**Microscopical identity of Picrorhiza tungnathii Pusalkar**

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**ABSTRACT**

*Picrorhiza tungnathii* Pusalkar (Plantaginaceae) presented here in view to the histology. The prime objective of the paper is to differentiate this species through microscopically to avoid misidentification of the raw materials and to use correct species in Ayurvedic formulation.

**Keywords:** Anatomy, Microscopy, Picrorhiza, Authentication, Ayurvedic formulation

**INTRODUCTION**

Two species of *Picrorhiza* viz. *P. kurroa* Royle ex Benth. and one species *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong are locally known in the western Himalaya as ‘Kutki (Kutaki), Kedar kutki, Kadwi and Karu’, being one of the most important medicinal group which are highly exploited for trade in Ayurvedic industries (Borah & Singh, 2021). Picrorhiza/Kutki is one of the important herbal drugs with an annual demand of the raw material of 4–6 tones in India (Olsen, 1998; Arya & al., 2013). Demand for Kutki is continuously increasing year by year. This medicinal herb group has recently been taken as high priority group for trade in Ayurvedic industries (Borah & Singh, 2021). Picrorhiza/Kutki is one of the important herbal drugs with an annual demand of the raw material of 4–6 tones in India (Olsen, 1998; Arya & al., 2013). Even after the discovery (inclusion and exclusion of the species) of *Picrorhiza tungnathii* Pusalkar. To provide a consolidated data, all the above mentioned parameters were carried out in the present study, which can be used to identify this plant group in crude herbal drugs for the authenticity and further conservation efforts too.

**MATERIAL AND METHODS**

Plants and plant parts were collected from Tungnath, Chandrashila and Ravanahila, district Rudarparyag, Uttarakhand. Herbarium specimens (116629 and 117212) were identified from Botanical Survey of India (BSD), Dehradun. Microtome sections were taken, stained and mounted following the usual plant micro-techniques (Khandelwal, 2012) and representative diagrams were taken through camera Axio Cam ICC5 at 10X and 40X magnification.

**RESULTS**

**Macroscopic Description**

*Habit: Picrorhiza tungnathii* Pusalkar is distinguished from the allied *P. kurroa* Royle by being 10–25 cm tall, having a moderately dense, (10–)15–25-flowered spike, a syzgomorphic, 2- lipped, glandular-ciliate corolla that is equalling or slightly exceeding the calyx and partly visible or sub-exserted between the calyx lobes, a conspicuously long (longer than the corolla lobes), curved corolla tube

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(1/2–4/5 the length of the calyx), unequal corolla lobes that are ovate- lanceolate to lanceolate, acute to sub-acuminate and moderately ciliate, a mid-lobe of the upper lip that is obliquely erect, galeate with retuse or emarginate apex, lateral corolla lobes that are slightly smaller than the mid-lobe of the upper lip, a lower corolla lip that is slightly shorter than the lateral lobes, obliquely erect or spreading, didynamous, long-exserted stamens, pollen of the Paederota -type and a style that is 2-3 times as long as the corolla (Pusalkar, 2013) (Fig. 1).

Root - Thin, cylindrical, 4–8 cm long, 0.05–0.1 cm in diameter, slightly curved, dotted scars, attached with rhizomes, dusty grey, fracture, short; odour, pleasant; taste, bitter.

Rhizome - 6–12 cm long and 2–2.5 mm thick, sub-cylindrical, straight or slightly curved, externally greyish-brown, on breaking it shows smooth black to dark brown, surface rough due to longitudinal wrinkles, fish like scales, circular scars of roots and bud scales and roots attached, tip ends in a growing bud surrounded by tufted crown of leaves, fracture, short; odour, pleasant; taste, bitter (Fig. 1 B).

**Microscopic Description**

Root - Mature root shows 2–5 layers of periderm surrounded by unicellular trichomes; the cortex is distinct from phelloderm which has wide, circular or elliptical, thin-walled parenchyma cells in 20 to 25 layered, compact tearing or usually fissures. Pericycle is developed with single layer. The vascular bundle has varied primary phloem elements, xylem consisting of vessels, tracheids and parenchyma; vessels have varying shape and size, some cylindrical, some drum shaped.
Pith consisting of circular, thin walled compact polygonal cells (Fig. 1 C).

Rhizome - The rhizome consists of several squarish, thin walled, suberized phellem cells arranged in regular radial files of Periderm or cork cell. Phellogen is distinctly seen and 8 to 16 layers of rectangular phelloderm cells with lignified pericycle fibres. The cortex is distinct from phelloderm which has wide, circular or elliptical, thin walled compact parenchyma cells. Cells are usually not compact but tearing with fissure appearance. The vascular cylinder has a wide, continuous, secondary phloem with radial files of phloem elements. The secondary xylem is broken into three wide segments and between the segments is seen thick band of parenchyma cells. The secondary xylem consists of vessels and tracheid fibres and is arranged in regular radial rows. The vessels are narrow, circular and are mostly in radial multiple of several cells. The xylem fibres are thin walled with narrow lumen and are lignified. Pith is wide and triangular in outline, consisting of circular, thin walled compact polygonal parenchyma cells (Fig. 1 D).

DISCUSSION

Transverse Section (TS) of *Picrorhiza*, rhizome revealed important parts like cork, cambium, cortex, endodermis, xylem, phloem, pith, starch grains, pigment cells, and cortical parenchyma (Meena & al., 2010; Selvam, 2012; Shilpa & Upadhyay, 2018). Physically, rhizomes of *Picrorhiza kurroa* were found cylindrical, straight or slightly curved in shape (Sarin, 1996). External colour was creamish-brown. Surface was rough due to the presence of various fish like scales. Taste was very bitter (Sarin, 1996). Fracture was short and clear showing large creamish vascular bundles arranged in a prominent
circular broken ring. In microscopic study transverse section of rhizome showed 5–15 layers of cork, 1–2 layered cork cambium and primary cortex. Phloem and xylem vessels constituted the vascular tissue with pith in the centre. Long narrow fragments of xylem elements with elliptical, scalariform and pitted thickenings, thick mass of parenchyma and squarish shaped periderm cells (Selvam, 2012). Cork cells, starch grains, lignified fibers, trachieds and pith cells with pitted wall thickenings were also found in the powder microscopy (Shilpa & Upadhyay, 2018).

Taxonomically, Picrorhiza tungnathii differs from P. kurroa and Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong by means of actinomorphic and zygomorphic flowers. Palynologically, P. tungnathii and Neopicrorhiza scrophulariiflora differs from P. kurroa by means of Paederota and Scrofella type pollens. Anatomically, Picrorhiza tungnathii differs from P. kurroa and Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong by means of periderm which consist of squarish, thin walled, suberized phellem cells about 8-16 layers of cork. Phellogen cells with phelloderm arranged in 2–4 layers. The cortex consists of thin parenchymatous cells and consists 15–25 of cortical layers. Cells are fissured with tearing appearance usually not compact as P. kurroa. The vascular bundles are arranged in 3–5 small vascular cylinders. The secondary phloem is composed of sieve tubes, companion cells and parenchyma. Vascular cambium is 1–2 layered. Secondary xylem consists of vessels, tracheids, fibres and parenchyma cells. Vessels vary in size and shape. The pith is characterized by the presence of parenchymatous cells. Diameters of each cells may be varied due to the age or months of the sample (Figs. 1–3).
Table 1. Comparative Microscopic characteristics of Picrorhiza tungnathii, P. Kurrooa and P. scrophulariflora

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Picrorhiza tungnathii</th>
<th>P. kurrooa</th>
<th>P. scrophulariflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cork</td>
<td>8–16 layers of cork consisting of</td>
<td>10–20 layers of cork consisting of</td>
<td>8–16 layers of cork consisting of</td>
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<tr>
<td></td>
<td>tangentially elongated, suberized cells.</td>
<td>tangentially elongated, suberized cells.</td>
<td>tangentially elongated, suberized cells.</td>
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<tr>
<td>Cortex</td>
<td>Single layered with multiple polygonal cells.</td>
<td>Single layered with multiple compact</td>
<td>Single layered with multiple polygonal</td>
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<tr>
<td></td>
<td>Layers are generally fissured.</td>
<td>polygonal cells. Layers are not fissured.</td>
<td>cells. Layer are generally fissured.</td>
</tr>
<tr>
<td>Vascular bundles</td>
<td>2 or 3 vascular bundles. Vascular bundles</td>
<td>1 or 2 vascular bundles. Vascular</td>
<td>2 or 3 vascular bundles. Vascular</td>
</tr>
<tr>
<td></td>
<td>surrounded by fibrous bundle sheath. Black</td>
<td>bundles surrounded by fibrous bundle</td>
<td>bundles surrounded by fibrous bundle</td>
</tr>
<tr>
<td>Vascular cambium</td>
<td>2–4 layered.</td>
<td>4–5 layered.</td>
<td>2–3 layered.</td>
</tr>
<tr>
<td>Phloem</td>
<td>Secondary phloem composed of</td>
<td>Secondary phloem composed of</td>
<td>Secondary phloem composed of</td>
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<tr>
<td></td>
<td>parenchyma cells.</td>
<td>parenchyma cells.</td>
<td>parenchyma cells.</td>
</tr>
<tr>
<td>Xylem</td>
<td>Secondary xylem consists of</td>
<td>Secondary xylem consists of</td>
<td>Secondary xylem consists of</td>
</tr>
<tr>
<td></td>
<td>vessels, tracheids, fibres and parenchyma</td>
<td>vessels, tracheids, fibres and parenchyma</td>
<td>vessels, tracheids, fibres and parenchyma</td>
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<tr>
<td></td>
<td>cells. Vessels vary in size and shape.</td>
<td>cells. Vessels vary in size and shape.</td>
<td>cells. Vessels vary in size and shape.</td>
</tr>
<tr>
<td></td>
<td>Tracheids, thick walled- and lignified.</td>
<td>Tracheids, thin walled and lignified.</td>
<td>Tracheids, thin walled and lignified.</td>
</tr>
<tr>
<td>Pith</td>
<td>Triangular in outline, pitted wall</td>
<td>Cells are compact and polygonal.</td>
<td>Triangular in outline. Compact and</td>
</tr>
<tr>
<td></td>
<td>thickening. Compact and polygonal.</td>
<td></td>
<td>polygonal.</td>
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</tbody>
</table>

Phytochemistry of P. kurrooa Royle ex Benth. and Neopicrorhiza scrophulariflora (Pennell) D.Y. Hong have been studied extensively, whilst P. tungnathii Pusalkar is yet not studied. There is no report after inclusion and discovery of P. tungnathii. In view of a recent delimitation of these species, the possibility of current available chemical analysis and histological appearances for P. kurrooa may belong to mixed samples (if done based on raw material available in the market). Picrorhiza species contain several bioactive compounds that have therapeutic properties. The active constituents are Kutkiol, Kutkisterol, D-Mannitol, Apocyanin: phenol glucosides; androsim and picein iridoid glycosides; Kutkin, Picroside I, II & III; Kutkoside, Minecoside, Picrorhizin, Arvenin, scrososide H, scrososide, picrogentioside, picrogentioside A, picrogentioside B, picrogentioside C, picrosecoside, picrosecoside A, picrosecoside B, scrocaffeside A, scrocaffeside B, scrocaffeside C, scrososide D, scrososide E, scrososide G, scrophenoside Avanillic acid, gallic acid, arbutin, androsin, 11-O-galloylbeneginin, 6-feruloylcatapol, veronicoside, sweroside, gentiopicroside, isofeuralic acid, plantamajoside, plantainoside D, vanillin, β-sitosterol, daucosterol and suberic acid (Tong & al., 2008; Yin & al., 2010; Tiwari & al., 2012; Shah & Vashney, 2013).

Katoch & al. (2011) inferred correlation with altitudes with picroside-I and kutkoside content of the Picrorhiza kurrooa. Highest concentration of picroside-I was accumulated in the population from Rohtang (3978m, 3.5%), followed by Keylong (3350m, 2.56%), Manikaran (1737m, 1.86%), Khoksar (3160m, 1.76%), Chamba (996m, 1.69%), Manali (2050m, 1.51%) and Morhi (3300m, 1.38%). The concentration of kutkoside was also found to be the highest in the population from Rohtang (3978m, 2.0%), followed by Keylong (3350m, 1.6%), Chamba (996m, 1.13%), Manali (2050m, 1.05%), Khokasar (3160m, 1.04%), Morhi (3300m, 0.83%), Manikaran (1737m, 0.83%). The best population for collection of the botanical drug was found to be from Rohtang (3978 m) with highest percentage of picroside-I and kutkoside, which is superior to other populations of picroside content. The concentration of picroside-I was determined to be higher than the kutkoside in all the populations (Katoch & al., 2011).

If we will consider the natural habitats of Picrorhiza species as per their elevation after inclusion and discovery of P. tungnathii, the possibility of current available chemical spectra/analysis might be mixed. Hence further studies are needed to isolate chemical constituents and biological active markers to get clear idea of correct chemical spectrum (Pusalkar, 2013; Rana & al., 2018). During the bulk collection of the raw materials in the nature it is tough to measure the age of the rhizome by naked eyes. The prime requirement is to use of the correct species for efficacy of single drug in herbal industry and avoids any sort of intra species adulteration.

Ground parts of Picrorhiza kurrooa is generally pale brown with heavy scales while as Picrorhiza tungnathii ground parts are black brown, lesser scaly and furrows are wrinkled with fish/crocodiles like scales/appearance. Histologically, cortex persists in some cases, not compact
tearing or wrinkled appearance and deep black to brown in coloured. When root/rhizomes parts collected from wild habitats instead of cultivated source there will be few possibilities of the collection; if plants were collected from timberline ecotone or sub-alpine region it will be _P. kurroa_, if it is collected from rocky crevices of glacial-fluvial area or alpine region the chances of assemblage might be _Neopicrorhiza scrophulariiflora_ (Pennell) D.Y. Hong and interestingly, if the plant parts are to be collect from high alpine meadows then it will be _P. tungnathii_ Pusalkar. Although, for naked eyes it is not possible to differentiate/identify rhizomatous roots of all species in the fields but at the time of flowering it is easy to assemble and identify them (Pusalkar, 2013; Rana & al., 2018). Using key characteristics feature of the anatomy, we can identify all raw material of the species (Table 1).

**CONCLUSION**

We hope this study will facilitate use of the correct species/raw material with an appropriate active marker compound in herbal industry to avoid high scale substitutions of inter and intra species. And to increase efficacy of the herbal drugs (single or multiple) in Ayurvedic system of medicine or traditional system of medicine.

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**REFERENCES**


