

Standardization and Quality Evaluation of 'Banafsha'

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Abstract – 'Banafsha' is an important herbal drug of indigenous systems of medicine. Flowers of *Viola odorata* L. (Violaceae) are considered to constitute the genuine drug 'Banafsha'. However, due to limited distribution of this plant, another species of *Viola*, i.e. *V. pilosa* having almost similar medicinal properties, is mostly used under this vernacular instead. Ethnobotanically also different species of *Viola* i.e. *V. odorata*, *V. pilosa* and *V. betonicifolia* are used for various ailments at different places in our country. In the present study two species of *Viola*, namely *V. pilosa* and *V. betonicifolia* along with the commercial samples of 'Banafsha' were studied and authenticated. It was observed that the market samples procured from Dehradun, Mumbai, Lucknow, Palampur, Ramnagar and Ranikhet showed close resemblance with *Viola pilosa* in having similar morphological characters like uniseriate hairs on the ovary, geniculate and clavate style, truncate stigma and almost similar TLC profiles. On the other hand the Almora sample was identified as *Viola betonicifolia* by the presence of large purple flowers, clavate style and convex stigma forming hump like structure. However, the market sample procured from Pathankot was found to be a mixture of two species of *Viola* namely, *V. pilosa* and *V. odorata* having dominance of the former species.

Key words – Quality evaluation, *Viola pilosa*, 'Banafsha', Authentication, Commercial samples.

Introduction

'Banafsha' is an important herbal drug of indigenous systems of medicine and is attributable to *Viola odorata* L. belonging to the family *Violaceae*. The whole plant is considered to be antipyretic, diaphoretic, febrifuge and the flowers as emollient, demulcent, which are used in biliousness and lung troubles (Chopra et al., 1956, Nadkarni, 1940, Kirtikar and Basu, 1933). Originally the Greeks used this drug and acquainted the Muslims with its properties (Watt, 1893). However, later this drug also became popular in Ayurvedic system of medicine. In the Unani system this plant is the main ingredient of 'Joshanda' consisting of a mixture of drugs used mainly

for cough and cold in the form of a decoction and is also administered for improving the complexion (Anonymous, 1950).

Several species of *Viola* are used ethnobotanically also by different tribes viz. *Viola odorata* leaves and flowers are applied externally in throat-ache (Dar et al., 1984, Virjee et al., 1984). Flowers made into Khambir are taken in bed in cold and headache during winter by the people of Sind valley (Dar et al., Loc cit). Similarly the flowers of *Viola odorata* and *V. pilosa*, boiled in water are used as purgative in Kumaon hills (Virjee et al., Loc cit, Shah and Joshi, 1971). The whole plant of *V. pilosa* is considered as antipyretic and dry flowers are given with tea in fever, biliousness in Garhwal hills (Nautiyal, 1981). The leaves of *V. betonicifolia* are cooked and taken as vegetable (Pal, 1984).

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In India *V. odorata* L. grows wild in Kashmir and Simla hills at an altitude of 1,600-2,000 metres (Hooker, 1886). Another species of *Viola* i.e. *V. pilosa* Blume grows abundantly throughout the hilly district of India and has similar medicinal properties with those of *V. odorata*. It is in this context that *V. pilosa* was collected from Ranikhet and studied in detail and one more species of *Viola* growing side by side and identified as *V. betonicifolia* Smith was also collected with a view to make comparison with the market samples.

The present communication deals with the standardization of the two species of *Viola* i.e. *V. pilosa* and *V. betonicifolia* and authentication of eight commercial samples of 'Banafsha' procured from the market of Almora, Dehradun, Mumbai, Lucknow, Palampur, Pathankot, Ramnagar and Ranikhet. The study includes morphological characters of flowers, scanning electron microscopic studies of pollen grains and the stigma of the genuine as well as the market samples. Besides, the percentages of different physico-chemical values and extractive values (From non-polar to polar) were determined along with the thin layer chromatographic studies of various extractives. An attempt has also been made to compare the densitometric scans of genuine 'Banafsha' with the market samples procured from various drug markets of the country for quality evaluation. Phytochemical aspects of *V. betonicifolia* and market sample of Almora could not be studied in detail due to the paucity of the material.

Materials and Methods

The plants of *Viola pilosa* and *V. betonicifolia* were collected from Ranikhet (U.P.) at the height of 1,800 metres and lodged in the institute's herbarium. The scanning electron microscopic studies were performed with Phillips Scanning Electron microscope and procedure followed for the preparation of sample

was according to the method described by Mehrotra and Shome (1993).

Physico-chemical values like, total ash, acid insoluble ash, percentage of glucose and tannins were calculated according to the methods described in Anonymous (1965) and Anonymous (1984) respectively.

Observations

General morphology of the flowers –

The flowers of *Viola pilosa* are white or pale violet, pedicellate, bracteate, spurred and zygomorphic. The pedicel is about 7.5 cm long and 1 mm in diameter. The bracts are borne in pairs nearly 2.5 cm below the flowers. The spurs are about 2.5 cm long and 1.5 cm broad; sepals are appendiculate, hairy, pentamerous, imbricate, free, the apex pointed and margin entire. Stamens are pentamerous, filaments short, anthers basifixed and four lobed. The anterior lobes are smaller than the posterior ones. The connective is broad and bears an up growing corona on the dorsal side. The corona of all the stamens unite to form an arch over the gynoecium. The two anterior stamens are larger and possess spur rising from the dorsal side of the connective and running into the spur of the petal.

The gynoecium varies in size 1-1.5 cm long. The ovary is sessile and highly pubescent. Style is swollen subclavate, subtruncate and slightly beaked at the apex, stigma truncate (Plate I, Fig. 1a).

Viola betonicifolia flowers are purplish white with darker veins and pedicellate. The pedicel is about 1.5 mm broad and 12.5-13.5 cm long. The bracts are coming out alternately about 6 cm below flowers almost midway and are 7 mm long and 1mm broad. The sepals are 4-8 mm, ovate to obovate, lanceolate acute or acuminate; the petals are up to 1.5 cm, obovate-oblong. The lateral petals are usually bearded at the base. The spur is 2-6 mm, cylindrical straight or slightly upcurved. No hairs are observed on the ovary

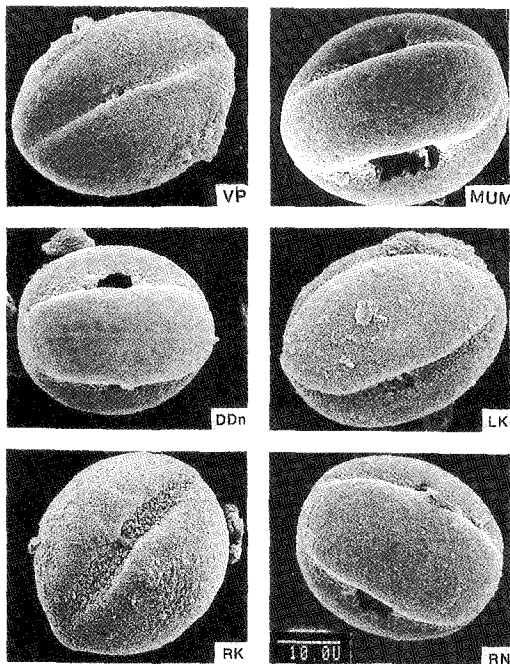


Plate I. SEM Photographs of Pollen grains of *Viola pilosa* and market samples of Banafsha.

wall. The style is 3 mm, geniculate at base, clavate above, stigma convex forming a hump like structure with an opening on the lateral side (Fig. 1b).

The comparative morphological characters of the two species of *Viola* i.e., *Viola pilosa* and *V. betonicifolia* along with *V. odorata* (after Singh, 1965) are given in Table 1.

Phytochemical studies

A known quantity of dried plant material was extracted in Soxhlet apparatus with hexane, chloroform, acetone, alcohol and water successively and percentage of each solid extractive was calculated from the respective extractive by evaporating the solvents. The results are depicted in histogram (Fig. 2).

Thin layer chromatography of each extractive was carried out with different solvent systems. The characteristic finger print profiles of thin layer chromatograms can be used as markers for the quality evaluation of a particular sample. For instance the acetone

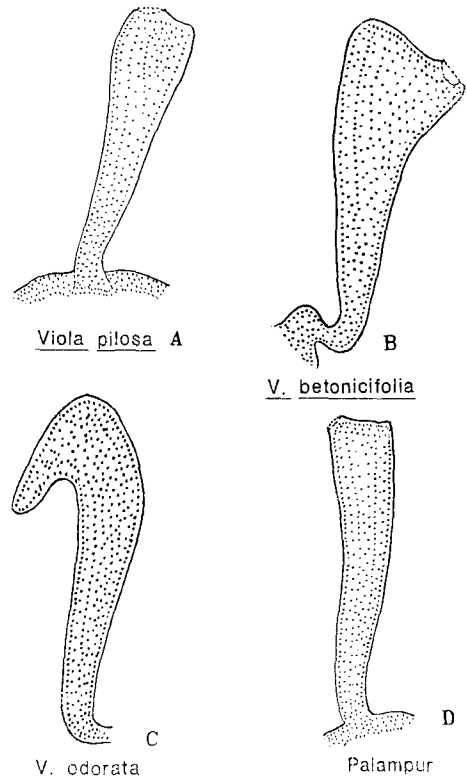


Fig. 1. Showing different types of stigmas and styles. Abbreviations used in Figs. 2-5 DDN, Dehradun sample; λLko, Lucknow sample; Mum, Mumbai sample; Palm, Palampur sample; Pkot, Pathankot sample; Rk, Ranikhet sample; Rn, Ramnagar sample.

extractive of all the samples when resolved in ethylformate:toluene:formic acid:and water (80:100:20:2) showed characteristic spots at different Rfs. For the development of chromatograms the 10% ethanolic-sulphuric acid used as spraying reagent. Majority of the commercial samples viz. Lucknow, Dehradun, Mumbai, Palampur, Ramnagar and Ranikhet showed very close resemblance with *V. pilosa* in having almost more or less the same spots at the same Rfs. However, the sample procured from Pathankot shows different type of TLC profile in having absence of spots at Rfs 0.14, 0.20, 0.37, 0.73, and presence of 2 more spots at Rfs 0.12 and 0.42 of yellow and light purplish colours respectively.

The percentage of total ash, acid insoluble

Table 1. Morphological Characters of *Viola* spp

Characters	<i>V. betonicifolia</i>	<i>V. odorata</i>	<i>V. pilosa</i>
Stem	Plants without a subterranean stem	Short; stolons 15-20 cms slender.	Stems or stolons usually long, prostrate leafy.
Lamina	1.5-8.0×0.5-3.0 cm, roundish obtuse, sub-acuminate, crenate.	1.5-3.5×2.0-4.0 cm, broadly ovate-cordate obtuse crenate, glabrous	1.5-8.0×1.0-6.0 cm, ovate to deltoid serrate, deeply cordate at base, mostly hirsute or pilose to glabrous
Petiole	2.0-10.5 cm & winged above	4.0-8.0 cm, slightly pubescent & has two wing like growth along the sides	2-10 cm, pubescent
Stipule	0.5-1.5 cm, ovate, lanceolate, acuminate, adnate up to middle point.	Subulate, lanceolate, glandular, toothed.	0.6-1.5 cm, ovate-acuminate, sub entire to dentate
Peduncle	Equalling or exceeding leaves, bibracteate, 12.5-13.5 cm long & 1.5 mm in diameter.	Bibracteate 8.5-10.0 cm & 2.0-2.5 mm in diameter.	3-8 cm, bibracteate & pilose, 7.5 cm long, 1 mm in diameter
Flower	White to purple with darker veins.	Violet or White tinged.	White or pale-violet
Sepals	Long & curved, 4-8 mm	Short & straight, 1.5-4 mm	Long & curved, 2-5 mm
Spur	2.7×3-4 mm	2.4×2.0 mm.	2.5×1.5 mm.
Style	Geniculate at base, clavate above, 3 mm size.	Inflated, broad & smooth.	Subclavate, subtruncate & shortly beaked at the apex, 2 mm in size.
Stigma	Convex, forming hump like structure and opening on the lateral side. No hairs are observed. (Fig. 1B)	Decurved & hairy (Fig. 1C).	Stigma truncate opening sublaterally, ovary highly pubescent. (Fig. 1A).
Capsule	Up to 1 cm ellipsoid to oblong & glabrous.	5 mm globose, hirsute	Up to 1 cm globose & pubescent.

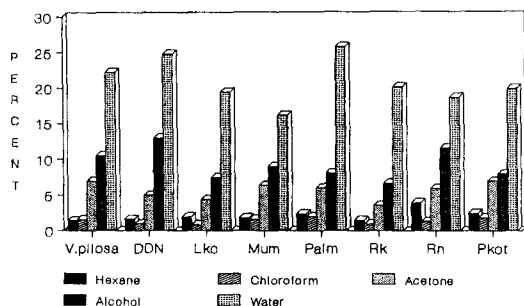
Successive Extractive Values Of Drug 'Banafshah' (Av. Percentage)

Fig. 2.

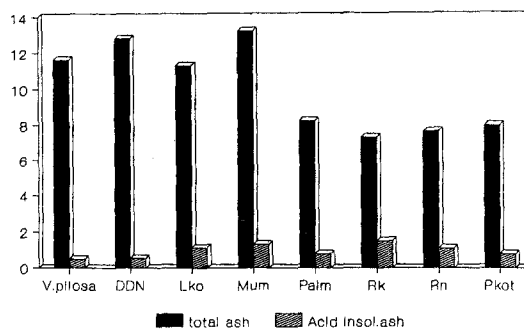
Percentage of Total and Acid insoluble ash of 'Banafsha'

Fig. 3.

ash, tannins and glucose were also calculated and the results are depicted in Fig. 3, 4

and 5 respectively.

As an additional parameter for characteri-

Tannin percentage in 'Banafsha'

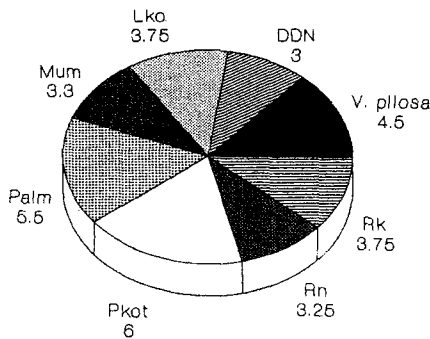


Fig. 4.

Using different market samples the thin layer chromatographic plates of various extractives were scanned through Hitachi 650-60 fluorescence spectrophotometer at different excitation and emission wave lengths.

The finger prints (Fig. 6) of alcoholic extractive also support the observation obtained through morphological and chromatographic studies.

SEM Studies: (Plate I, II, III and IV)

Scanning electron microscopic study of the pollens and stigmas of the two species along with the market samples of 'Banafsha' was carried out and some note worthy differences observed are:

1. The pollen grains oval in shape in *V. pilosa*, (Plate I V.P.), while these are elongated in *V. betonicifolia* (Plate II Vb.). The size ranges from 35.84×33.28-33.28×30.74

Sugar percentage of different samples of 'Banafsha'

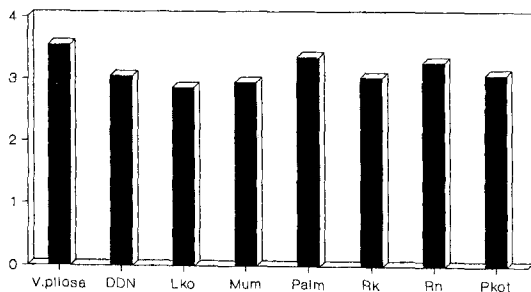


Fig. 5.

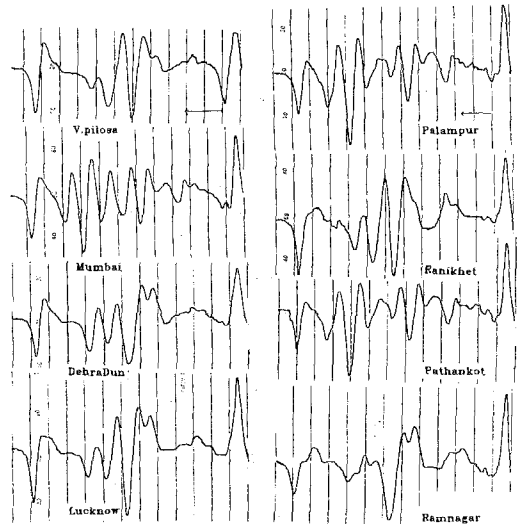


Fig. 6. Densitometric scans of alcoholic extractive of *V. pilosa* and market samples of 'Banafsha'. Excitation wavelength, 370 nm; Emission wave length 740 nm; Excitation and Emission slit, 7.5 and 7 nm; Chart and plate speed, 30 mm/min; Energy Range 0.5 V; Solvent system used, Butanol : Acetic acid : Formic acid and Water (103 : 11 : 25:1).

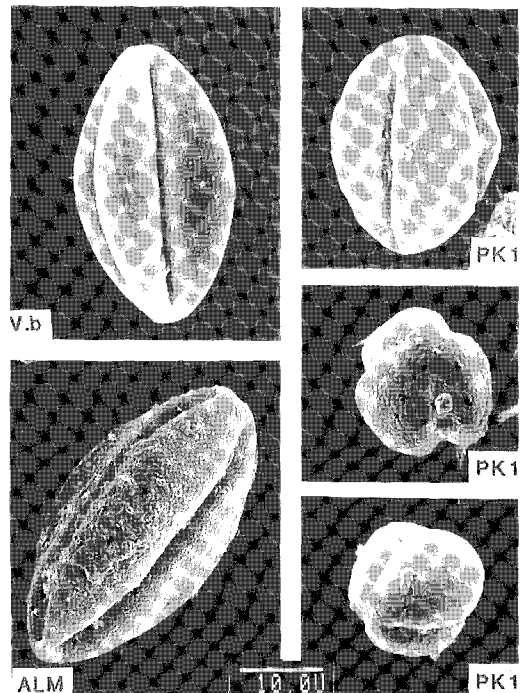


Plate II. SEM Photographs of Pollen grains of *Viola betonicifolia* and market samples of 'Banafsha'.

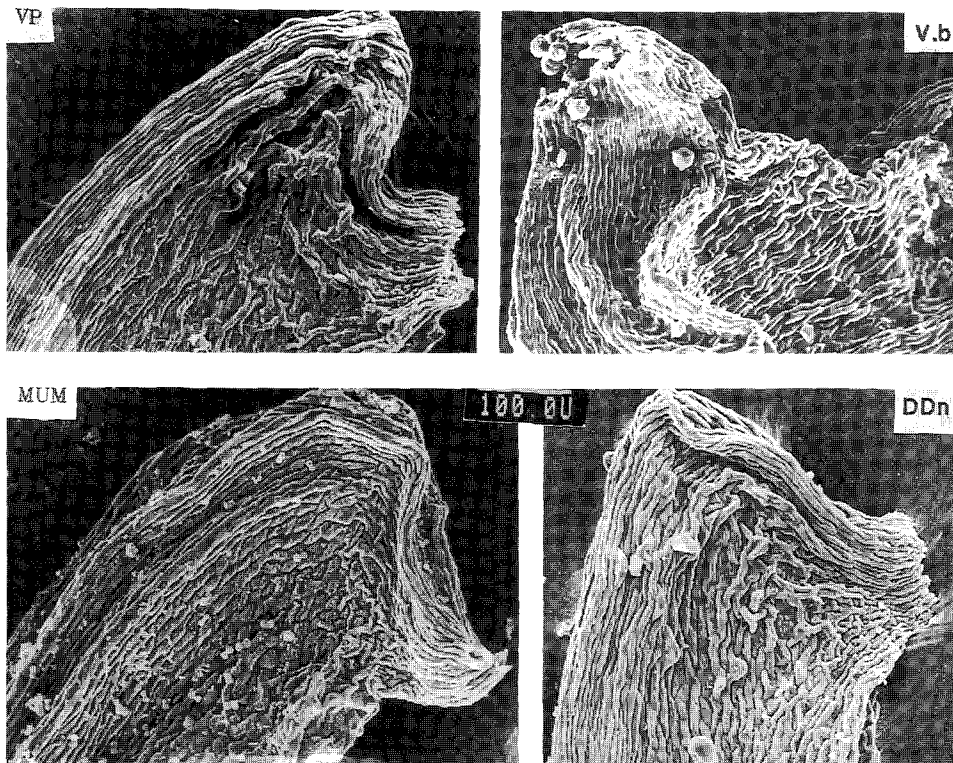


Plate III. SEM Photographs of stigmas of *Viola pilosa*, *V. betonicifolia* and market samples of 'Banafsha'.

μm in *V. betonicifolia* while *V. pilosa* the range is from 28.1×25.60 – $33.28 \times 30.72 \mu\text{m}$. The stigma is subclavate and forming hump like structure in *V. betonicifolia* (Plate III V. b.) and truncate in *V. pilosa* (Plate III V.P.). The ovary wall is hairy in *V. pilosa* and hairs are absent in *V. betonicifolia*.

Discussion and conclusions

A detailed pharmacognostic study of *Viola betonicifolia*, *V. pilosa* and the market samples procured from Almora, Mumbai, Dehradun, Lucknow, Palampur, Pathankot, Ramnagar and Ranikhet markets was carried out & it was observed that the market samples from Dehradun, Mumbai, Lucknow, Palampur, Ramnagar and Ranikhet were conclusively identified as *Viola pilosa* on the basis of uniseriate hairs on the ovary, geniculate and clavate style and truncate stig-

ma while the market samples procured from Almora was identified as *Viola betonicifolia* in view of the close resemblance with it in having white to purple large flowers; clavate style, larger in size (3 mm); convex stigma, forming hump like structure and elongated pollen grains. However, the market sample procured from Pathankot is a mixture of two *Viola* spp. namely *V. pilosa* and *V. odorata* having dominance (more than 90%) of the former one. After surveying the different drug markets of Northern India it has been concluded that in the majority of places *V. pilosa* is being sold as 'Banafsha'.

The scanning electron microscopic study also substantiates the above observations except the Almora sample which resembles *Viola betonicifolia*.

The inference drawn on the basis of morphological observations was also well supported by the densitometric study of the al-

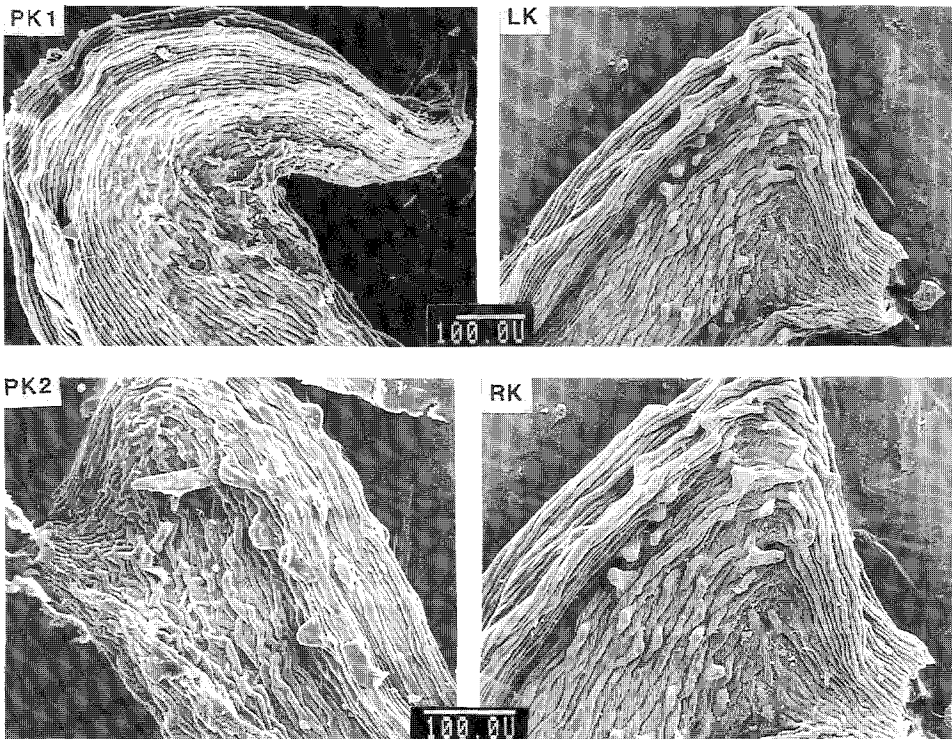


Plate IV. SEM Photographs of stigmas of different market samples of 'Banafsha'.

coholic extractive of all the materials studied. This extractive produces the similar finger prints in *V. pilosa* and majority of the commercial samples i.e. Dehradun, Lucknow,

Palampur, Ranikhet and Ramnagar. However, the sample procured from Mumbai market shows some differences in the finger prints with *V. pilosa*. This may probably be due to the lesser amount of flowers in the sample. Being the mixture of two species of *Viola* the finger prints of Pathankot sample in all together different from the rest of the samples (Fig. 6).

The conclusion drawn on the basis of the above studies is very well depicted in flow chart (Fig.7).

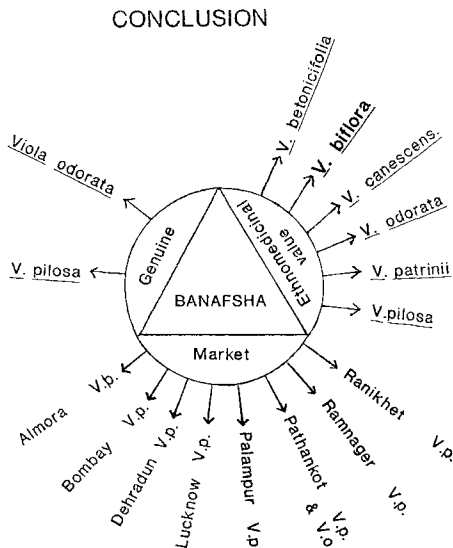


Fig. 7.

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