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Histology and ash analysis of *Gentiana Kurroo* Royle – an endangered medicinal plant

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ABSTRACT

Gentiana kurroo Royle, a potent temperate Indian bitter drug, is valued as bitter tonic, antiperiodic, expectorant, antibilious, astringent, stomachic, anthelmintic, blood purifier and carminative. Because of high demand and limited supply from natural resource, it is known to be adulterated with the roots of *Picrorrhiza kurroa*, *Gentiana tenella*, *G decumbens* etc. A perusal of literature reveals that no pharmacognostic tool is available for proper diagnosis of crude drugs of karru. Keeping this in view the present study was carried out in the Department of Forest Products, College of Forestry, Dr YS Parmar university of horticulture and forestry, Solan, HP. On macroscopic study, it was observed that after secondary growth, rhizome splits anomalously into four parts at a distance of 2.02+0.435 cm near collar region. Leaf was found to be dorsiventral, stomata anamocytic, and present only on the lower surface. Stomatal index ranged between 24.26 and 25.78 and pallisade ratio was 5.68. Xylem elements in roots were polyarch and radialy arranged. The air dried rhizome after incerneation gave 4.06 per cent of total ash (cremish colour), 2.78 per cent of acid soluble ash and 8.03 per cent of sulphated ash. Calcium content was 0.28 per cent. These paramerters can be used as identification tool for testing the purity of raw material of *G kurroo*.

Keywords: Anatomy, stomatal index, palisade ratio, ash analysis

INTRODUCTION

Gentiana kurroo Royle (family Gentianaceae) is an endangered bitter drug plant in indian system of medicine (Fig 1A). Rhizomes and roots of this plant is valued as bitter tonic, antiperiodic, expectorant, antibilious, astrigent, stomachic, anthelmintic, blood purifier and carminative (Kirtikar and Basu 1935). It is also reported to be used for curing skin diseases (leucoderma), bronchial asthama and urinary infections (Chopra et al 1956, Anon 1956). Because of its high demand in international market and limited supply from natural resources, it's known to be adulterated/ substituted with roots of *Picrorrhiza kurroa*, *G tenella*, *G decumbens* etc (Datta and Mukerji 1949, Anon 1956). Anatomical details and ash analysis are pharmacognostically important parameters for judging the purity and authenticity of

crude drugs (Trease 1949). Such studies along with other parameters help in correct identification of the drugs. A perusal of literature reveals that complete data regarding the aforesaid parameters of official parts of G kurroo are scanty. Keeping this in view the present study was contemplated.

MATERIAL AND METHODS

Plant materials was collected from healthy plants from natural habitat, washed thoroughly, cut into small pieces and preserved in Formalin Acetic Alcohol (FAA) solution at room temperature. Hand sectioning method (with the help of sharp razor) was adopted. Thin and uniform sections were stained as per the schedule given by Johanson (1940), mounted in DPX mountant, covered by cover slip and observed under microscope. Type and arrangement of cells were studied as per the details given by Esau (1953) and Fahn (1967). The different types of cells were measured by using ocular and stage micrometer and reported in micron. The stomatal studies were made from freshly peeled epidermal (lower) layer. The fresh peels were mounted in plain water covered with cover slip and sealed with paraffin wax. The size of the stomata was measured with the help of ocular and stage micrometer. The stomatal index was determined using the following formula based on 50 readings:

$$I = S/ (E+S) \times 100$$

Where I = Stomatal index,

E = No of epidermal cells in the same area,

S = No of stomata/unit area

The type of stomata was determined on the basis of stomatal classification as given by Fahn (1967). The palisade ratio was determined by counting the number of palisade cells below four continuous epidermal cells in a dechlorophylled leaf. The palisade ratio was calculated on single epidermal cell basis and was based on 40 observations. Palisade cells which were less than half inside the observed epidermal cells were not counted while cells which were more than half were counted.

For getting total ash percentage plant materials (root and rhizome) were washed thoroughly, cut into small pieces, sundried for four days and oven dried at 70^0C till consistency in weight was observed. Three gram of this dried powdered mass was kept in a silica crucible and burned in a muffle furnace maintained at a temperature of 800^0C for 24 hour. After cooling, the crucible with ash was weighed, ash content was calculated and reported in percentage.

For calculating acid insoluble ash, crucible containing plant material (3 gm air dried powdered mass) was saturated with dilute hydrochloric acid (10% w/w), boiled over water bath for few minutes and then filtered by using Whatman-1 filter paper. The residue along with filter paper was ignited at a temperature of 450^0C for four

hours. After cooling acid insoluble ash was calculated and reported in percentage. Similarly, sulphated ash percentage was calculated by digesting the plant material with concentrated sulphuric acid. The calcium content of ash was calculated by using Elmer atomic absorption spectrophotometer model 2380 and reported in percentage.

RESULTS

The anatomy of root, rhizome and leaf showed the typical dicotyledon pattern. Transverse section of rhizome of *G kurroo* revealed epidermis, hypodermis, cortex, vascular region and pith occurring in that order (Fig 2A). The epidermis consisted of single layer of wavy rectangular or flattened closely packed cells. The outer walls of these cells were thickened and cutinized. Below the epidermis, hypodermis formed a more or less distinct zone consisting of four to five layers of thin walled living cells. These cells were oval to rectangular in shape and tightly packed without any intercellular space. Cortex were multilayered (14-16 layered) and consisted of more or less round in shaped parenchymatous cells with intercellular air spaces. Endodermis and pericyle layer were not distinguishable. Cells of outer layer of cortex were randomaly arranged while as those of inner layers are radially arranged. The vascular system consisted of xylem and phloem forming a distinct vascular ring. Xylem elements are polyarch and radially arranged while as phloem elements were tangentially arranged. Pith consisting of oval, round or polygonal shaped living thin walled cells, wasmore elaborate and occupied more space at the centre of rhizome. The qualitative and the quantitative characters of different kind of cells in rhizome are given in Table 1. On macro-observation, the older rhizome (more than 3-4 yrs old plants) showed an interesting feature of forking at a distance of 2.02 +0.435 cm from collar region (Fig 1B). The transverse section of rhizome just above the splitting zone and the individual splits revealed that before splitting cambial growth was more concentrated at four corners. The medullary extension grew outward extending up to the point where the cambial growth was less. As the vascular cambium sufficiently grew enough, schizogenous splitting by the separation of cork cambium started and extended towards the medular zone making a 'u' shaped notch. Both splitting and epidermal layer formation went simultaneously and the rhizome was divided into two parts. The same process was continued in the two newly formed segments and finally rhizome was forked into four parts (Fig 2B & C). In cross section the individual splits of rhizome were characterized by a thin single layer of epidermis, hypodermis, vascular cambium with xylem and phloem elements towards the inner side. The pith cells were present towards one side below xylem/ phloem elements (Fig 2D).

Microscopic study of root of *G kurroo* in cross section showed that anatomically it can be distinguished into epiblema, hypodermis, cortex and vascular cylinder (Fig 3A). Epiblema, the outermost layer of root consisted of single layered thin walled rectangular to oval shaped cells. These cells were closely packed and cutinized with a wavy outline. Hypodermis was characterized by four to six layers of square or rectangular shaped closely packed thin walled living cells. Cortex consisted of 18 to 26 layers of thin walled round, polygonal shaped living cells. Cells in outer layers of cortex were randomly arranged while as those of inner layers were radially arranged. Vascular cylinder was characterized by a distinct cambial ring with xylem elements distributed in a scattered fashion through closely packed wood parenchyma. Xylem was broad and consisted of large tracheae. Tracheids were arranged singly or in groups. The qualitative and quantitative characters of different kinds of cells in cross section of root are given in Table 2.

Cross section of dorsiventral leaf of *Gentiana kurroo* revealed that palisade cells occurred towards the upper surface and spongy parenchyma towards the lower surface (Fig 3B). The stomata were present only on the lower surface and were of anamocytic or ranunculaceous type (Fahn 1967) and the stomatal index ranges between 24.26 to 25.78. The palisade ratio (ie number of palisade cells corresponding to one epidermal cell) was found to be 5.62.

Table 1. Qualitative and quantitative characters of different kinds of cells in cross section rhizome of *G kurroo*

Region	Type of cells in cross section	Dimension (µm)
Epidermal layer	Single layered	28.87+1.21
Epidermal cell	Rectangular flattened, closely Packed	27.75±1.98 x 31.45±4.13
Hypodermal layer	Four-five layered	103.45+3.72
Hypodermal cells	Oval, rectangular, tightly packed	17.50.51 x 20.0±0.71
Cortical portion	Fourteen-sixteen layered	262.12±0.03
Cortical cells	Round, loosely radially arranged	15.00±0.79 x 22.00±0.84 to 37.50±0.71 x 49.00±1.82
Endodermis and pericycle	Not clearly distinguished	—
Xylem	Rectangular, polygonal	18.132.22 x 19.34+1.75
Phloem	Rectangular, squarish, polygonal	15.252.23 x 15.75+2.19
Pith	Oval, round	52.07±1.51 x 59.54±3.43

Table 2. Qualitative and quantitative characters of different kinds of cells in cross section of root of *G kurroo*

Region	Type of cells in cross section	Dimension (μm)
Epiblema	Single layered, wavy outline	—
Epiblema cell	Rectangular, oval shaped	30.25±1.95 x 46.75±3.18
Hypodermis layer	Four-six layered	139.81+2.14
Hypodermal cell	Rectangular, squarish shaped	18.00±4.15 x 37.50±1.17
Cortex	Eighteen-Twentysix layered	297.62+0.14
Cortical cell	Round, polygonal shaped	11.07±0.85 x 15.35±0.61 to 22.00±0.44 x 33.00±1.09
Trachid	Rectangular, polygonal	36.50±1.79 x 38.50±1.95
Xylem	Polygonal, scattered in wood parenchyma	30.0+1.0 x 31.00±1.14
Wood parenchyma	Round, closely packed	24.22±0.95 x 25.10±0.79
Phloem	Polygonal	15.8+1.2 x 16.00±1.14

Table 3. Qualitative and quantitative parameters of different kinds of cells in cross section of leaf of *G kurroo*

Region	Type of cells in cross section	Dimension (μm)
Cuticle layer		
Upper surface		10.93±0.70 to 12.80±0.84
Lower surface		4.00± 0.65 to 7.25± 0.41
Epidermal cells		
Upper surface	Oval, round, compactly arranged	39.14±1.79 x 50.93±2.33 to 44.57±1.67 x 54.57±1.89
Lower surface	Oval round, compactly arranged	21.6±0.44 x 39.75±1.88 to 14.75±0.98 x 18.00±1.91
Palisade parenchyma	Two-three layered, elongate, prismatic and compactly arranged	14.720.92 x 34.20±1.42
Palisade ratio	—	5.62+0.53
Spongy parenchyma	Oval, round, loosely arranged	30.00±1.41 x 32.40±1.54
Xylem	Polygonal, diarch	9.40±0.33 x 15.50±0.44
Phloem	Polygonal	6.50±0.57 x 16.00±1.13
Stomata	Present on lower surface only anomocytic/ranunculaceous	37.86±0.99 x 50.40±0.91
Stomatal index	—	25.2±0.58

The qualitative and quantitative characters of different kinds of cells present in cross section of leaf are given in Table 3.

The rhizome ash obtained as a result of incernation of known quantity of air dried rhizome was analysed for total ash, sulphated ash, acid soluble, water soluble ash percentage and calcium content and the results are presented in Table 4.

Table 4. Rootstock ash analysis of *G kurroo*

Parameters	Quantity (%)
Total ash	4.06+0.36
Sulphated ash	8.03+0.77
Acid soluble ash	2.78+0.06
Water soluble ash	0.37+0.21
Calcium	0.28+0.01

DISCUSSION

The anatomical studies of rhizome, root and leaf of *G kurroo* follow the typical dicotyledonous pattern (Esau 1953, Fahn 1967). On macro-observation, it was found that the older rhizome was split into four parts seemingly merging into a single structure near the collar portion, enclosed in an outer layer of periderm. The presence of oil globules and small calcium oxalate crystals in the cortical cells of rhizome, as reported by Satyavati et al (1976) could not be observed in the present study. However, their observation of more or less rectangular shaped endodermal cells is confirmed. There is some confusion regarding the type of xylem present in its roots. While as Satyavati et al (1976) reported tri or tetrarch xylem, Datta and Mukherji (1949) reported xylem strands scattered through wood parenchyma. In the present study, xylem appeared to be scattered without any arch condition (Fig 3A). Details regarding stomata type, stomatal index and palisade ratio in this species are being reported for the first time.

The quality and quantity of ash obtained as a result of incerenation of air dried crude drugs is an important pharmacognostic parameter to judge the authenticity and purification of crude drugs (Trease 1949). Often such data are presented to test the purity of a drug sample. In the present investigations, total ash percentage of 4.06 was obtained from the rootstock of *G. kurroo*. The acid soluble, water soluble and sulphated ash percentage was found to be 2.78, 0.37 and 8.03 per cent respectively (Table 4). The calcium content of rootstock was found to be 0.28 per cent. Data regarding such parameters are being reported for the first time except for total ash percentage. Earlier reports (Anon 1956, Chopra et al 1958, Datta and Mukerji 1949, Nadkarni 1976) give a total ash percentage of 0.7 per cent. However, a perusal of these reports reveals that the figure of 0.7 per cent has been reproduced by Anon (1956), Chopra et al (1958) and Nadkarni (1976) following the observation of Datta and Mukerji (1949). Doubt

regarding the authenticity of this observation due to improper drying of the rhizome sample has been raised by Anon (1956) and Nadkarni (1976). The present observation of 4.06 per cent of ash content may appear to be quite large as compared to 0.7 per cent reported by earlier workers (Anon 1956, Chopra et al 1958, Datta and Mukerji 1949, Nadkarni 1976) but the doubt regarding the proper drying of the rhizome sample raised by Anon (1956) and Nadkarni (1976) indicates that the earlier observation of 0.7 per cent may be erroneous. Although this observation was made several times during the present study but further confirmation would be needed to establish the total ash percentage in this species.

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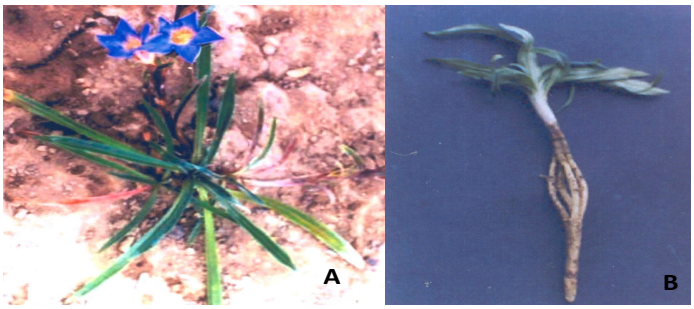

Fig 1 A : *G kurroo* at flowering B Rhizome splitting

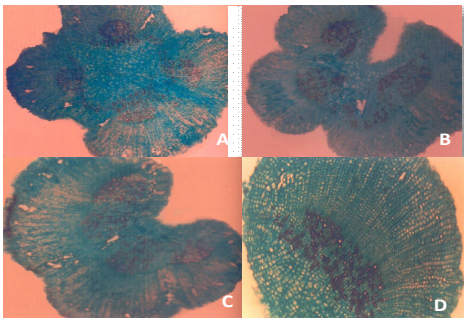

Fig 2 A. T S Rhizome B. T S Rhizome through splitting zone stage-I
C. T S Rhizome through splitting zone stage-II
D. T S Rhizome (single split)

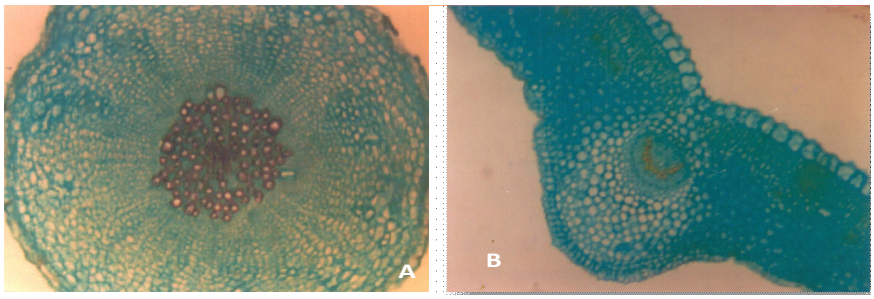

Fig 3 A. T S root B. T S leaf (close up at mid rib region)