

Full Length Research Paper

Pharmacognostic studies of *Viola betonicifolia*

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The pharmacognostic profile of crude drug has key role in standardization for quality, purity and drug identification. Various pharmacognostic studies of *Viola betonicifolia* were carried out in order to establish parameters for its identification and to check adulteration or contamination by other species of *Viola*. Morphological characteristic of the whole plant and its anatomic parts were studied by organoleptic evaluation. Transverse sections of the root and petiole were prepared and studied. Powder drug study of the whole plant was also carried out and various structures of the powder drug were observed. This study also includes the numerical and quantitative leaf microscopy, ash values and extraction values. The pharmacognostic profile of *V. betonicifolia* was first time studied in this research paper.

Key words: *Viola betonicifolia*, morphological, organoleptic evaluation.

INTRODUCTION

Medicinal plants are the mainstay of about 75 to 80% of the world population, mostly in the developing countries. In Pakistan, major research work on medicinal plants is mostly conducted in universities and these research activities are dependent on the documented ethno-botanical uses. But the local communities of Pakistan have the knowledge of medicinal plants which is still not documented; such indigenous knowledge of plants uses has been transferred from generation to generation (Shinwari, 2010). In Pakistan, large populations are using the medicinal plants for various diseases like asthma, fever, hypertension, etc., such treatments are provided mostly by local hakims. The major problem for these hakims is the identification of plants, because in the same genus a large number of species are present and these species are very similar with a little morphological difference, therefore, the pharmacognostic studies of these plants have great importance for the identification of plant, especially in same genus.

Viola betonicifolia belongs to the genus *Viola* and family Violaceae; it is widely distributed in Pakistan, Australia, Sire Linka and India. In Pakistan, it is mostly found in Swat, Hazara, Dir and locally it is known as Banafsha or Banosha. It is found in relatively open and shady places.

This species is distinguished by long, slender arrow-shaped leaves that grow from the base of the plant, which has no stem as such and are up to 6 cm (2.4 in) long with a v-shaped sinus at the base, and are usually rather bright, fresh green. Its striking bright purple flowers are 1 to 1.5 cm in diameter and occur in spring and summer. Flowers are followed by small pale brown pods with tiny blackish seeds (Flora of Pakistan). In the traditional system of medicine, it is mostly used as astringent, diaphoretic, antipyretic, anticancer, febrifuge and purgative, and also, it is used for cough, epilepsy and nervous disorders (Hamayun, 2005; Husain et al., 2008). Some of the uses are sinusitis, skin and blood disorders and pharyngitis (Bhatt and Negi, 2006). Roots and fruits are used for kidney diseases, pneumonia and bronchitis. Flowers are used in lung troubles, cough and colds. In continuation to our research work on Pakistani medicinal plants (Saeed et al., 2010, 2011; Barkatullah and Muhammad, 2011; Rahman et al., 2011), we investigated *V. betonicifolia* for its pharmacognostic profile as there is no published data on the pharmacognosy of this plant.

MATERIALS AND METHODS

Plant materials

Whole plant of *V. betonicifolia* (Figure 1) was collected from Swat, Khyber Pakhtunkhwa in April 2010. Plant specimen was identified by Professor Dr. Muhammad Ibrar, Department of Botany,

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University of Peshawar and a specimen was deposited there in the herbarium under voucher number 6410/Bot. The whole plant was powdered for microscopic study and fresh plant was used for cross section study.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste (Wallis, 1985; Evans and Trease, 2002).

Microscopy

The microscopy of powder drug and cross section of the petiole and root was carried out using digital microscope attached with computer system (Olympus Mic-D). The outer epidermal membranous layer (in fragments) were cleaned with chloral hydrate, mounted with glycerin and observed under microscope. Observations were noted for the following parameters: epidermal cells, stomata and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh plant as well as a small quantity of the powdered leaves were also cleared, mounted and observed under microscope (Pharmacopoeia, 1986).

Pharmacognostic features of leaves

Leaf microscopy was carried out for determination of palisade ratio, stomata number, epidermal cells, vein islet number and veinlet termination number.

Physicochemical characteristics

The plant powder was also tested for moisture content, total ash, acid insoluble ash and water soluble ash (Pharmacopoeia, 1980).

Extractive values

10 g of the air-dried powdered whole plant was taken for successive maceration with methanol (3 × 100 ml), hexane (3 × 100 ml), chloroform (3 × 100 ml), ethyl acetate (3 × 100 ml), butanol (3 × 100 ml) and water (3 × 100 ml) at ambient temperature for 24 h and vacuum filtered. The filtrates were concentrated to dryness under vacuum at 40°C. Yield of extracts were determined.

RESULTS AND DISCUSSION

Macroscopy

V. betonicifolia that is often found in shady damp places, may occur in open grass lands. It is a perennial herb having tufted appearance, occur mostly in clusters but may be found in solitary. Perennial creeping roots are present originated from the short stem. Stem is short, light green in color, straight, glabrous or somewhat pubescent. Leaves are long, slender arrow-shaped with dark green color. Leaves are lanceolate to oblong with

long petiole ranges from 2 to 10 cm. Lamina, mostly a length of 1 to 8 cm and width of 5 to 25 mm. Leaf margins are entire or slightly serrate. Linear stipules fused to petiole are present, which may be entire or lacinate. Flowers are violet-shaped having purplish-blue color, some time paler. Cleistogamy phenomena are present in the flowers, that is, produce viable seeds without opening. Sepals are 3.5 to 7 mm long. Petals are violet or whitish with 8.5 to 14 mm long. Fruits are pods having pale brown color. There are many tiny blackish seeds in each pod. The taste of the plant is light spice and order is spice like.

Microscope

The microscopic study of the plant consists of the powder drug study of the whole plant and the cross sectional study of the petiole and root. The results of the powder drug study are given in the Figure 2. The prominent parts of the powder drug are epidermis, cortex, spong parenchyma, anisocytic stomata, vascular bandals, parenchymatous cells, Ca-crystals, xylem vessels, unicellular trichom, cholenchymatous cell of stem, bast fiber, schlerenchyma fiber, palisade parenchyma, single schlarenchyma, lower epidermis with stomata, xylem and phloem of root, cholenchymatous cell, bast fibers, fibers, trichom, parenchymatous cells, stem xylem and stem phloem. The microscopy of the cross sections of the root and petiole is as shown in Figure 3.

Pharmacognostic features of leaves

The pharmacognostic investigations of some physical parameters are helpful in setting standards for a crude drug as these parameters are mostly constant for a plant. The palisade ratio, stomata number, epidermal cell, vein islet number and veinlet termination number were determined for this plant. The detail is given in Table 1. These parameters are valuable in evaluation of a crude drug and prevention of adulteration.

Physicochemical characteristics

In order to protect the crud drugs from microbial growth, the moisture content should be considered. The lower the moisture content the higher will be the stability of that drug and chance of microbial growth will be less and vice versa. The shelf life of the drug also increases with lowering the moisture contents (Ehiabhi, 2010). The *V. betonicifolia* in its dried form is expected to have a long shelf-life with reduced chance of microbial growth due to its low moisture content of 5.2%. Mean ash values (%) was found to be 9.8 (total), 0.7 (acid insoluble ash) and 1.05 (water soluble ash). Total ash value was relatively

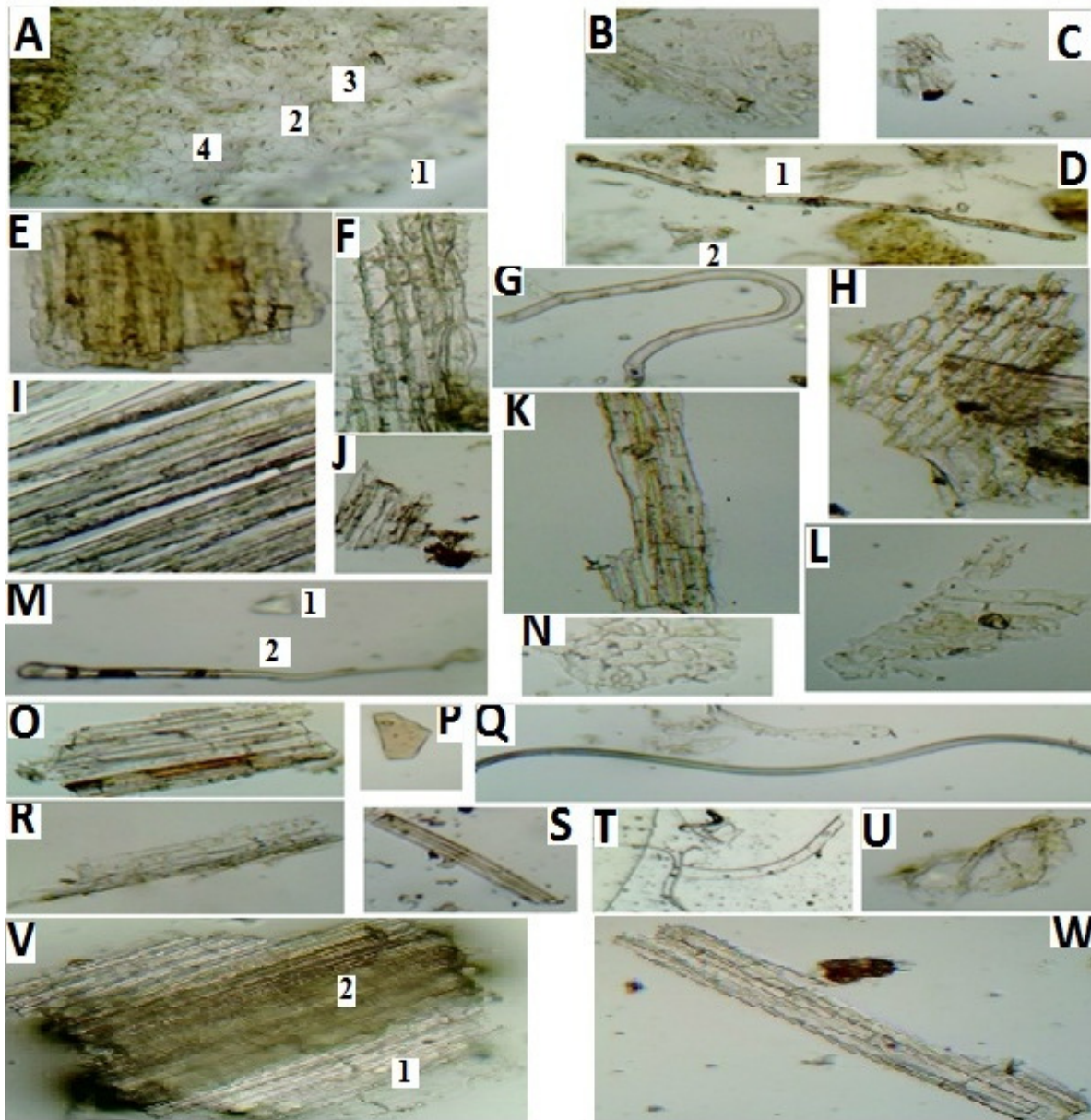


Figure 1. (A) 1, Epidermis, 2, cortex, 3, spong parenchyma, 4, anisocytic stomata, 5, vascular bandals; (B). parenchymatous cells with Ca-crystals; (C) Ca-crystals; (D) 1, trichom, 2, Ca-crystals; (E) xylem vessels; (F) tracheids; (G) unicellular trichom; (H) chlorenchymatous cell of stem: (I) Bast fiber; (J) stem tissue; (K) sclerenchyma fiber; (L) palisade parenchyma; (M) single sclerenchyma; (N) lower epidermis with stomata; (O) xylem and phloem of root; (P) chlorenchymatous cell; (Q) bast fibers; (R) sclerenchyma fiber; (S) fibers; (T) trichom; (U) parenchymatous cells; (V) 1, stem xylem, 2, stem phloem; (W) tracheids.

low, which may be due to low inorganic components. Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Acid insoluble ash value indicates high digestibility when the plant is consumed.

Extractive values

Six solvents were used for investigation of extraction. The

heights extractive value was 18% w/w for methanol followed by 11 and 8.5% w/w for hexane and water, while the extractive values of chloroform, ethyl acetate and butanol were 4, 3 and 5% w/w, respectively. The results of all extractive values are given in the following graph. Using of plants as such is not good therapeutically in compression to use plants extract, therefore the selection of such solvent with good extractive value is most important. The best solvents for extraction of

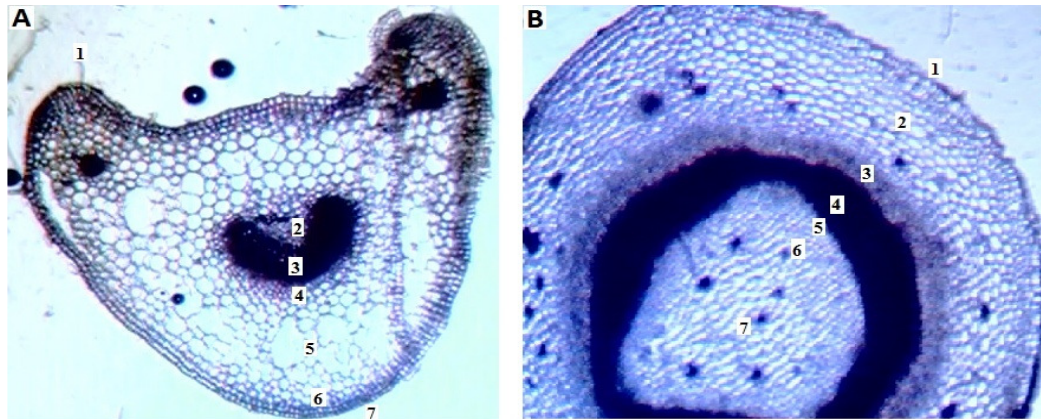


Figure 2. Transverse section of root. A (1, Glandular hairs, 2, xylem, 3, phloem, 4, endodermis, 5, cortex, 6, epidermis, 7, cholenchymatus cell) transverse section of petiole. B (1, epidermis, 2, cartex, 3, endoderm, 4, pericycle, 5, phloem, 6, xylem, 7, pitch).



Figure 3. Whole plant of *V. betonicifolia*.

Table 1. Quantitative leaf microscopy and physical parameters of *V. betonicifolia*.

Morphological feature	Range	Means
Palisade ratio	9 - 8.25	8.62 ± 0.23
Stomata number, upper epidermis	12 - 19	16.70 ± 0.31
Epidermal cells, upper epidermis	52 - 73	60.70 ± 0.39
Stomata number, lower epidermis	54 - 62	55.23 ± 0.28
Epidermal cells, lower epidermis	142 - 154	140.12 ± 0.43
Vein islet number	7.5 - 10	8.40 ± 0.25
Veinlet termination number	5 - 6	5.50 ± 0.11

Ash value	Percentage
Total ash	9.8
Water soluble ash	0.7
Acid in-soluble ash	1.05
Moisture content	5.2

V. betonicifolia would be methanol and n-hexane.

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