

Pharmacognostical and Phytochemical studies of *Viscum articulatum* Burm. f.

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Abstracts: Pharmacognostical and Phytochemical studies of *Viscum articulatum* Burm. f., Evaluation of *Viscum articulatum* Burm.f. Pharmacognostic investigation and determination of some of physical constants of the stem and powdered whole plant *Viscum articulatum* Burm.f. The stem and dried coarsely powdered whole plant *Viscum articulatum* Burm.f. was used for macroscopic and microscopic diagnostic evaluation using Motic microscope. Determination of quantitative standards and preliminary phytochemical screening were done as per the standard guideline of World health organization. Observation of organoleptic characters confirmed that *Viscum articulatum* Burm. f. was a leafless, much branched, hemiparasitic shrub. The transverse section of stem shown to consist of epidermis covered with cuticle as well the presence of the parenchymatous cells, lignified fibres, calcium oxalate crystals and vascular bundles. The microscopical examination of powdered whole plant has also shown the presence of lignified fibres, vascular bundle, starch grains, epidermis and parenchyma. The results of analytical parameters of powdered whole plant material were total ash-11.45% w/w, water soluble ash-2.9 %w/w, acid insoluble ash-0.5 %w/w, loss on drying- 6.4 % w/w and water soluble and alcohol soluble extractive values were found to be 28.72 % w/w and 13.76 % w/w respectively. The qualitative chemical tests of powdered whole plant revealed the presence of triterpenoids, flavonoids, steroids, saponins and glycosides. The present study might be helpful for identification and standardization parameters of specific species of *Viscum articulatum* Burm.f.

INTRODUCTION

The plant *Viscum articulatum* Burm. f. (Loranthaceae) is commonly known as Pudu. In Ayurveda the plant is used as bitter, acrid, cooling, sweetish, alexipharmic, aphrodisiac, alterative and useful in kapha, vata, diseases of the blood, ulcers, epilepsy and biliousness.^[1] In Chinese medicine the plant *Viscum articulatum* Burm. f. has commonly been used as a curative for a number of ailments such as hemorrhage, pleurisy, gout, heart disease, epilepsy, arthritis and hypertension.^[2,3] The present investigation was planned to study the morphoanatomy, histology and phytochemical characters of plant *Viscum articulatum* Burm. f. which could be helpful for its identification and standardization

MATERIALS AND METHODS

Plant Material

The whole plant *Viscum articulatum* Burm. f. (Loranthaceae) parasitic on *Grewia tilifolia* (Tiliaceae) was collected from the Satpuda hills of Toranmal, Dist. Nandurbar (M.S.), India. The material was taxonomically identified and authenticated by Botanical Survey of India, Pune. A voucher specimen (No.GURHAV6) was deposited in Botanical Survey of India, Pune.

Pharmacognostical Studies

Systematic morphological observations such as shape and size of stem and leaves, arrangements of flowers, nodes on branches, colour, taste and odour were carried out. Histological studies were accomplished by using Motic microscope instrument and photodocumentation was made. The average thickness of the section was 10-12 µm.

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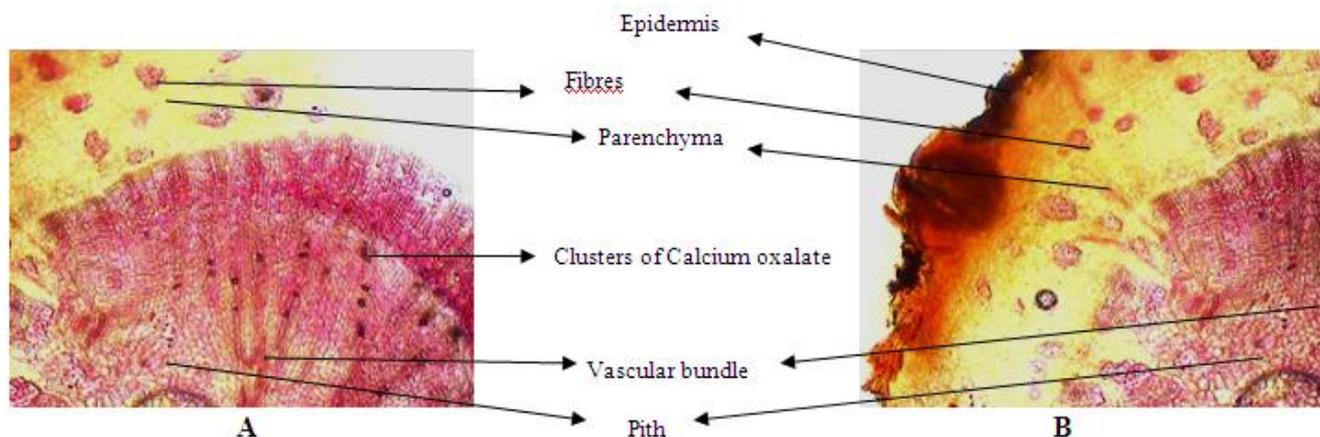
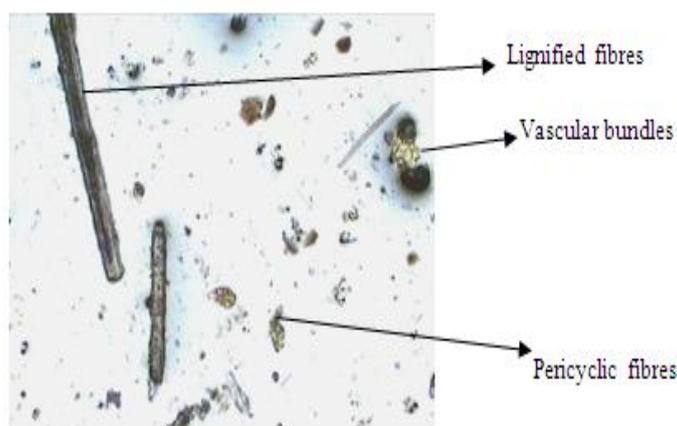
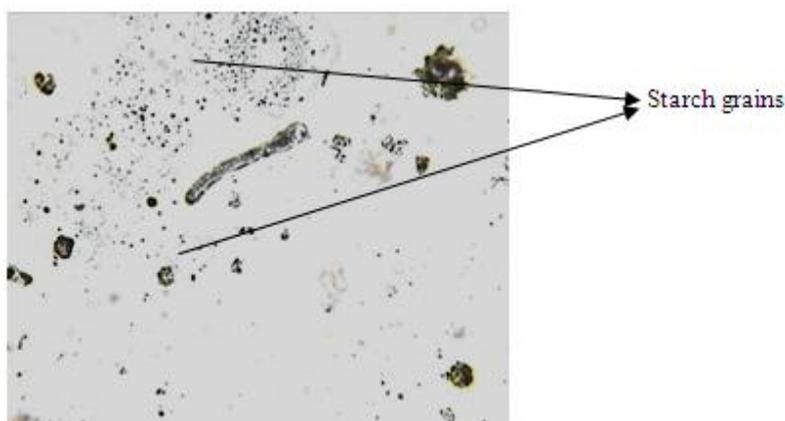
The sections were stained with Phloroglucinol and concentrated HCl (1:1). It rendered pink colour to the lignified tissue. ^[4] The total ash, water soluble ash, acid insoluble ash, alcohol and water soluble extractive values and loss on drying of powder of the whole plant material was carried out. ^[5] For microscopc examination of powdered drug, three slides were prepared one in water, one in iodine and one in phloroglucinol and concentrated HCl (1:1).^[6] Also the preliminary phytochemical screening for identification of various secondary metabolites were performed.

HPTLC Studies of Bioactive Crude Extract

Crude drug extract standardization is the first step towards standardisation of herbal formulations. Only standardised input generated standardised output. Various tests for the standardization are well described e.g. in the Indian Herbal Pharmacopoeia and discussed here, will be limited to HPTLC characterization, the the purpose of routine quality control. In the eastern hemisphere, the purpose of analysis and its quantification as Quantification of known fraction (QKF). The western countries, on the other hand, believe in the "active principle" or "marker" concept i.e. the medicinal properties are attributed to a compound(s) solely. Since these terms can also lead to controversy "known fraction(s)" may be used as a more accurate description of the purpose of analysis. The "total extract" can only be characterised by "fingerprint (FP)" technique, in which a large amount of chromatographic data of "standard" is compared with the data from the sample(s). This characteristic data is obtained from multiwavelength scanning in UV, fluorescence scan, in-situ UV spectra, image comparison and where applicable, data after post chromatographic derivatisation. The "standard" extract would be a certified plant material, extracted in a well-documented way and whose biological activity has been proved by pharmacologists for the intended use. Before formulations can be standardized, the raw materials need to be standardized. Except in dry powder formulations, fingerprint

Table1: Analytical parameters of powder of whole plant of *Viscum articulatum* Burm. f.

Parameter	Result (%w/w)
Total ash	11.45
Water soluble ash	2.9
Acid- insoluble ash	0.5
Loss on drying	6.4
Alcohol Soluble extractive value	13.76
Water soluble extractive value	28.72

**Figure 1:** Transverse Section of stem of *Viscum articulatum* Burm. f. by staining with Phloroglucinol and concentrated HCl (1:1) at 4x objective**Figure 2** Microscopic examination of powdered drug of *Viscum articulatum* Burm. f. stained with Phloroglucinol and concentrated HCl (1:1) at 10x objective**Figure 3:** Microscopic examination of powdered drug of *Viscum articulatum* Burm. f. stained with iodine solution at 10x objective

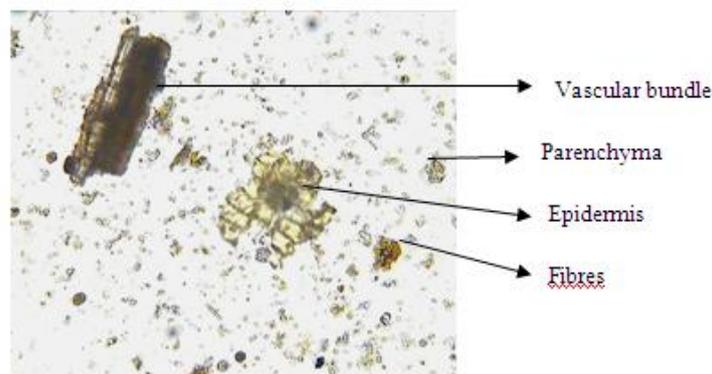
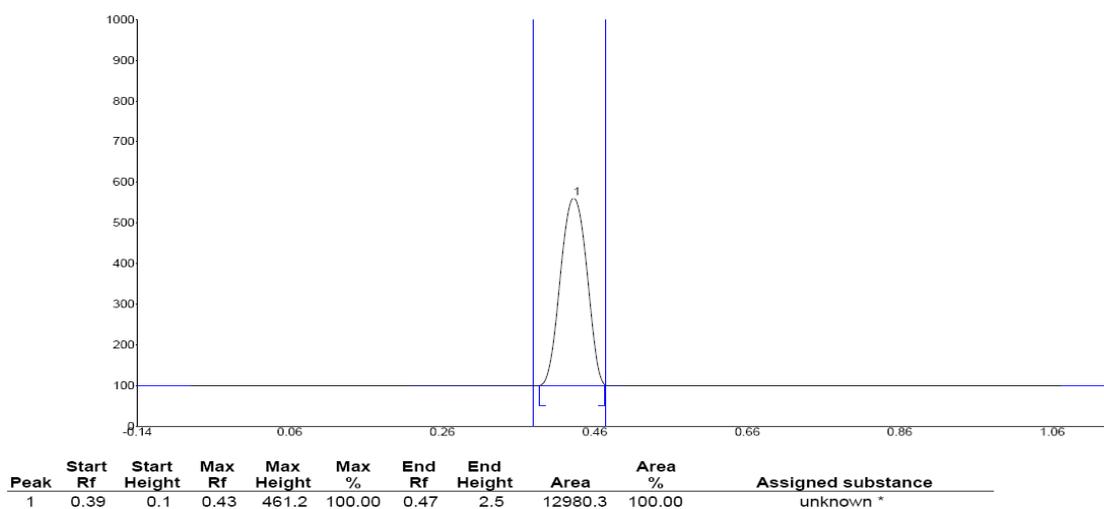
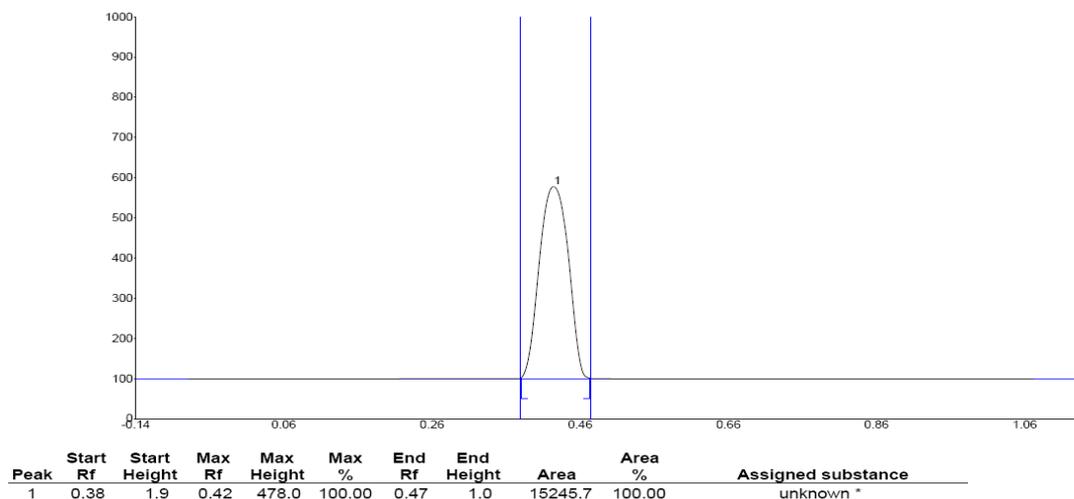


Figure 4: Microscopic examination of powdered drug of *Viscum articulatum* Burm. f. in glycerin and water at 10x objective



Track of Standard: Oleanolic acid (5 µl)

Figure 5: HPTLC chromatogram of standard solution of oleanolic acid (5 µl), Track of standard



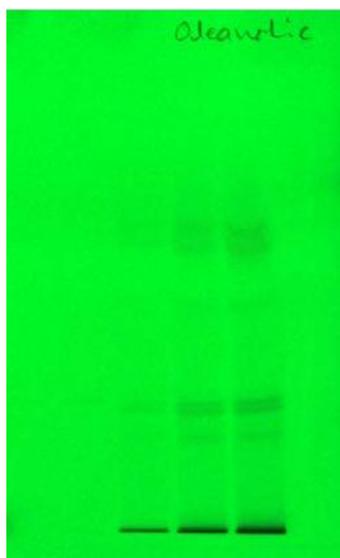
Track of Sample: Chloroform extract (100 µl)

Figure 6: HPTLC chromatogram of sample solution of Chloroform extract (100 µl), Track of Sample

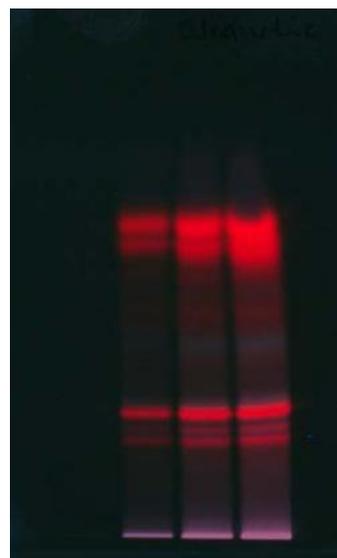
is perhaps, the practical solution to characterize complex herbal formulations, for routine quality control.

High Performance thin Layer Chromatographic (HPTLC) fingerprint data comparison of such a "standard" with that of a sample can be accepted as the rapid, reliable and modern procedure for routine quality control. The FP

method of analysis requires the complete "standard extract" and not just one fraction. These standard and sample extracts are HPTLC chromatographed side by side. Then the TLC scanner is used to multiwave scan the plate, record UV absorbance spectra of all fractions, fluorescence if any, images documented by a digital camera in UV 254 and



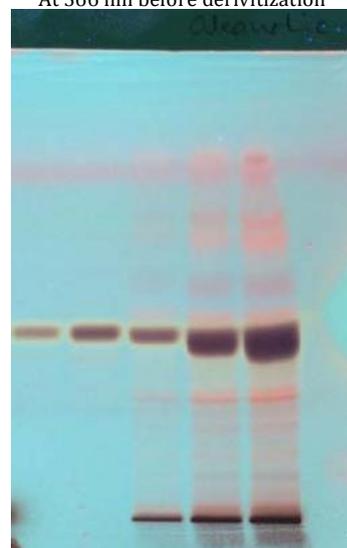
At 254 nm before derivitization



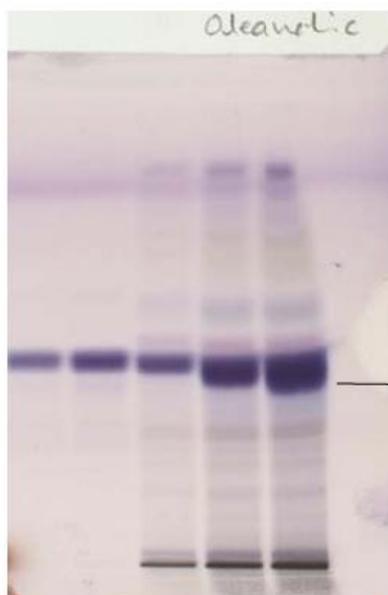
At 366 nm before derivitization



White remission



At 366 nm after derivitization



→ Violet colour spot corresponding to Oleanolic acid

White remission: after spraying anisaldehyde- Sulphuric acid reagent

Photograph 1: HPTLC chromatogram of chloroform extract of *Viscum articulatum* Burm

UV 366 nm. Occasionally, post chromatography derivatisation may be carried out for specificity or sensitivity reasons.

HPTLC Profile of Chloroform Extract of Whole Plant *Viscum articulatum* Burm.F.

- **Sample Preparation:** 10 mg of Chloroform extract was dissolved in 10 ml chloroform.
- **Standard Solution:** 10 mg of oleanolic acid was dissolved in 10 ml of chloroform. Oleanolic acid as a marker was provided by Anchrom Labs.
- **Stationary Phase:** Precoated TLC plates of Silica gel G 60 F254 (E. Merck), 5 x 10 cm in size were used as stationary phase.
- **Mobile Phase:** Toluene: Ethyl acetate : Formic acid (7:3:0.3) was used as a mobile phase.
- **Procedure:** 100 µl of the sample solution and 5 µl of standard solution were applied as band length 5 mm to 8 mm from lower edge of the plate using 100 µl syringe on CAMAG LINOMATE V automatic sample applicator.
- **Development:** Plate was developed in 20 x 20 cm twin trough (CAMAG) chamber. Developing distance was 8 cm from lower edge of the plate. Plates were then dried by air for 10 min.
- **Derivatization:** The plate was sprayed with anisaldehyde- sulphuric acid reagent and heated at 105°C till the spots appeared. The Rf value and colour of the spots were recorded.
- **Densitometer Scans:** Plates were scanned at 580 nm using scanner-3 (CAMAG)
- **Documentation:** The profile obtained was photo documented at 254nm, 366 nm visible before derivatization and after derivatization through camera.
- The HPTLC chromatogram of chloroform extract of *Viscum articulatum* Burm.f. at 366 nm (Track of sample 100 µl) shown the spot of oleanolic acid at Rf value 0.47 compared to standard. The concentration of oleanolic acid in the chloroform extract was calculated as 5.87% w/w.

RESULTS AND DISCUSSION

The leaves of the plant were reduced and appeared scale like. It was a much branched parasitic shrub. The branches were jointed and the internodes are 2.5-5 cm long. They were flattened and narrowed at each end, readily disarticulating. It formed pendulous tufts 15 cm to 0.9 m long. The shrub was pale green when fresh and yellowish brown when it is dried. The flowers were axillary and sessile and male flowers usually in a pair below involucre of female, subtended by acute bracts 0.7 mm long; subsequent flowers mostly female, developing below

earlier ones. Male flower pyramidal, 1 mm long. Female flower broad-cylindrical, tepals 0.5 mm long, ovary 0.5 mm long. Fruit was globose, 4–6 mm in diameter, somewhat translucent, white, yellowish or pink.

The transverse section of stem (fig.1) showed an epidermis with thick cuticle. Below the epidermis cortex was present which consist of thin walled parenchyma cells and lignified fibres. The vascular bundle consists of xylem and phloem. The pith region consists of calcium oxalate crystals as well as thin walled lignified polygonal parenchyma with intercellular spaces.

The microscopic study of powder of whole plant *Viscum articulatum* Burm. f. showed the presence of lignified fibres, parenchyma, starch grains, vascular bundle, epidermis and calcium oxalate crystals (figure2,3,4).

The analytical parameters such as total ash, water soluble ash, acid insoluble ash, loss on drying, alcohol (90%) and water soluble extractive values of powder of whole plant *Viscum articulatum* Burm. f. are given in Table 1.

The qualitative chemical tests of the powdered whole plant of *Viscum articulatum* Burm.f. revealed the presence of triterpenoids, flavonoids, steroids, saponins, glycosides and proteins.

The HPTLC of the bioactive crude extract (chloroform extract) of *Viscum articulatum* Burm.f. at 366 nm (Track of sample 100 µl) shown the spot of oleanolic acid at Rf value 0.47 compared to standard. The concentration of oleanolic acid in the chloroform extract was calculated as 5.87% w/w.

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