



(RESEARCH ARTICLE)



Phyto-pharmacognostical evaluation and HPTLC finger printing profile of Gulbanafsha (*Viola odorata* L.) flower

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Abstract

Viola odorata Linn. (Family Violaceae) is popularly known as Sweet Violet or English Violet and Gulbanafsha in Hindi. *V. odorata* is used as remedy for coughs, sore throat, hoarseness and tonsillitis. It is valued as an expectorant, antioxidant, diaphoretic, antibacterial, antipyretic and diuretic. Pharmacological studies have revealed the role of *V. odorata* in Unani drugs for treatment of common cold, asthma and cough associated diseases. The present study focuses on the phyto-pharmacognostical evaluation and HPTLC finger printing profile of Gulbanafsha (*Viola odorata* L.) flower - white variety. Physicochemical parameters were determined and phytochemical screening done by standard procedures. HPTLC finger-printing profile of the methanolic extracts of dried flowers, were performed on precoated TLC silica gel 60F₂₅₄ plates with salicylic acid as marker. Phytochemical screening revealed presence of flavonoids, glycosides, phenolic compounds/tannins.

Keywords: *Viola odorata* L. – flowers; Gulbanafsha, quality standards; Pharmacognostical studies; Physico-chemical Parameters; Phytochemical screening; HPTLC finger-printing

1. Introduction

Medicinal herbs used in Asian schools of traditional medicine have received much importance in recent years due to their extensive applications in healthcare on a global scale. Different strategies have been utilised for the selection of specific herbs for study. The herbs selected are screened for the active phytoconstituents, which are isolated by different techniques. Ayurveda, the traditional school of Indian medicine, lists a huge number of single drugs and formulations. In continuation of our studies on the investigation of Ayurvedic drugs, we took up the phyto-pharmacognostical investigations and HPTLC fingerprinting of the dried flowers (white variety) of *Viola odorata* Linn. acclaimed in Ayurveda as Gulbanafsha. [1-8]

The Violaceae family contains about 900 species belonging to 22 genera; this family is a medium-sized of perennial or rarely annual herbs or shrubs, including the violets or pansies. The *Viola* Linn. is the largest genus of the family and has about 400 species in the world. *Viola odorata* Linn. belonging to the family Violaceae, is an ethnobotanical herb of India. It is popularly known as English Violet, Sweet Violet, Common Violet, Blue Violet, March Violet or Garden Violet in English, and Gulbanafsha in Hindi. The sweet, unmistakable scent of this flower has been popular for generations, and has consequently been used in production of perfumes and cosmetic fragrances. *V. odorata* is used as remedy for hoarseness, sore throat, coughs, and tonsillitis. It is valued as an expectorant, antioxidant, diaphoretic, antibacterial, antipyretic and diuretic. Pharmacological studies have revealed the role of *V. odorata* in certain Unani drugs for treatment of common cold, asthma and cough associated diseases. [1-8]

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1.1. Botanical Synonyms

Viola odorata f. *odorata*, *Viola wiedemannii* Boiss.[2]

1.2. Classical Synonyms

Jvarapaha, Nilapushpa, Sukshmapatra, Vanapsa.[1]

1.3. Other /Regional Language Names

Bengali: Banafsha, Banosa; *English*: Apple-leaf, Bairnwort, Banwort Beassy Banwood, Blaver; *Kannada*: Violethoo; *Marathi*: Bagabanosa; *Tamil*: Vauilettu; *Telugu*: Vialettu; *Urdu*:Banafshah. [1, 3]

1.4. Phylum

Plantae; Division - Magnoliophyta; Class - Magnoliopsida; Order - Violales; Family - Violaceae.

Genus - *Viola*; Species - *V. odorata* Linn.

1.5. General Information about the plant in brief

Perennial stock short, but sometimes branched, and usually emitting creeping scions. It spreads with stolons. Leaves in radical (or rather terminal) tufts, broadly cordate, rounded at the top, and crenate, downy or shortly hairy, with rather long stalks. Stipules narrow - lanceolate or linear, and entire. Peduncles about as long as the leaf stalks, with a pair of small bracts about way up. Flowers are nodding sweet-scented, of two varieties - the bluish purple colour that gives the name of the plant after them, or white. Sepals obtuse. Spur of the lower petal short. Stigma pointed, horizontal or turned downwards. It occurs in Kashmir at 5000-6000 ft. and Uttarakhand, and is planted in many hill stations in North West Asia, North Africa and Europe. The plant is bitter and pungent; hot, antiperiodic; cures malarial fevers, bronchitis, asthma, and 'tridosha' (Ayurveda). [1-8]

The following pharmacological activities of extracts of different parts of *V. odorata* the plant have been reported [5, 6, 7]: Antioxidant, anti-bronchitis, sedative and pre-anesthetic, antipyretic, anticancer, antitumor, cytotoxic, anti-inflammatory, antimicrobial, antifungal, antidyslipidemic, antihypertensive, antidyslipidemic, laxative, molluscicidal activity; as well as activity against headache and insomnia, kidney and liver disorders.

Antibacterial and phytochemical aspects of *Viola odorata* Linn. extracts against respiratory tract pathogens have been investigated by Gautam *et al.* [9]. The authors concluded that their investigation supports a good response to the use of *V. odorata* in herbal medicine and as a base for the development of new drugs and phytomedicine in rationale for its use in treatment of respiratory infectious diseases.

Noor Nihad Baqer *et al.* [8] have reported Gas Chromatography-Mass Spectroscopy analysis of methanol extract of *V. odorata* flowers, collected in April and July in Iraq, which showed 84 phytochemical constituents. Results of GC-MS investigations of Egyptian and French varieties have been summarised by Mittal [6]. GC-MS analysis of the essential oil of *V. odorata* flowers revealed high percentages of monoterpenes and sesquiterpenes [5]. Analysis of essential oil composition of the leaves of *Viola odorata* L. revealed the presence of 25 identified compounds [5]. Reports about different types of phytoconstituents, *viz.* have been collected by Mittal [6] and Singh [7] in their reviews. Phytochemical screening of extracts of different parts of *V. odorata* has shown that plant contains salicylic acid; *trans*-caffeic, protocatechuic, gentisic, *p*-hydroxybenzoic, 4-hydroxyphenylacetic, *trans*- and *cis*-coumaric acids; flavonoids; glycosides; alkaloids; steroids; terpenes; saponins and tannins [7]. It is reported that about 30 cyclotides have been identified from the aerial parts and roots of *V. odorata* [6].

Extensive work has been done on various aspects of the *Viola odorata* plant; the work has been reviewed [5, 6, 7]. However, to our knowledge, no pharmacognostical studies and HPTLC profiling studies have been reported. These are also not mentioned in the multi-volume Ayurvedic Pharmacopeia of India published by ICMR and then CCRAS. Hence, we took up investigations on these aspects of *Viola odorata* flowers - white variety.

2. Material and methods

2.1. Collection and authentication of Plant material

Viola odorata L. whole plant, including flowers, were obtained from well-reputed suppliers in Kolkata. The plant was identified by Professor N. D. Paria, Indira Gandhi National Open University, and formerly of Botany Department, University of Calcutta.

2.1.1. Plant sample processing

The flowers were dried at ambient temperature in the shade for 7 days. After drying, approximately 20g of the dried flowers were obtained. Approximately 3g in total were ground with a grinder and sieved to obtain 60-mesh size powder, these were used for organoleptic and microscopical studies. Rest of the dried flowers was separately ground to coarser powder, which was used for determining physicochemical parameters including extractive values and carrying out preliminary phytochemical analysis and HPTLC analyses.

2.1.2. Physicochemical Parameters

These were determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/WHO protocols [10, 11].

2.1.3. Preliminary Phytochemical Analysis

The phytochemical analysis of methanol extract of *V. odorata* was carried out by using standard chemical tests [12, 13].

2.1.4. Solvents and Chemicals

The solvents and chemicals were of GR grade (E. Merck Ltd., Mumbai, India).

2.2. Pharmacognostic Investigations

2.2.1. Macroscopy of plant material

The organoleptic parameters, viz. texture, shape, size, colour etc. of the plant material were noted by naked eye observation with a simple microscope Olympus OIC DM.

2.2.2. Cytomorphology of plant material

For powder microscopy, finely powdered samples (~1 g) were separately treated with different reagents, viz. aqueous saturated chloral hydrate (for maceration), 50% glycerin, phloroglucinol in concentrated HCl (for staining lignified tissues) and 0.02 N iodine reagent (for starch grains), mounted on slides with glycerin following a standard protocol and observed under the binocular compound microscope (Olympus OIC- 07964) at 10X and 40X magnifications. Photomicrographs of the different cellular structures and inclusions were taken using Magcam DC14 camera attached with Olympus CX21i trinocular compound microscope. Measurement of different cellular inclusions were taken with the help of Magnus monocular student compound microscope, stage micrometer and ocular micrometer.

2.3. HPTLC Study - Equipment

A CAMAG HPTLC system (Switzerland), comprising a CAMAG ATS-4 applicator, CAMAG TLC scanner 3, CAMAG Wincats Software Version 1.44, CAMAG Reprostar 3, a CAMAG TLC plate heater, and a CAMAG UV cabinet, was used for this study.

3. Results and discussion

3.1. Pharmacognostical analysis of dried flowers of *Viola odorata*

3.1.1. Macroscopic features

The dried flowers shown in Figures 1, 2 are solitary, in the apical portion of long pedicel. Bracts are located in the middle of the scapes, opposite and linear lance shaped. There are glands along margin. Flowers are zygomorphic. Flowers are 0.5-0.8 cm in diameter. Sepals 5, oblong to ovate in shape and apex acuminate. Zonal appendages are present at base. Dried petals are yellowish brown in colour. Petals that are near the bottom are obcordate and longer than sepals, which

in the middle was bigger than two sides. Filaments are short and broad, superior ovary smooth, styles apically curved and rostellate. The flowers possess slightly aromatic odour and slightly pungent and bitter taste.



Figure 1 Dried flowers of *Viola odorata* L.



Figure 2 Enlarged view of flower through simple microscope



Figure 3 Powdered flowers (white variety) of *Viola odorata* L.

3.1.2. Powder features

Organoleptic characteristics of the fine powder of dried flowers (Figure 3): Colour - yellowish brown with flakes; texture - rough; odour - slightly aromatic; taste - slightly pungent and bitter.

3.2. Powder Microscopy

Yellow brown coloured powdered sample of dried flower (white variety) shows the following diagnostic feature: unicellular trichomes, some trichomes with warty wall; epidermal cells of petal with characteristic papillary projections; polygonal thin walled parenchymatous cells with rosette crystals; vessels with spiral thickenings individually scattered or with parenchyma cells; polygonal, closely packed fragmented cells of flower epidermis; few canary yellow coloured smooth walled tricolpate pollen grains with near-spherical shape; special thickening on endothecium cells (the lining of the cavity of anther) of clinandrium; group of beaded polygonal parenchyma cells; long aseptate fibres (Figures 4 and 5).

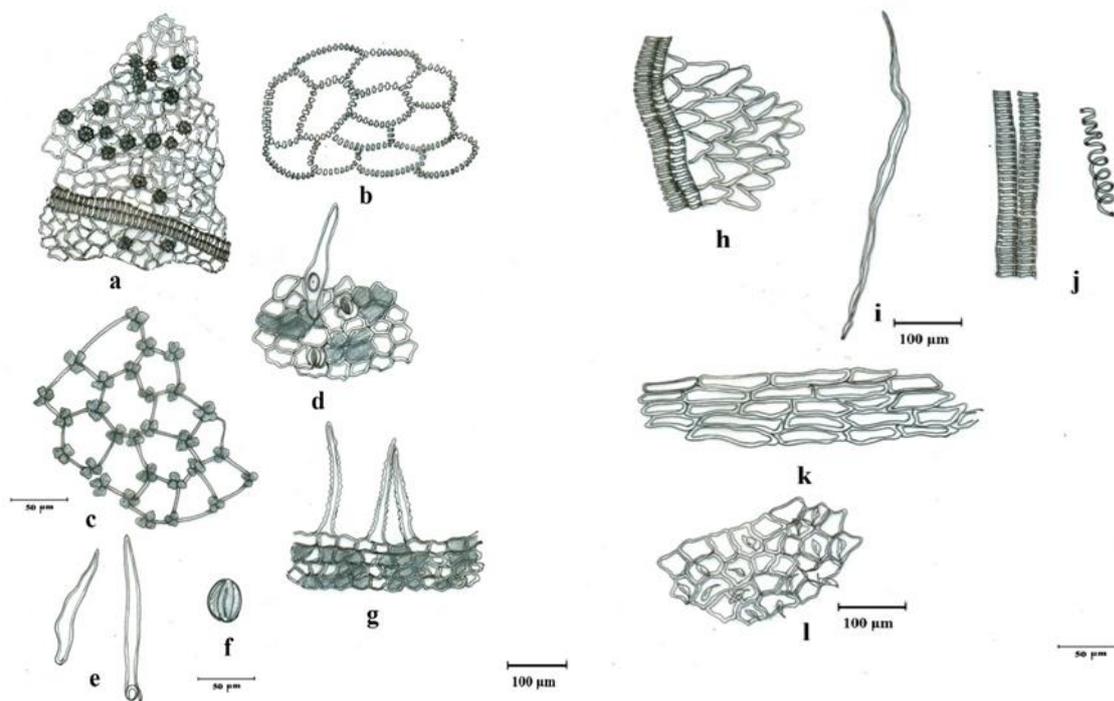


Figure 4 Camera Lucida drawing of powder microscopy of flowers of *Viola odorata* L.

a: Parenchymatous cells along with spiral vessels containing rosette crystals; **b:** Group of beaded polygonal parenchyma cells; **c:** Special thickening of endothecium cells of clinandrium; **d:** Origin of trichome from epidermis with stomata; **e:** Unicellular trichome; **f:** Tricolpate pollen grain; **g:** Origin of warty trichome from epidermis. **h:** Epidermal cells of petal with papillary projections; **i:** Long aseptate fibres; **j:** Vessel with spiral thickening; **k:** Elongated parenchyma; **l:** Fragmented cells of flower epidermis.

3.3. Parameters related to cellular inclusions

Rosette crystal diameter: 25.34 µm to 28.16 µm

Pollen grain diameter: 28.16 µm to 30.97 µm

Fibre (fragmented) diameter: 14.08 µm

Trichome diameter: 15.48 µm to 25.34 µm

Trichome length: 230.91 µm to 328.06 µm

3.4. Photomicrographs

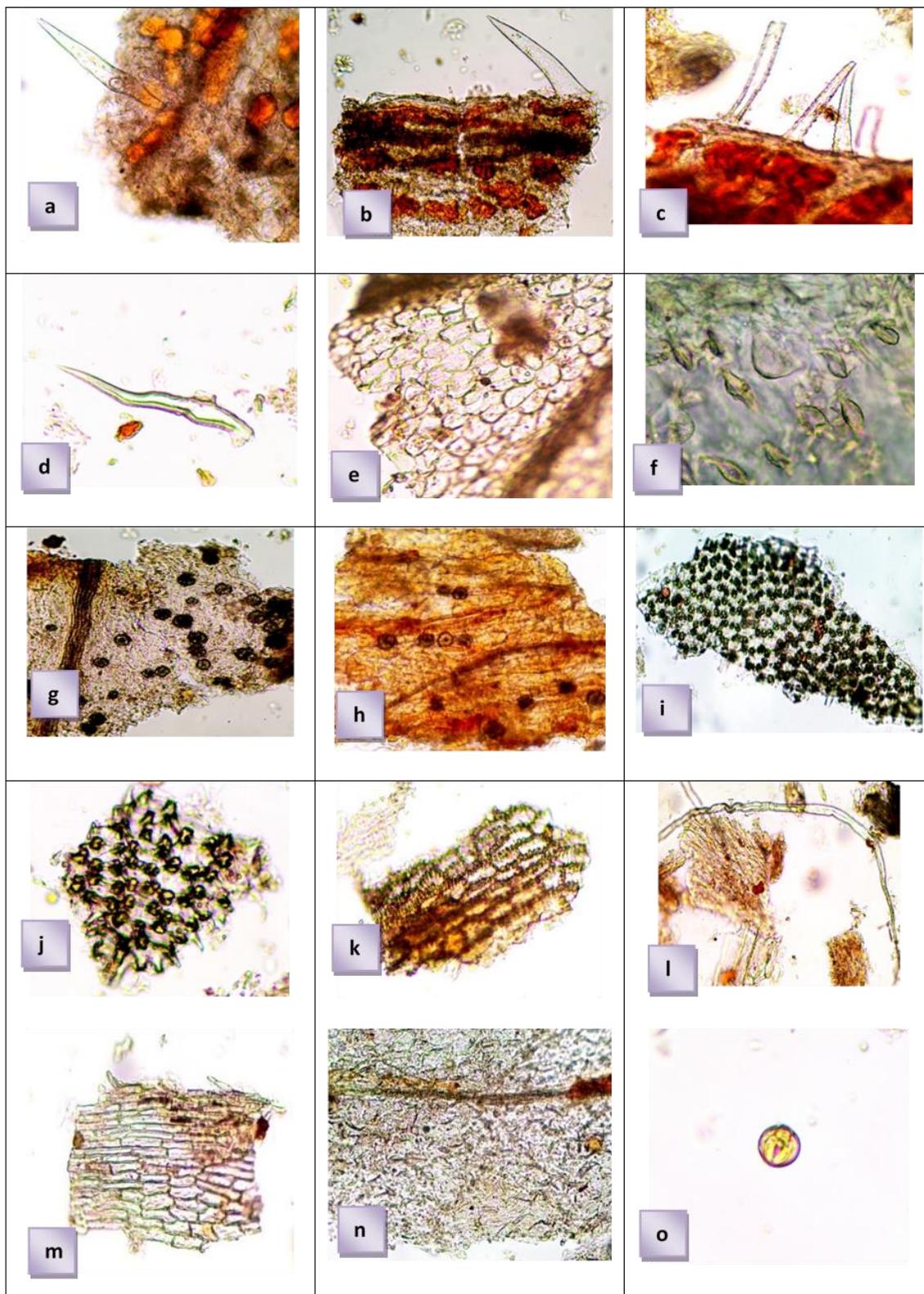


Figure 5 Photomicrographs of powder microscopy of flowers of *Viola odorata* Linn.

a, b c: Origin of trichome from epidermis; **d:** Unicellular trichome; **e,f:** Epidermal cells of petal with papillary projections; **g,h:** Parenchymatous cells along with spiral vessels containing rosette crystals; **i, j:** Special thickening of endothecium cells of clinandrium; **k:** group of beaded polygonal parenchyma cells; **l:** Long aseptate fibres; **m:** Elongated parenchyma; **n:** Fragmented cells of flower epidermis; **o:** Tricolpate pollen grain.

3.5. Physicochemical Parameters

Determination of the following physicochemical parameters were carried out - water-soluble extractive, 90% alcohol-soluble extractive, loss on drying, total ash, acid-insoluble ash, pH of 10% aqueous suspension (Table 1). These were determined in accordance with standard protocols as mentioned in Ayurvedic Pharmacopoeia of India/ WHO. [10, 11]

Table 1 Physicochemical Parameters

Parameters	Results (% w/w)
Loss on drying (at 105 °C)	18.26
Total ash	8.42
Acid-insoluble ash	1.52
Water-soluble extractive	28.59
90% Alcohol-soluble extractive	9.70
Volatile oil content	0.04
pH (10% aqueous suspension)	5.60

3.6. Preliminary Phytochemical Analysis

3.6.1. Preparation of extract for Preliminary Phytochemical Analysis

1 g of the coarse powder taken in a round bottom flask and 20 ml of CH₃OH was added to it. The mixture was boiled for one hour under reflux and filtered (Whatman No. 40 filter paper). The filtrate was taken for preliminary phytochemical analysis.

The methanolic extract, obtained as described in the previous section was utilised for preliminary phytochemical testing - 2 ml of the extract being taken for each test. The results are given in Table 2.

Table 2 Preliminary Phytochemical Tests of Methanolic Extracts of dried flowers of Gulbanafsha

Sl. No.	Test/Reagent used	Observation
1a.	Alkaloids - Mayer's test (in dil. HCl)	Negative
1b.	Alkaloids - Dragendorff's test (in dil. HCl)	Negative
2a.	Carbohydrates - Molisch's test	Negative
2b.	Reducing sugars - Fehling's test	Negative
3.	Flavonoids - Shinoda test	Positive
4.	Terpenoids/ Steroids - (Liebermann-Burchardt Test)	Negative
5.	FeCl ₃ test for Phenolic compounds/ tannins	Positive
6.	Glycoside (Acetic acid, FeCl ₃ , H ₂ SO ₄)	Positive

3.7. HPTLC Finger-printing profile of flowers of *Viola odorata*

3.7.1. Sample preparation

1 g of the sample was subjected to reflux with methanol (10 ml) for 1 hour and extract was filtered through filter paper (Whatman No. 40). The filtrate was concentrated ($c = 1 \text{ g}/10 \text{ mL}$) and taken for HPTLC profiling.

3.7.2. Preparation of standard

1.0 mg of salicylic acid (Marker) was dissolved in 10 ml of methanol in a volumetric flask.

3.7.3. Chromatography experimental

Stationary Phase: Precoated (support on Aluminum Sheets) Silica Gel Plate. Specification: TLC Silica Gel 60F₂₅₄. Mfg. by Merck, India; 26.09.2016, Batch No. 1.05554.0007.

3.7.4. Mobile Phase

Hexane: Ethyl acetate: Methanol: Formic acid = 4: 4.5: 1: 0.25 (v/v) [G R grade solvent used, mfg. by Merck, India]

3.7.5. Sample application

Applied volume - 5 μ L as 6 mm band and applied at 12 mm from the base of the plate with a CAMAG ATS4. Plate size was 10 x 5 cm.

3.7.6. Development

Developed up to 90 mm of the plate in CAMAG Twin trough chamber. Plate preconditioning - temperature 25°C; relative average humidity was 42%.

3.7.7. Observation

The chromatograms were visualised in CAMAG TLC visualiser and scanned using a CAMAG TLC Scanner 4.

Photography of Developed HPTLC Plate: 254 nm and 366 nm.

3.8. General Comments

The HPTLC chromatograms Visualised at 254 nm and 366 nm are given in Figure 6. Corresponding Densitometric finger print profiles are given in Figures 7 and 8. The more intense and sharper bands have been listed, along with their R_f values in Table 3

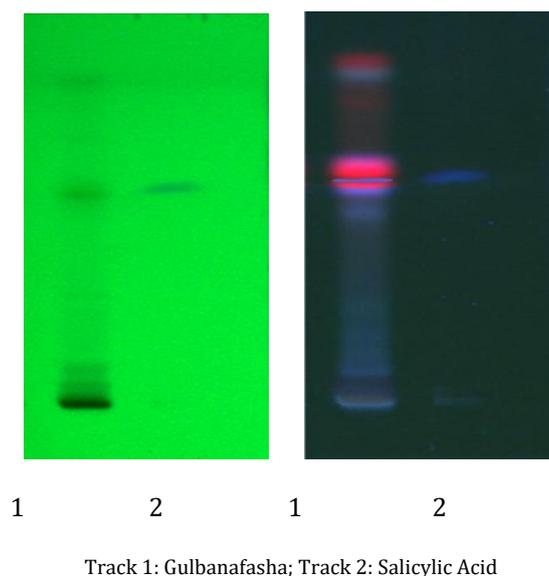


Figure 6 Photography of HPTLC Plate – (a) Visualisation at 254 nm; (b) Visualisation at 366 nm

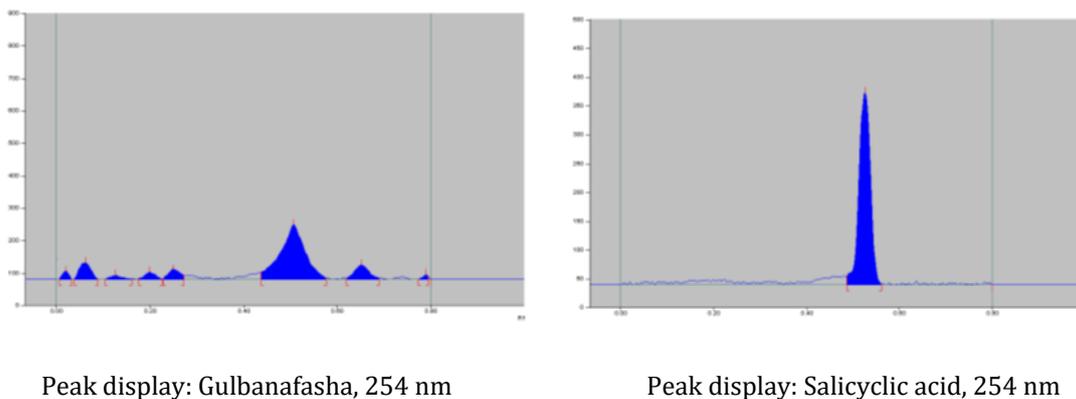


Figure 7 Densitometric finger print profiles at 254 nm of *Viola odorata* flowers (Gulbanafasha)

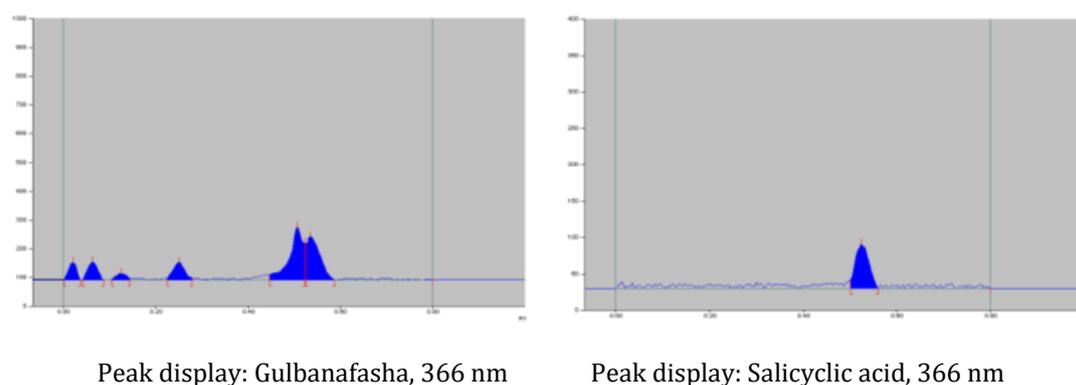


Figure 8 Densitometric finger print profiles at 366 nm of *Viola odorata* flowers (Gulbanafasha)

Table 3 R_f values of major bands

Sl. No.	Sample	R _f values	
		254 nm	366 nm
1.	GulBanafsha	0.05, 0.09, 0.15, 0.22, 0.27, 0.53, 0.54, 0.55, 0.67, 0.81	0.08, 0.12, 0.18, 0.24, 0.46, 0.52, 0.54, 0.58, 0.74, 0.81, 0.83
2.	Salicyclic acid	0.54	0.54

4. Conclusion

The present report on pharmacognostical profiling and HPTLC analysis of dried flowers of *Viola odorata* provides a vital diagnostic tool for identification, authentication and development of quality parameters of this botanical. Data obtained in present study may be considered to be the standard for future studies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest

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