

QUALITY ASSESSMENT PROFILE OF SEEDS OF *VERNONIA ANTHELMENTICA* (L.) WILLD.

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ABSTRACT

Vernonia anthelmintica (L.) Willd. (Syn: *Centratherun anthelminticum*, Family: Asteraceae) is a tall, robust, stout, erect annual herb, 60-90cm in height with pubescent branches. Seed is traditionally used as stomachic, anti-asthmatic, anthelmintic and treatment of various skin infections like leucoderma and psoriasis. Micromorphology and physicochemical analysis of the seeds of *V.anthelmintica* to be performed as per WHO and Pharmacopoeial methods. Seeds of *Vernonia* are actually one-seeded fruit. The pericarp of the fruit and the seed with the embryo are fused to form a single unit called cypsela also called achene. Fruits are achenes (pericarp fused with seed embryo) very small, truncate, oblong and cylindrical in shape. Microscopic evaluation of the fruit is circular in shape with

prominent thick ridges and deep furrows. The ridges are wide and semicircular; there are about 10 ridges alternating the equal number of furrows. The epidermal cells of pericarp are dilated, spherical bulbous bodies at regular intervals. Powder microscopy showed the presence of mesocarp cells, fibre, pollen- grains, pappus scales and calcium oxalate crystals. Preliminary phytochemical screening of appropriate solvent extracts showed the presence of

sterols, tannins, proteins and amino acids, flavonoids, fixed oil, terpenoids, saponin, carbohydrates and absence of alkaloids, mucilages, glycosides and volatile oil. Microscopic analysis and other parameters were informative and provide valuable information in the identification, standardization of *V.anthelmintica* seed.

KEYWORDS: *Vernonia anthelmintica*, *Centratherum anthelminticum*, Asteraceae, Microscopical evaluation.

1. INTRODUCTION

Vernonia anthelmintica (L.) Willd. (Syn: *Centratherum anthelminticum*) is commonly known as wild cumin or iron weed belonging to Asteraceae (Compositae).^[1, 2] It is a tall robust, leafy, stout, erect or diffusely branched annual herb and 60-90cm in height with pubescent branches widely distributed in all districts, often on road sides, well grown in black cotton soil and cultivated in India, Brazil, USA, Bangladesh and Nepal (Figure 1). This plant bear seeds in the month of May to June, distributed throughout India up to an elevation of 1700m in the Himalayas.^[3-5]



Figure 1: Habit and Habitat of *V.anthelmintica*

Taxonomy^[6]

| | | |
|------------|---|---------------|
| Kingdom | : | Plantae |
| Subkingdom | : | Tracheobionta |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Sub Class | : | Asteridae |
| Order | : | Asteridae |

| | | |
|---------|---|----------------------|
| Family | : | Asteraceae |
| Genus | : | <i>Vernonia</i> |
| Species | : | <i>anthelmintica</i> |

Vernacular Names ^[7, 8]

| | | |
|-----------|---|--|
| English | : | Purple fleabane, Wild cumin, Iron weed |
| Hindi | : | Kalijiri, Somraj, Baksi |
| Kannada | : | Kadujirage |
| Malayalam | : | Krimisatru, Kattujirakam. |
| Sanskrit | : | Aranyajiraka |
| Tamil | : | Cittilai, Kattuccirakam. |
| Telugu | : | Garitikamma, Adavijilakarra. |
| Bengali | : | Kalijiri, Somraj. |
| Marathi | : | Ranachajiri. |
| Gujarathi | : | Kalijiri |

The plant has been reported for anthelmintic ^[9, 10], analgesic & antipyretic ^[11, 12], diuretics ^[13, 14], anti-oxidant ^[15, 16], antidiabetic & hypolipidemic ^[17, 18], antiarthritic ^[19], antidepressant ^[20], nephrolithiasis ^[21], larvicidal ^[22, 23], antibacterial ^[24, 25], antifungal ^[26], antifilarial ^[27], anticancer ^[28] and traditionally used in the treatment of leucoderma, psoriasis and other skin infections. ^[29]

Various phytoconstituents has been reported in this plant such as Flavonoids 2', 3, 4, 4'- tetra hydroxy chalcone; 5, 6, 7, 4'-tetra hydroxy flavone and butin ^[30], sterols - Delta-7-avenasterol, 4-alpha-methyl vernosterol, vernosterol, avenasterol and stigmastadienol^[31, 32]; Epoxy oleic acid-vernolic acid and methyl vernolate ^[33, 34]; saponins ^[35]; bitter principles ^[36] etc.

As mentioned earlier several reports have been published regarding chemical constituents and different biological activities *in-vitro* and *in-vivo*. An investigation to explore its pharmacognostic examination is inevitable. The present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant *Vernonia anthelmintica* seeds to treat various diseases.

2. MATERIALS AND METHODS

2.1: Chemicals: Formalin, acetic acid, ethyl alcohol, chloral hydrate, toluidine blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were of analytical grade.

2.2: Collection of Specimens and authentication: The wild, healthy cumin seed were collected from commercial market in Madurai, Tamil Nadu and was authenticated by Dr. P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamil Nadu, India.

2.3: Macroscopic analysis: Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc was noted.^[37]

2.4: Microscopic analysis ^[38, 39]: The seeds were fixed in FAA (Formalin - 5 ml + acetic acid - 5 ml + 70% ethyl alcohol - 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C), until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning: The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. After dewaxing the sections were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc.,

Photomicrographs: Photographs of different magnifications were taken with Nikon lab-photo 2 microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

2.5: Powder microscopy: Coarse powder of the seed was used to study the microscopical characters of the seed powder.^[40, 41]

2.6: Physicochemical analysis: Total ash, acid insoluble ash, water soluble ash, loss on drying and extractive values were determined.^[42, 43]

2.7: Preliminary phytochemical screening: Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure.^[44, 45]

3. RESULTS

3.1 Macroscopy: Seeds are oblong shaped and the surface of the seeds comprised of about 10 ridges which are covered with trichomes. It has dark brown colour with characteristic odour and intensely bitter taste. It is about 5mm long and 1.2mm breadth. What is known as seeds of vernonia are actually one-seeded fruit. The pericarp of the fruit and the seed with the embryo are fused to form a single unit called cypsela also called achene. Fruits are achenes (pericarp fused with seed embryo) very small, truncate, oblong and cylindrical in shape. Outer surface of fruits are 10-ribbed, pubescent in nature. Pappus is copious and reddish, usually inner pappus is long and outer is short (Figure 2).

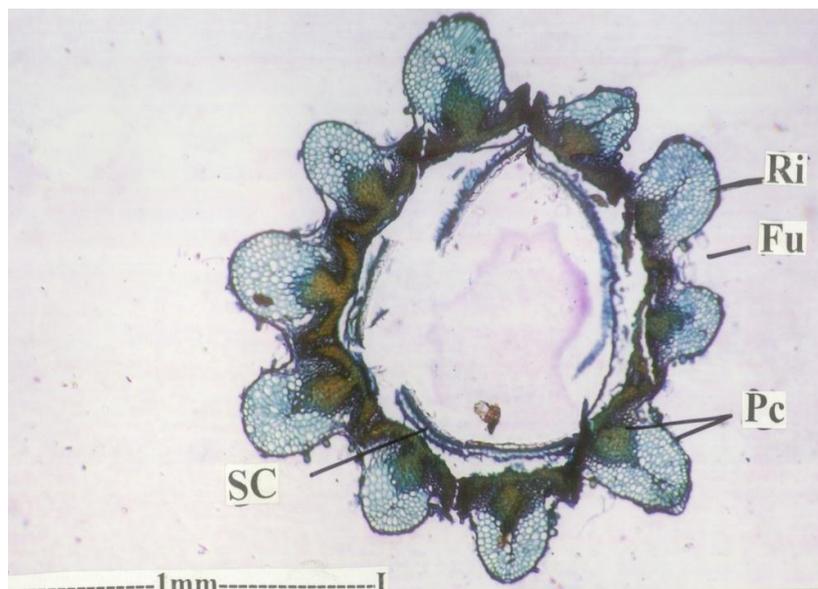


Figure 2: Seed (Fruits) of *V. anthelmintica*

3.2 Microscopy of the Seed

Transverse sections of the seed (cypsela)

TS of fruit are circular in shape with prominent thick ridges and deep furrows. The ridges are wide and semicircular; there are about 10 ridges alternating the equal number of furrows (Figure-3). The fruit is 1.4 mm in diameter along the ridges. The ridges are 300 μ m in height.



Ri-Ridges, Fu-Furrow, Pc-Pericarp, SC-Seed coat

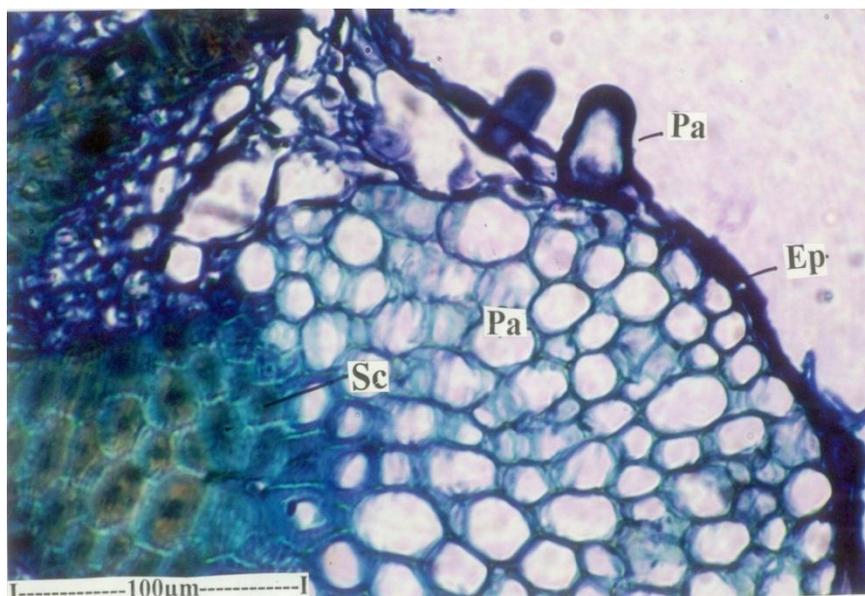
Figure 3: T.S of *V.anthelmintica* Fruit - Entire view (4×)

Pericarp: The pericarp consists of the following regions; Epidermis, mesocarp and endocarp (Figure- 4, 5). The epidermis is thin, thick walled and darkly stained. The epidermal layer is about 8µm thick.



Ep-Epidermis, MC- Mesocarp, EC- Endocarp, SC- Seed Coat

Figure 4: TS of *V.anthelmintica* Fruit - A Portion enlarged (10×)



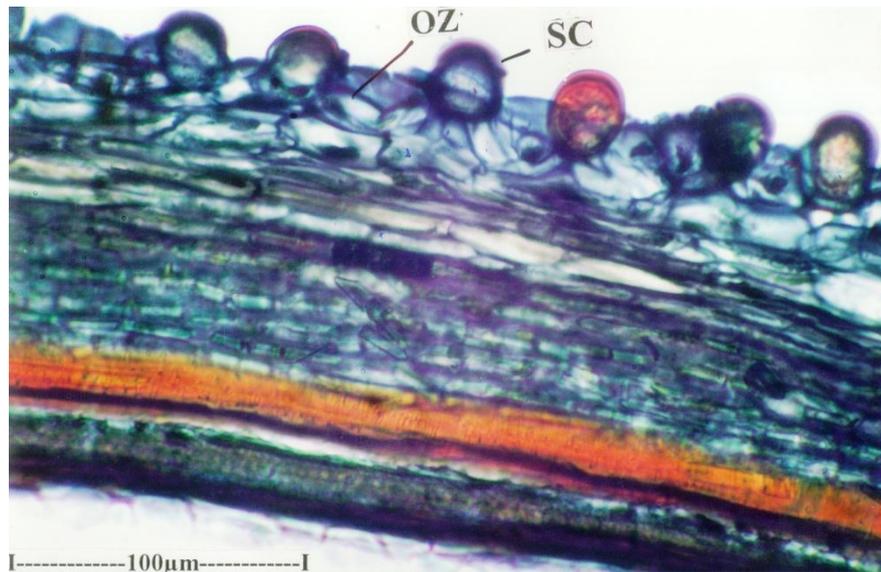
Pa- Papillate epidermal outgrowth, Ep- Epidermis, Pa- Parenchyma, Sc- Sclerenchyma

Figure 5: T.S of *V.anthelmintica* Fruit - Ridge Portion enlarged (40×)

Mesocarp: Inner to the epidermis is a wide zone of about 8 layers of parenchymatous mesocarp. The cells of the mesocarp are fairly thick walled angular, compact and 150µm in wide.

Endocarp: It is the inner layer of the pericarp and extends all around the fruit; along the ridges the endocarp extends in the form of semicircular body with narrow stalk. The cells of the endocarp are sclerenchymatous with thick walls and reduced lumen (Figure-5). The semicircular body measures 200µm thick.

The epidermal cells of pericarp produce, dilated, spherical bulbous bodies at regular intervals. These spherical bodies arise from deeper part of the epidermis. The spherical cells of the epidermis are 100µm in diameter (Figure-6). At certain places, these are wide thick walled vertically rectangular cells produced by the epidermal cells. These elongated cells occur mostly along the lateral side of the ridges of the pericarp.

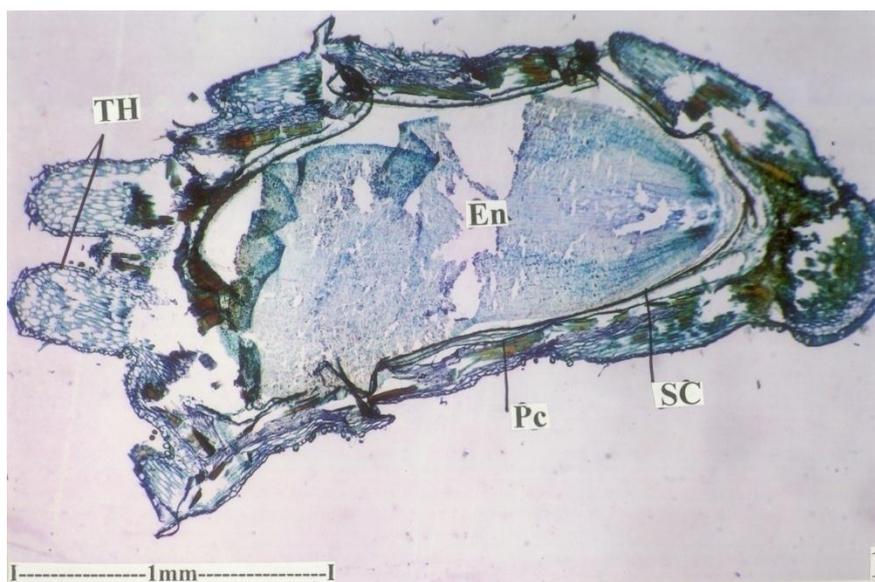


OZ – Outer Zone, SC – Spherical Cell

Figure 6: Pericarp showing outer zone and spherical bodies on the surface (40×)

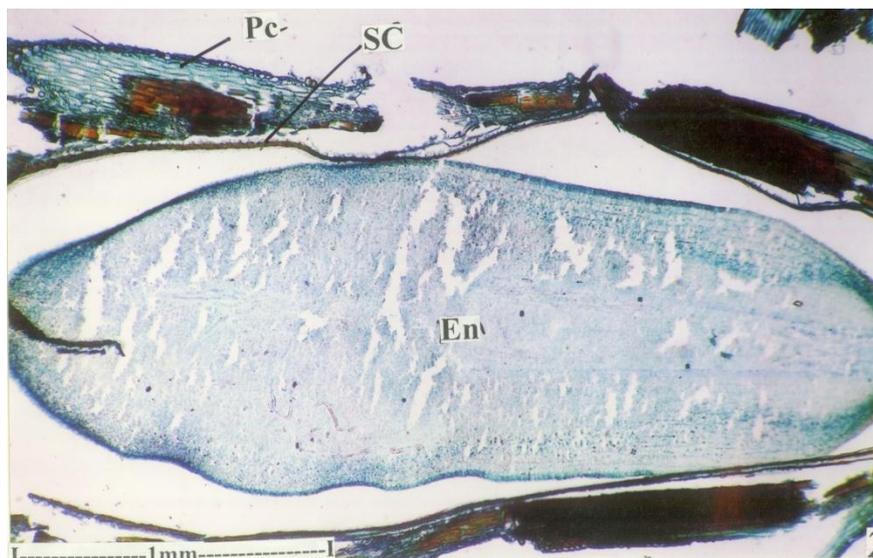
Longitudinal section of the seeds

The fruit has slightly narrow cylindrical part and wider upper end consists of two thick cylindrical horns (Figure 7). The pericarp encloses wide, long cotyledons of the embryo (Figure 7 & 8).



En- Endosperm, TH – Terminal Horns, Pc-Pericarp, SC-Seed coat

Figure 7: LS of Fruit (4×)

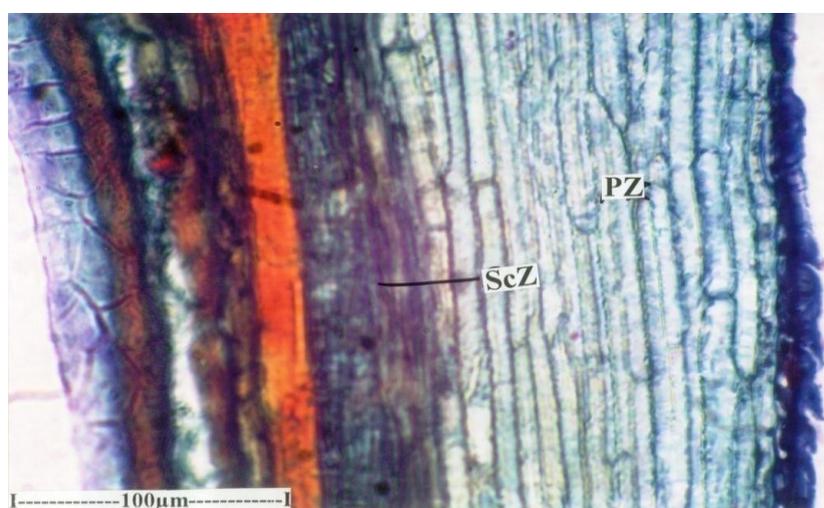


En- Endosperm, Pc-Pericarp, SC-Seed coat

Figure 7: LS of Fruit - Endosperm & Pericarp (4×)

The pericarp has narrow, darkly stained epidermis. The mesocarp has narrow vertically elongated tubular parenchyma cells.

Inner to the parenchymatous mesocarp is the sclerenchymatous endocarp. The sclerenchymatous cells are vertically elongated thick walled sclereids. In the median part of the sclerotic endocarp, there is wide, thin walled canal which runs all along the length of the fruit. The canal has no cross walls. It stains different from the adjacent sclereids (Figure 8). The canal is 50µm wide.



PZ- Parenchymatous Zone, ScZ- Sclerenchymatous Zone

Figure 8: Pericarp showing epidermis, Parenchymatous mesocarp, Sclerenchymatous mesocarp & secretory canal (40×)

3.3: Powder Microscopy

Organoleptic characters

| | | |
|--------|---|-------------------|
| Nature | - | Coarse powder |
| Colour | - | Brown |
| Odour | - | Agreeable odour |
| Taste | - | very bitter taste |

The powder microscopy of the seeds shows the following characters.

Fragment of **mesocarp cells** are common in the powder. In surface view, the cells are vertically elongated, wide or narrow and thick walled. The cells have thick spiral wall thickenings. The cells are 30-70 μ m long and 20 μ m wide (Figure 9).

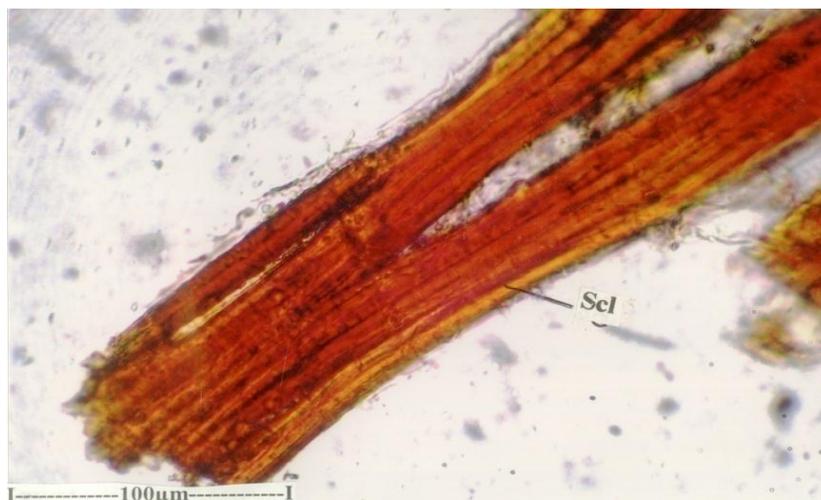


Spiral Wall Thickenings

Figure 9: Pericarp Cells in surface view (40 \times)

Fibre – sclereids

Broken pieces of fibre – sclereids are abundant in the powder, they are long, narrow fibre-like cells; they resemble the sclereids having thick, lignified walls and abundant simple pits. The fibre sclereids form the inner part of the pericarp (Figure 10).



Scl: Sclereids

Figure 10: Bundles of Sclereides of the Pericarp (40×)

Pollen- grains

Large, spherical pollen-grains are very frequent in the powder. They are dark and have smooth surface. They are about 60µm diameter (Figure 11).

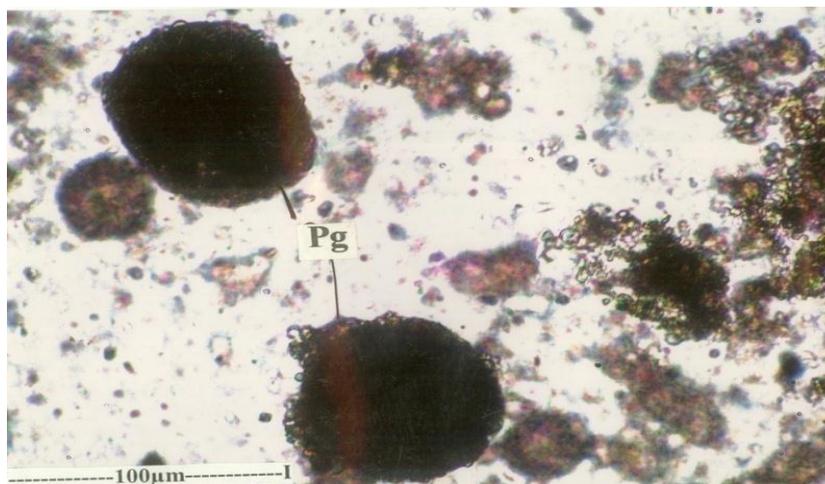


Figure 11: Pollen grains (40×)

Pappus scales

Membranous, triangular pappus scales are common in the powder, they consists of long thick walled cells. The tip and margin have spiny, pointed projecting cells. The pappus occurs all around the terminal part of the achene (Figure 12).

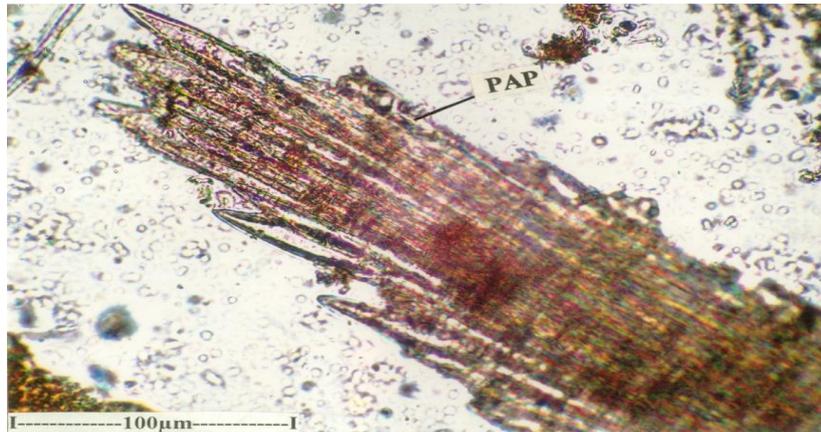
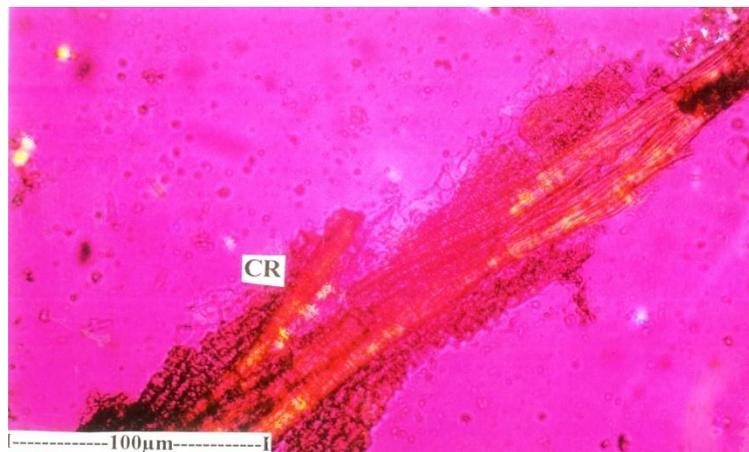


Figure 12: Pappus plate: Needle shaped cells (40×)

Crystals

Calcium oxalate crystals are abundant in the powder. They are prismatic type ranges from cuboidal, rectangular to styloid (seale) types. The crystals occur both in the pericarp and endosperm. The sizes of crystals are also variable (Figure 13, 14, 15).



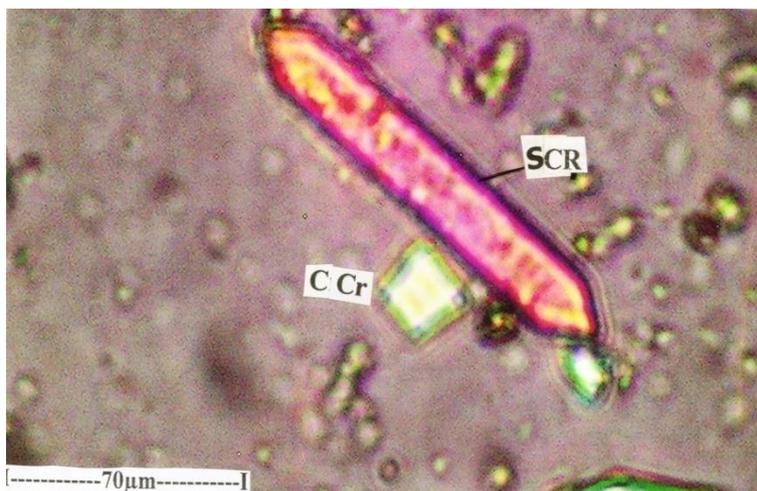
CR-Crystal

Figure 13: Sclereids enclosing crystal



Pr Cr- Prismatic Crystals

Figure 14: Prismatic crystals of different shape and size



S Cr-Styloid Crystal, C Cr- Cuboidal Crystal

Figure: Styloid and cuboid crystals

3.4: Physical parameter

3.4.1: Ash Value and LOD

Ash Values and LOD of the Seeds of *Vernonia anthelmintica* L.

| Range | Total Ash (%)w/w | Acid Insoluble ash (%)w/w | Water soluble Ash (%)w/w | LOD |
|---------|------------------|---------------------------|--------------------------|------|
| Minimum | 5.75 | 1.05 | 1.75 | 0.9 |
| Average | 6.135 | 1.295 | 2.115 | 1.01 |
| Maximum | 6.55 | 1.7 | 2.45 | 1.2 |

3.4.2: Extractive Values of crude drugs

| Solvent | Extractive value (% w/w) |
|-----------------|--------------------------|
| Petroleum ether | 14.90 |
| Benzene | 16.68 |
| Chloroform | 20.82 |
| Ethyl acetate | 16.56 |
| Ethanol | 15.98 |
| Water | 9.78 |

3.5: Preliminary Phytochemical Screening

Preliminary Phytochemical Screening of Different Solvent Extracts

| Tests | Petroleum ether extract | Chloroform extract | Ethyl acetate extract | Ethanol extract | Aqueous extract |
|----------------------|-------------------------|--------------------|-----------------------|-----------------|-----------------|
| Alkaloids | | | | | |
| Mayers Reagent | - | - | - | - | - |
| Dragendorffs reagent | - | - | - | - | - |
| Hagers reagent | - | - | - | - | - |
| Wagners reagent | - | - | - | - | - |
| Carbohydrates | | | | | |

| | | | | | |
|---------------------------------------|---|---|---|---|---|
| Molishch's Test | - | - | - | + | + |
| Fehlings Test | - | - | - | + | + |
| Benedicts Test | - | - | - | + | + |
| Glycosides | | | | | |
| General Test | - | - | - | - | - |
| Anthraquinone | - | - | - | - | - |
| Cardiac | - | - | - | - | - |
| Cyanogenetic | - | - | - | - | - |
| Coumarin | - | - | - | - | - |
| Phytosterols | | | | | - |
| Salkowski Test | + | + | + | + | - |
| Liebermann Burchard Test | + | + | + | + | - |
| Saponins | - | - | - | + | + |
| Tannins | - | - | - | - | - |
| Proteins & Free Amino Acid | | | | | |
| Millons test | - | - | - | + | + |
| Biuret test | - | - | - | + | + |
| Gums & Mucilage | - | - | - | - | - |
| Flavonoids | | | | | |
| Shinoda test | - | - | + | + | + |
| Alkaline Reagent test | - | - | + | + | + |
| Terpenoids | + | + | + | + | - |
| Fixed Oil | + | + | + | + | - |

4. DISCUSSION

Organoleptic evaluation of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile.^[46] Hence we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of seeds of *Vernonia anthelmentica*.

Adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs.^[47] Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials.^[48]

The microscopical investigation revealed that Pericarp is consists of epidermis, mesocarp and endocarp. Epidermis is consists of dilated spherical bulbous bodies at regular interval. At certain places these are appeared as vertically rectangular cells which occur mostly along the lateral sides of the ridges of the pericarp. Mesocarp consists of about 8 layers of parenchymatous cells which are thick walled and compactly arranged. Endocarp consists of

sclerenchymatous cells which are thick walls with reduced lumen about 200µm thick. In the previous report^[49] some important observations like spherical bulbous bodies, cylindrical horns and longitudinal section characters have been not mentioned. All the observations and powder microscopic characters are included in this study and moreover clear microscopical plates are displayed.

Powder microscopy showed Mesocarp cells, Sclereids are fibre like cells having thick lignified walls, Large spherical pollen grains, Pappus scales consists of membranous triangular thick walled cells, Prismatic types of calcium oxalate crystals are present in both pericarp and endosperm. They range from cuboidal, rectangular and styloid types, Uniseriate trichomes are abundantly seen.

The ash values are particularly important to find out the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter).^[50] Acid insoluble ash provides information about non-physiological ash produced due do adherence of inorganic dirt, dust to the crude drug.

Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery.^[51] Preliminary phytochemical screening of appropriate solvent extracts showed the presence of sterols, flavonoids, terpenoids, saponins, protein and aminoacids, carbohydrates and absence of alkaloids, mucilage, volatile oil and glycosides.

5. CONCLUSION

Many unknown plant are used in folk and tribal medicine practices as a source of medicine. The medicinal values of these plants are not brought in to the light of scientific world. One such plant is *Vernonia anthelmintica*. Keeping in this view an attempt was made for standardization of purity and quality of commonly occurring plant *Vernonia anthelmintica*.

Conflict of interest statement: We declare that we have no conflict of interest.

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