on a water bath. After evaporation of Ethyl Acetate, the beaker was allowed to cool at room temperature in a dessicator. After cooling, the weight of beaker containing the residue was noted down. The residue obtained was the neutral extract and consisting of *Fats and Waxes* from *Azadirachta indica* A. Juss leaves and *Vernonia cinerea* Linn whole plant.

C. The filtrate obtained from step 1 was evaporated to approximately $1/10^{th}$ its volume by heating in a water bath. It was then acidified with 2 M $H_2SO_4$. The acidified filtrate was extracted using 75 ml chloroform in a separating funnel. It then transferred to a dry pre weighed beaker. Chloroform layer was evaporated to dryness on a water bath.

D. After evaporation of Chloroform, the beaker was allowed to cool at room temperature in a dessicator. After cooling the weight of this beaker containing the residue was noted down, the residue obtained was moderately polar extract and consisting of *Terpenoids and Phenolics* from *Azadirachta indica* A.Juss leaves and *Vernonia cinerea* Linn. whole plant powder.

E. The aqueous acid layer obtained from step 4 was basified (using a pH paper). It was then further extracted with 60 ml of mixture of chloroform and methanol in the volume ration 3:1, followed by extraction with 40

ml chloroform in a separating funnel. The aqueous basic layer was transferred to a dry pre-weighed beaker. The aqueous basic layer was evaporated to dryness on a water bath. After evaporation of the solvent, the beaker was allowed to cool at room temperature in a dessicator. After cooling, the weight of the beaker containing the residue was noted down. The residue obtained was polar extract consisting of *Quaternary Alkaloids* and *N-oxides* from *Azadirachta indica* A.Juss leaves and *Vernonia cinerea* Linn. whole plant powder.

F. The organic layer i.e. chloroform and methanol was transferred to a dry, pre weighed beaker on a water bath maintained at $45^{\circ} \pm 5^{\circ}$ C after evaporation of the solvent, the beaker was allowed to cool at room temperature in a dessicator. After cooling, the weight of this beaker containing the residue was noted down. The residue obtained was the *Basic extract* consisting of *Alkaloids* from *Azadirachta indica* A.Juss leaves and *Vernonia cinerea* Linn. whole plant powder. [1]

**Separation by Chromatography**

After the different fractions were collected by the above procedure, they were then separated by using High Performance Thin Layer Chromatography (HPTLC)

**Chromatographic conditions**

Chromatographic analysis of phytoconstituents of *Vernonia cinerea* Linn. whole plant was done on silica gel 60 $F_{254}$ HPTLC plates. 10 µL of all the samples extracted as per Harborne method were applied on the plates. The mobile phase constituted of Toluene: Methanol 8:1(*v/v*). The plates were developed to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 15 min. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After development, plates were dried under air current at room temperature. The plate was developed and scanned at 550 nm after derivatization in Liebermann- Burchard reagent using Tungsten lamp with a Camag Scanner II in conjunction with Cats 3 software.

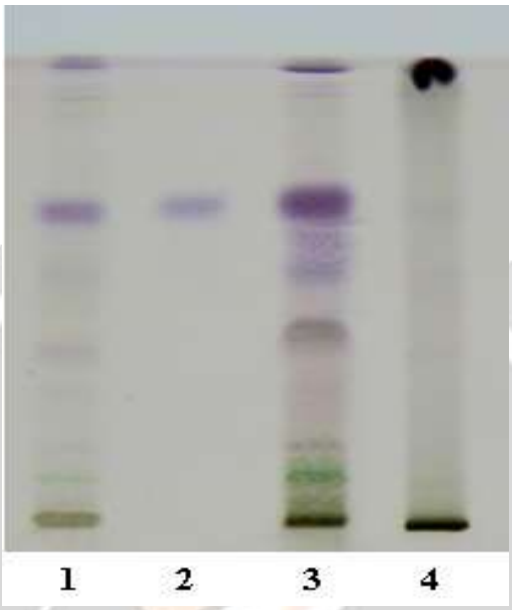
**RESULT**

**Table 1:** Percentage of phytochemical Constituents of *Vernonia cinerea* Linn. whole plant

| **Phytochemical extract** | **% Extract** |
|---|---|
| Neutral extract - Fats & waxes | 0.44 |
| Moderately polar extract-Terpenoids and Phenolics | 2.75 |
| Basic extract-Most alkaloids | 0.34 |
| **Polar extract-Quaternary alkaloids and N-oxides** | **9.67** |

| | |
|---|---|
| Fibers | 85.90 |

**Plate 1:** Chromatographic plate of Vernonia cinerea Linn. whole plant with phytoconstituents




**Track 1** – Fats & waxes

**Track 2** – Terpenoids and Phenolics

**Track 3** – Basic extracts (Most alkaloids)

**Track 4** – Polar Extract (Quaternary alkaloids and N-oxides)

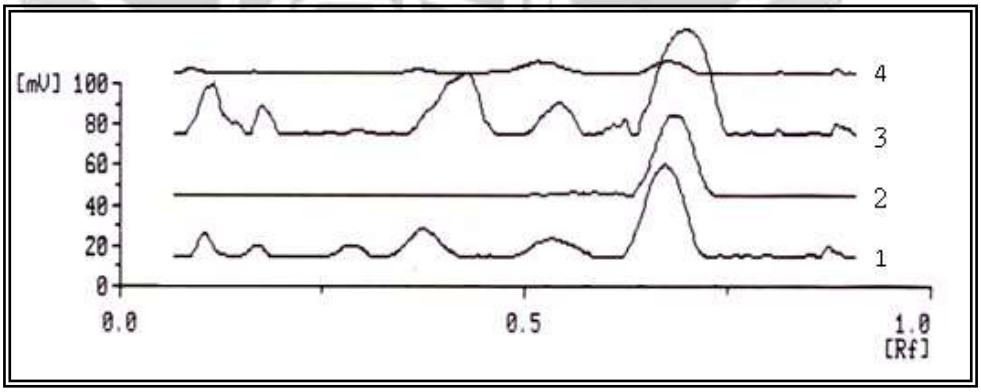



**Figure 1:** Chromatographic overlay of *Vernonia cinerea* Linn. whole plant with phytoconstituents

**DISCUSSION**

The profile showed that *Vernonia cinerea* Linn. whole plant powder has maximum amount of Polar extract-Quaternary alkaloids and N-oxides (9.67%). The other constituents includefat, waxes, terpenoids, phenolics, alkaloids and fibers. The results are expressed in terms of percentage of the different phytoconstituents present in five grams of plant powder used. The phytochemical constituents from *Vernonia cinerea* Linn. whole plant powder were extracted as per Harborne method and separated using HPTLC and it was found that the whole plant contained maximum amount of Polar extract (Quaternary alkaloids and N- oxides). The method can be used as a reference to check the quality of the plants during bulk collection.

**CONCLUSION**

The phytochemical constituents from Vernonia cinerea Linn. whole plant powder were extracted as per Harborne method and separated using HPTLC. The different phytoconstituents were identified by phytochemical analysis. Among the different phytoconstituents extracted Vernonia cinerea Linn. whole plant contained maximum amount of Polar extract (Quaternary alkaloids and N- oxides). The values obtained can be used as a reference to check the quality of the plants during bulk collection. HPTLC method developed in the current work is reliable, fast and economic. The fingerprint pattern developed by HPTLC is distinctive for Vernonia cinerea Linn. whole plant powder.

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