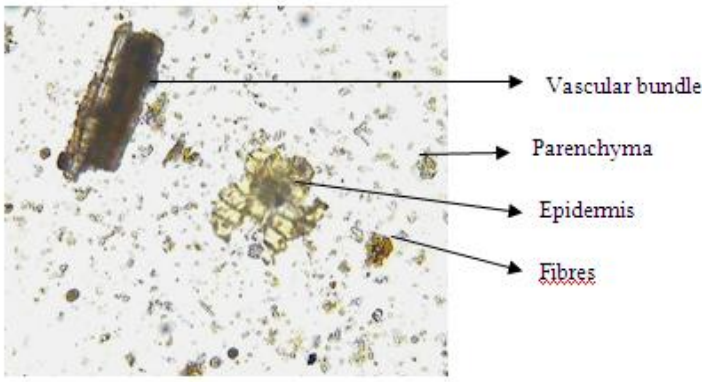
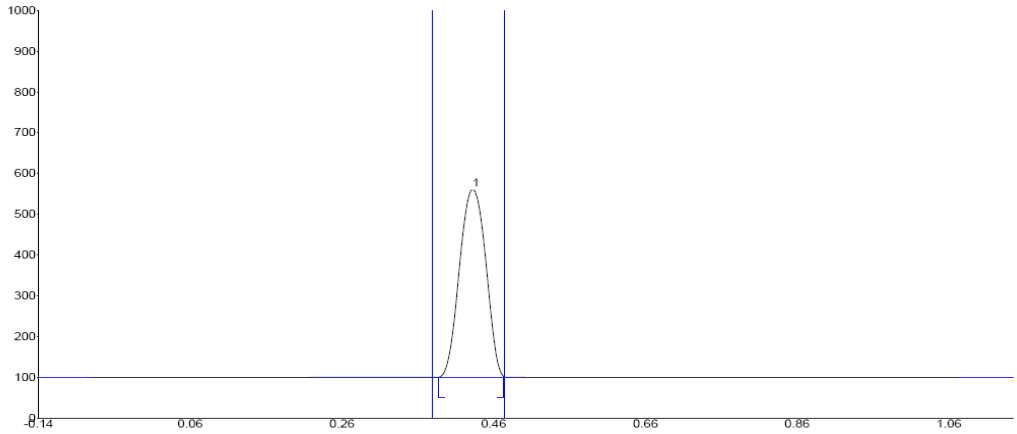


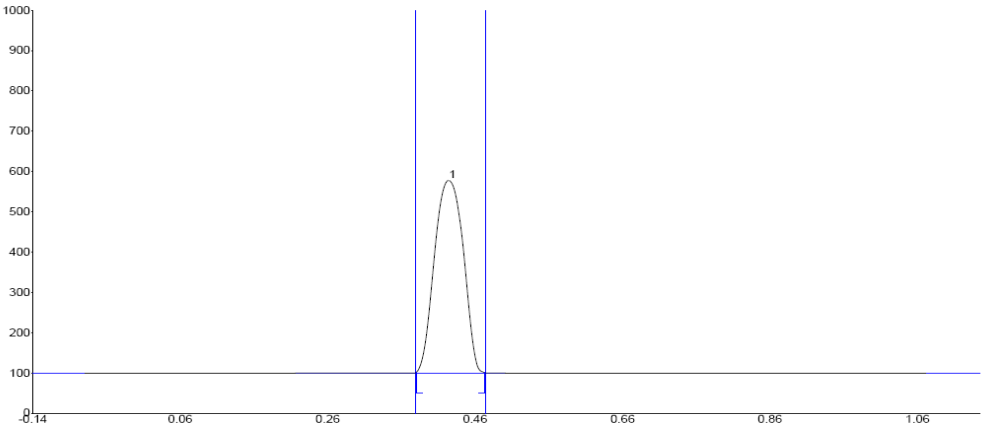
Figure 4: Microscopic examination of powdered drug of *Viscum articulatum* Burm. f. in glycerin and water at 10x objective



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	0.1	0.43	461.2	100.00	0.47	2.5	12980.3	100.00	unknown *

Track of Standard: Oleanolic acid (5 µl)

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



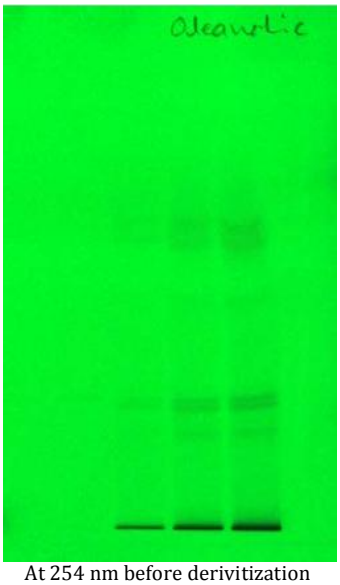
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.38	1.9	0.42	478.0	100.00	0.47	1.0	15245.7	100.00	unknown *

Track of Sample: Chloroform extract (100 µl)

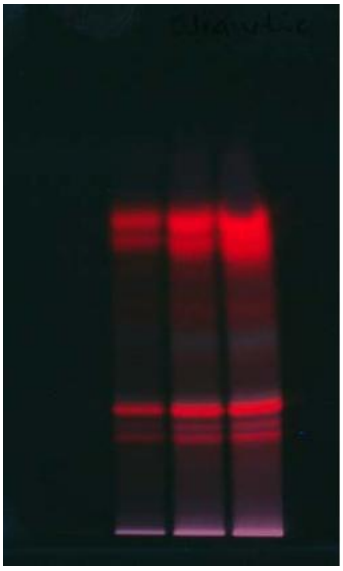
Figure 6: HPTLC chromatogram of sample solution of Chloroform extract (100 µl), Track of Sample

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 HPTL 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

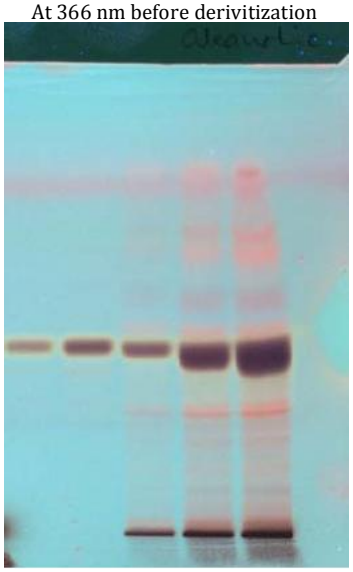


At 254 nm before derivitization

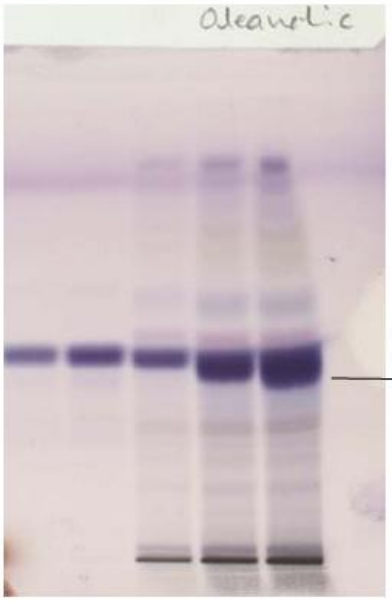


At 366 nm before derivitization

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

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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^{0}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 *Visccum 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 articulatu*m 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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