

Full Length Research Paper

Pharmacognostic investigation of the leaves and rhizomes of *Geranium wallichianum* D. Don ex Sweet

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Pharmacognostic investigation of the fresh powdered and anatomical section of the leaves and rhizomes of *Geranium wallichianum* was carried out to determine its morphological, histological and physicochemical characteristics. Macro and microscopic studies indicated that leaves are large, simple, petiolate, stipulate and palmatisect with five lobes. Lamina was 4 to 11 cm in diameter and green on the upper surface and light green on the lower surface. Venation was reticulate, multicostate and divergent. The upper epidermal cells were slightly larger than the lower ones. Orbicular anomocytic stomata were present on the lower epidermis. Below the upper epidermis was a single layered columnar celled palisade parenchyma filled with chloroplast pigments and contain aggregates of calcium oxalate crystals. Rhizomes were brown, stout, vertical, and 0.3 to 0.7 cm in diameter. Vascular bundles were about 6 to 8 groups, each consisting an outer phloem and inner radiate xylem. Physicochemical parameters such as total ash, water soluble ash and acid insoluble ash values were calculated. The results of the study will possibly provide diagnostic parameters for the quality, purity and correct identification of this plant material.

Key words: *Geranium wallichianum*, pharmacognostic, microscopy, physicochemical parameters.

INTRODUCTION

It has been reported that about 80% of the world's population rely on traditional system of medicine, particularly of plant source drug for their primary health care (Dubey et al., 2004). In recent years, the number of reports of negative health consequences caused by the use of herbal medicine has increased. A variety of reasons have been reported for such problem, one of the reasons of negative consequences is directly linked to the poor quality and purity of herbal drug and medicinal raw material (Gami and Parabia, 2010).

Therefore, the traditional medicine require intensive pharmacognostic, chemical and pharmacological investigations in order to avoid adverse events related to the use of the plant medicine for the prevention of diseases

particularly in developing countries. *Geranium wallichianum* is a perennial herb belongs to family Geraniaceae. It's very common in mountainous areas of Pakistan like Khyber Pukhtunkhwa, Azad Jammu Kashmir and Muree. Roots of the plant are up to 15 cm long and up to 5 mm in diameter. Leaves are simple, large and petiolate. Flowers are 3 to 4 cm broad. The plant acts as a powerful astringent.

The literature study revealed that the infusion or decoctions of the plant with hydrastine are used in conditions like gonorrhoea, leucorrhoea, diabetes etc (Nadkarni, 1954). The plant is also used in the treatment of toothache (Watt, 1972). The rhizomes of the plant are used to treat ulceration, dysentery, diarrhea, hemorrhage and leucorrhoea (Zabta et al., 2003). Paste of the plant is used for superficial skin infections while its infusion is used for treatments of diarrhea by local community. No earlier work is reported on *G. wallichianum*, regarding its Pharmacognostic characteristics / parameters therefore

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the present research work was carried out, in order to investigate the morphological, histological and physico-chemical parameters of the leaves and rhizomes of this plant, which will be useful in establishing pharmacognostic standards and diagnostic indices for the quality, purity and monograph of the plant.

MATERIALS AND METHODS

Plant material

The plant *G. wallichianum* was collected in July 2006 from Bara Gali, KPK, Pakistan. The plant was positively identified and a voucher specimen (No: 10706-Bot) was deposited at the herbarium, Department of Botany, University of Peshawar for ready reference. For powder drug study the leaves were dried in shade, while the rhizomes were dried in sun light. Both these parts were then powdered by electric grinder for microscopic and physicochemical examinations.

Morphological Studies

Different morphological parameters like size, shape, color, incision, venation and length of petiole of fully grown leaves were carefully recorded. Similarly morphological characteristics of the rhizomes including color, size, shape, texture, odor and taste were recorded as per standard procedure (Evans, 2002; Brain and Turner, 1975).

Microscopic studies

The microscopic studies include both qualitative and quantitative parameters and were carried as per standard procedures.

Qualitative microscopic studies

Qualitative microscopic examination was carried out by taking transverse section of the fresh leaves and rhizome in chloral hydrate, mounted in glycerin and observed under compound microscope (Evans, 2002).

Quantitative microscopic studies

The quantitative microscopic studies of the leaf was carried out using epidermal strips, pretreated with the chloral hydrate solution to determine the palisade ratio, stomatal index and vein islet number as described in the British Pharmacopoeia (1980).

Physicochemical studies

Total ash of the powder leaves and rhizome along with their water soluble and acid insoluble ash values were calculated as per standard procedure (Evans, 2002).

RESULTS AND DISCUSSION

Morphological studies

Leaves of *G. wallichianum* were large simple, petiolated, stipulated, palmate partite to plasmatisect with five lobes,

each lobe again incised in plamatifed manner. The petioles were 5 to 10 cm long. Lamina was 4 to 11 cm in diameter, green on the upper surface and light green on the lower side. Venation is reticulate, multicostate and divergent. Stipulate were 8 to 20 in number and 6 to 12 mm long, elliptic, obtuse, pilose-pubicent, apex some time 2-fid or irregularly toothed. The Organoleptic evaluation of the powdered leaves of the *G. wallichianum* revealed that powder was light green in colour, with a slight aromatic odor and a slightly bitter taste. The rhizome of *G. wallichianum* was brown, stout, vertical, 0.3 to 0.7 cm in diameter and coarsely annulated. The Organoleptic evaluation of the powdered rhizome showed that powder was dusty, light brown in colour, with a slight odor and a slightly bitter taste.

Qualitative microscopic studies

Transverse section of leaf

The results of the transverse section for the qualitative microscopic studies of the fresh leaf were showed in Figure 1. The results indicated a bifacial arrangement of cells. Both the upper and lower surfaces were covered by epidermal cells, with thin cuticle. Below the upper epidermis was a single layered columnar palisade parenchyma, with rich chloroplast pigments and many of them also contain rosette aggregate of calcium oxalate crystals. The spongy parenchyma cells were roughly arranged in 2 to 3 layers with little interspaces. Occasional calcium oxalate crystals were also present in the cells of spongy parenchyma. Below the midrib region, on both the surfaces collenchymatous tissue was present. Pericycle was in the form of an arc, surrounding the vascular bundles in which the radiate xylem was on the upper side and phloem on the lower side.

Transverse section of rhizome

Transverse section of the rhizome was shown in Figure 2. The transverse section of the rhizome was almost circular in out line. The outermost region observed was cork, consisting of 4 to 6 layers of thick, brown colored dead cork cells. Below the cork was the cortex region, consisting of many layers of rounded, parenchymatous cells some containing calcium oxalate crystals. Below the cortex was 3 to 4 layered pericycle and about 6 to 8 groups of vascular bundles arranged in a ring in the outer region of pith just below the pericycle. Each vascular bundle consists of an outer phloem and inner radiate xylem. Xylem has vessels and intervening xylem fibers in small groups. Medullary rays are 2 to 3 cells broad and radially elongated. The central region of the rhizome was occupied by thin walled parenchymatous cells, constituting the pith.

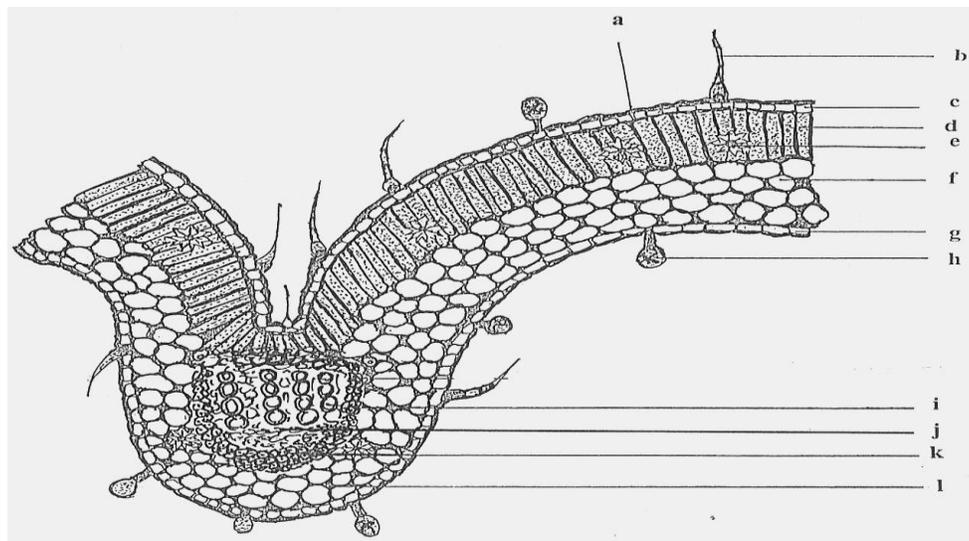


Figure 1. Transverse section of *Geranium wallichianum*. (a) cuticle (b) Nonglandular trichome (c) Epidermis (d) Palisade parenchyma (e) Calcium oxalate crystals (f) Spongy parenchyma (g) lower epidermis (h) Glandular trichome (i) Xylem (j) Phloem (k) Pericycle (l) Collenchyma.

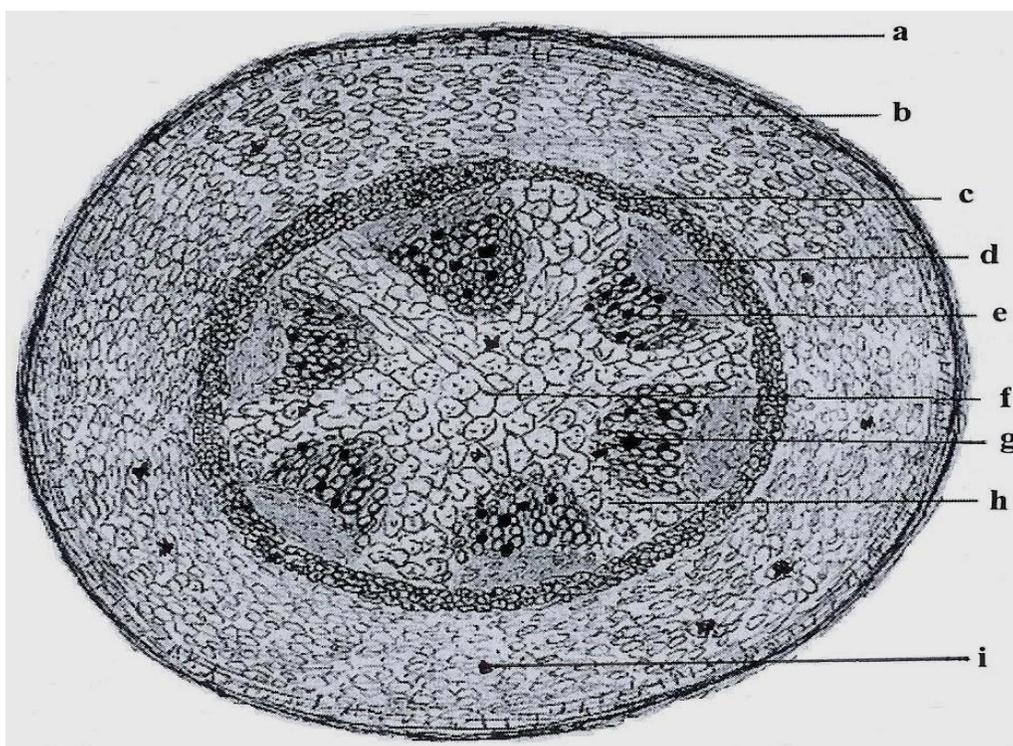


Figure 2. Transverse section of rhizome of *Geranium wallichianum*. (a) Cork (b) Cortex (c) Pericycle (d) Phloem (e) Xylem (f) Pith (g) Xylem fibers (h) Medullary ray (i) Calcium oxalate crystal.

Microscopic study of the chloral hydrate treated leaves powder showed numerous fragments of upper and lower epidermal cells (Figure 3a and b). Both epidermal cells were somewhat irregular, with wavy walls. The upper

epidermal cells were slightly larger than the lower epidermal cells. Orbicular anomocytic stomata were present on the lower epidermal only. Glandular and nonglandular trichomes were also present on both the

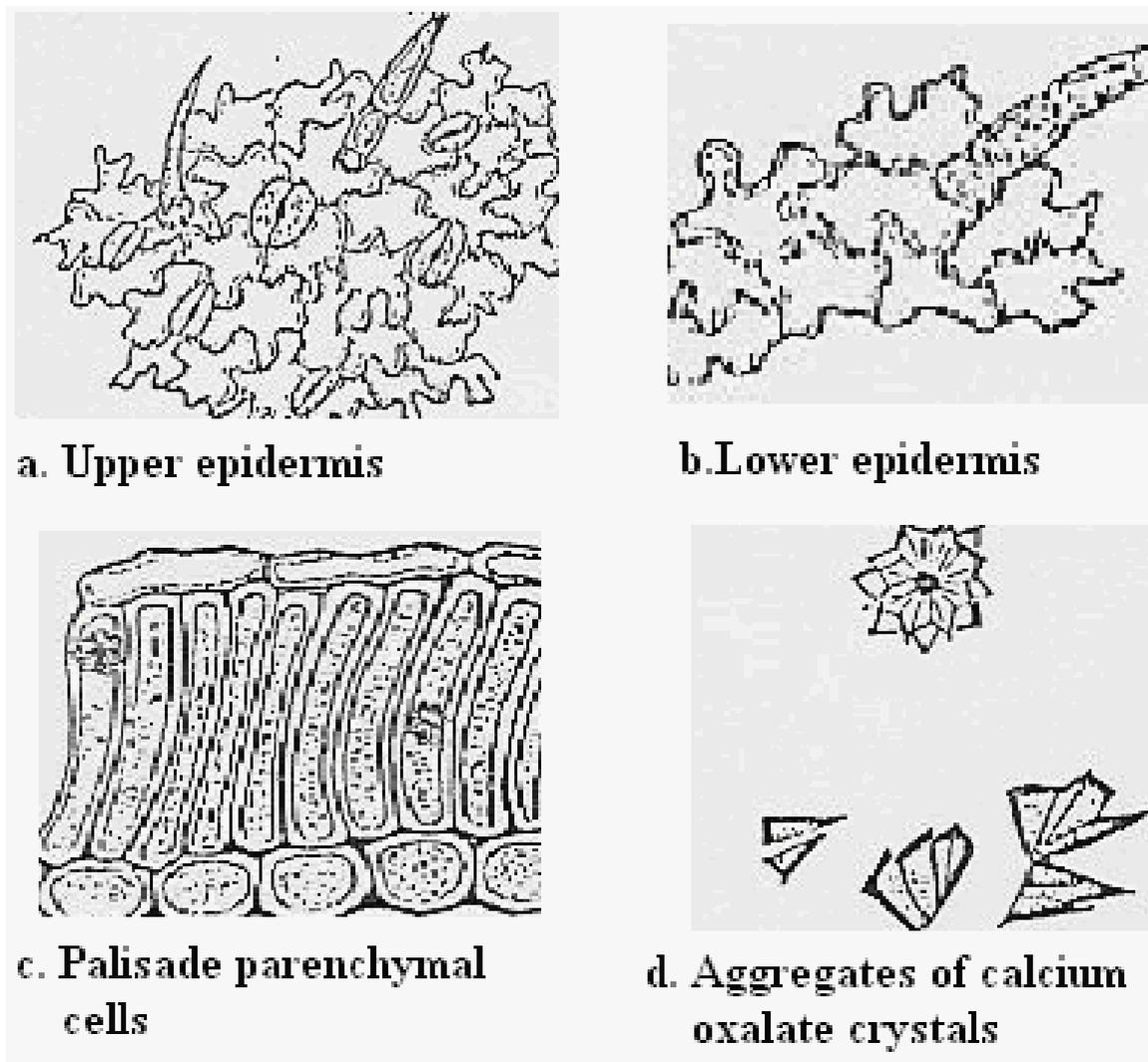


Figure 3. Powder drug study of leaves (a, b, c and d).

epidermises. Some of the fragments of upper epidermis with underlying elongated chloroplast-rich palisade parenchyma cells were observed, many of which also contained rosette aggregate of calcium oxalate. Some calcium oxalate crystals were also present extracellularly either intact or in fragments (Figure 3d). Fragments of palisade parenchyma cells were also seen in powder (Figure 3c). These cells were rather compact and more or less rounded in outline. Fragments of collenchyma with angular thickening and fragments of tracheids with annular and spiral thickenings were also observed in the powder. Fragments of pericycle fiber with rather thin walls and larger lumen were also present in the powder.

Powdered drug study of rhizome

The microscopic examination of the powdered drug

rhizome revealed the presence of brown colored thick cork cells (Figure 4a), fragments of thick walled more or less rounded parenchymatous cells form the cortex (Figure 4b) some containing rosette aggregates of calcium oxalate crystals (Figure 4c). Pericycle fibers (Figure 4d), xylem tissues fibers (Figure 4e) and fragments of radially elongated medullary rays were also observed.

Quantitative microscopic studies

The results of the quantitative microscopic studies of leaves are given in Table 1, 2 and 3. Quantitative determinations are useful for setting standards for crude drugs. Palisade Ratio, vein islet number and Stomatal index determined in the quantitative microscopy were relatively constant for plants and can be used to

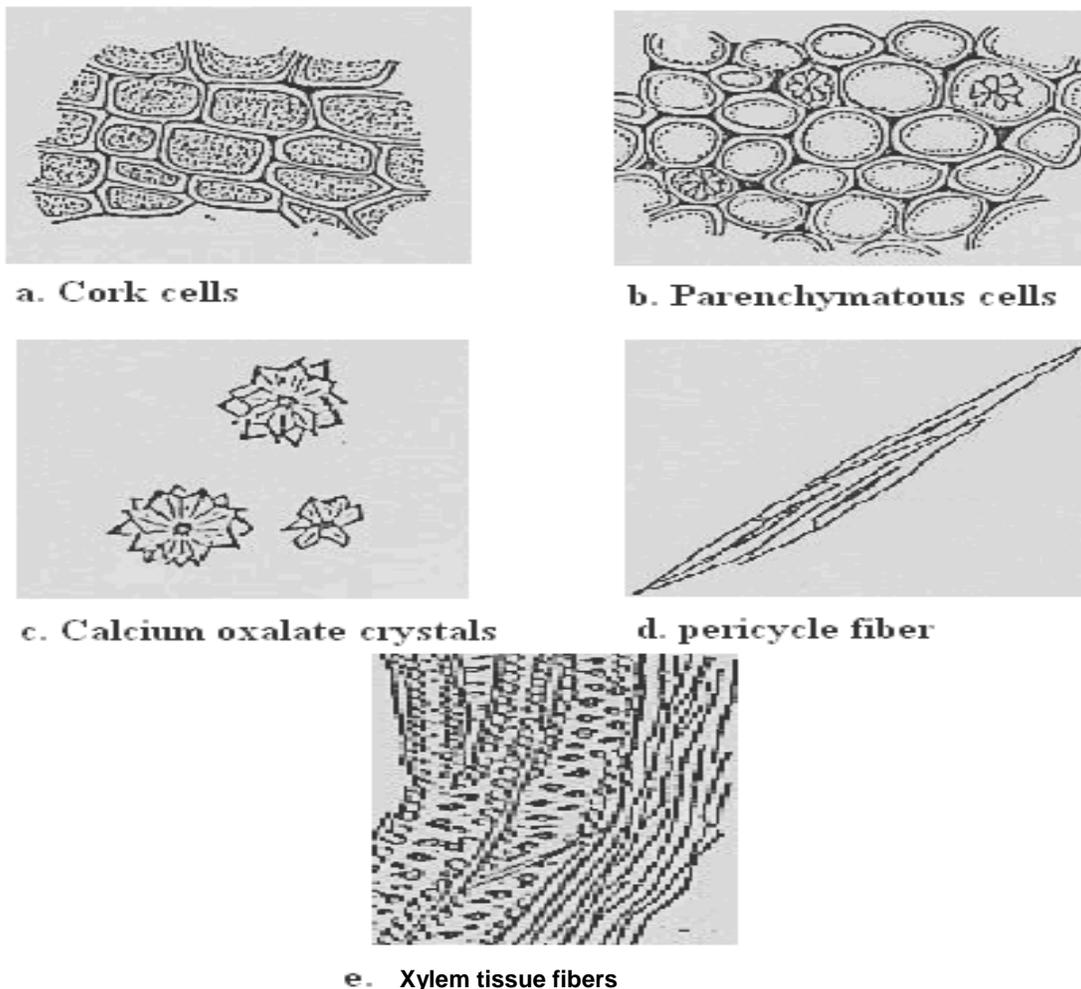


Figure 4. Powder drug study of Rhizome (a, b, c, d, and e).

Table 1. Palisade ratio of *Geranium wallichianum* Leaf.

S/N	No. of palisade cells	No. of epidermal cells	Average palisade ratio
1	9	4	2.25
2	9	4	
3	9	4	
4	9	4	

differentiate closely related species (Wallis and Forsdike, 1938; Chen and Chen, 2002; Evans, 2002; Jegede et al., 2005; Suresh et al., 2006). Organoleptic and microscopic examination of drugs is important in detection adulteration or improper handling of drugs during its collection, drying and storage (Evans, 2002).

Physicochemical studies

The results of physicochemical studies of leaves and

rhizomes are shown in Table 4. These parameters are helpful in prevention of adulteration and in authentication of crude drug. Ash value, particularly the acid insoluble ash value indicating the digestibility of plant when consumed (Ismail et al., 2011).

It is also important in evaluation of purity of drug by detecting the presence or absence of sandy earthy materials in the drug. Since *G. wallichianum* is very useful in traditional medicine for the treatment of various ailments, so it is important to standardize it for use as a drug.

Table 2. Stomatal index at different positions of *Geranium wallichianum* leaf.

S/N	Position	Number of stomata/mm ²	Number of epidermal cells / mm ¹	Stomatal index
1	Base	20	104	16.1
2	Margin	18	107	14.4
3	Center	19	105	15.3
4	Apex	19	109	14.9

Table 3. Average vein islet numbers at different position of *Geranium wallichianum* leaf.

S/N	Position	Leaf					Average
		1	2	3	4	5	
1	Base	12	14	8	9	11	10.8
2	Margin	14	12	15	12	11	12.8
3	Center	10	9	12	11	9	10.0
4	Apex	11	10	12	14	12	11.8

Table 4. Physicochemical parameters of the powdered leaves and rhizomes of *Geranium wallichianum*.

Evaluative parameter	leaves (value %, w/w)	Rhizome (value %, w/w)
Ash value	7	9.1
Water soluble ash value	1.96	2.36
Acid insoluble ash value	0.56	0.7

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