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Root Anatomical Characters of Herbal Drug *Sophora* (Fabaceae) Using Resin Embedding

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Abstract

Sophora root has been used for many years in traditional herbal medicine both as a pain reliever and also as a treatment for cancer and thus there is a need for rapid and accurate identification of individual species. Other workers have shown that stem xylem characteristics of *Sophora* are informative at intraspecific levels. As stem and root woods share similar properties, we report studies based on anatomical characteristics of root specimens from *Sophora* to develop an identification tool for individual *Sophora* species. Light microscopy was employed. A resin embedding approach was chosen instead of wax embedding because herbal drug materials are very dry and difficult to section thinly**.** It is shown that root xylem characteristics of *Sophora* may be used for identification. Root anatomical characters of *Sophora* can be used for herbal drug identification at intra- (e.g. species) and infra (e.g. varieties)-specific levels. Quantitative data will be gathered in further studies.

Keywords: *Sophora*, xylem, resin embedding, anatomy, herbal drug

1. Introduction

1.1 Developing Authentic Identification Tool

The genus *Sophora* is composed of about 70 species (Lai, He, Jiang, & Chen, 2003), belonging to the tribe Sophoreae of the legume family Fabaceae (Leguminosae). They are widespread in warm and dry habitats including Asia, North and South Americas, and New Zealand (Cumbie & Mertz, 1962; Lai, 2003). The roots of medicinal species of *Sophora* (Fabaceae) contain matrine and oxymatrine, and these compounds have been shown to be effective for the relief of abdominal pains and the treatment of tumors, and to act as antidotes, *etc*. (Chock, 1956).

One important area of pharmacognosy has been the collection of reliable data classifying plants accordingly to the disease types for which they are effective remedies (Dahanukar, Kulkarni, & Rege, 2000). For the correct identification of herbal drugs, plant anatomy has proved to be an effective scientific tool (Li & Liu, 2011).

1.2 Xylem Caracteristics

Root xylem characteristics have been analyzed in the current study, based on previous work by Cumbie and Mertz (1962) on the xylem characteristics of *Sophora* stems of different taxa at interspecific level. According to these authors (Cumbie & Mertz, 1962), stem xylem systems of *Sophora* differed consistently between herb, shrub, and tree habits. They reported that the decrease of vascular cambium activity is correlated with an increase in multiple layering of vessels and narrower rays (Cumbie & Mertz, 1962). The distribution patterns of vessels of *Sophora* species show characteristic differences (Cumbie & Mertz, 1962); for instance ring-porous taxa have solitary pores and multiple pores in early woods and late woods respectively (Cumbie & Mertz, 1962).

1.3 Observation of Anatomical Characters of Sophora

In general, the stem and root woods of plants show qualitative and quantitative similarities in their fundamental structures including fibers, parenchyma tissues, rays, xylem and phloem, and tracheids (Denne & Gasson, 2008). However, they can also exhibit differences including the absence of distinct growth rings in root woods. Therefore, root wood xylem characters could be very useful for comparison with those of stem woods (Denne & Gasson, 2008). In the current work, we report histological studies of the wood root systems of *Sophora* species which are being used as medicinal materials, in order to develop a tool for their unambiguous identification.

2. Method

2.1 Sample Collection

The six different root materials used were either collected direct from cultivation or purchased in herbal drug markets in the following areas; KwangSuhSung in China (*Sophora tonkinensis*); Jingxi County of Guangxi Province, China (*S. tonkinensis* var. *tonkinensis*, *S. tonkinensis* var. *polyphylla*); Korean herbal drug market in SuhChun, ChungNam (*S. flavescens*); YunNam in China (*S. glauca* var. *albescens*); and Nemongo in China (*S. alopecuroides*). They are kept as voucher specimens in the herbarium of the Natural Product Research Institute of the Dept. of Pharmacy at Seoul National University (NPRI).

2.2 Resin Embedding

The herbal materials were dried roots and very hard; therefore the glycol methacrylate)-based resin (Technovit 7100, Heraeus Kulzer GmbH, Germany) embedding technique was used. Tissues were softened by treatment overnight with 10% ammonia solution (McLean, 1916). Samples were then dehydrated sequentially for 12 hours or longer with each of 10%, 20%, 35%, 55% and 75% *t*-butanol in water, and finally with 100% *t*-butanol. Samples were subsequently infiltrated with resin by sequential treatment for 8 hours at each stage with ethanol:Technovit 7100 mixtures (75:25, 50:50 and then 25:75) followed by immersion in 100% resin. The samples were then glued to Histoblocks (Heraeus Kulzer GmbH) with Technovit 3040 (Heraeus Kulzer GmbH, Germany). Finally, thin (3 - 5 μm) sections of the tissues were made under wet conditions using a rotary microtome (Model 820, Reichert-Jung Co., Germany). Softeners were not employed. Sections were then transferred to glass slides and stained for 30 seconds with 0.1% Toluidine Blue O dye (O'Brien et al., 1964), freshly prepared from a 1.0% stock solution, before rinsing with double-distilled water.

2.3 Light Microscopy

Images were acquired with a digital camera (DPx26, Olympus Co.) attached to a light microscope (Olympus BX-50), using the accompanying software CellSense (Olympus Co.). Magnifications of x40, x100, and x200 were used.

3. Results

The stained cross-section samples are shown in Figures 1-3. All the six root samples (different taxa) had common internal structures including xylem, phloem, cortex, and cork layers. They had starch grains stored in the parenchyma tissues in the cortex area (Figures 3.1, 3.2, 3.3, 3.5) and frequently also had crystal cells in the border regions between cortex and cork layers (Figures 3.4, 3.6). However, they also showed characteristic secondary xylem arrangements, either single (Figures 1-3), or multiple groups (Figures 4-6). The individual descriptions are given below.

3.1 Sophora tonkinensis Gagnepain

Due to the well-developed vascular cambium, multi-layered structures including xylem, phloem, cortex, and cork cambium were distinct (Figure 1.1; Figure 2.1). The xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 1.1; Figure 2.1). The shape of the vessel elements was roundish; there were no angles at the edges (Figure 2.1). The vessels were arranged as single, double, or multiple layers interspersed with fibers (Figure 2.1). In the case of xylem parenchyma tissues, a single or double layer of rays was observed (Figure 2.1). Inside the parenchyma cells, starch grains were accumulated (Figure 3.1). The cortex was composed fundamentally of parenchyma cells and fibers (Figure 3.1). Fibers were found dispersed throughout the cortex although they occur less frequently than those in xylem tissues (Figure 3.1). Crystals were found in the cortex area, usually in the boundary between cortex and cork layers (Figure 3.1).

3.2 Sophora tonkinensis var. tonkinensis Gagnepain

Tissues such as xylem, phloem, cortex, and cork cambium were distinctly developed (Figure 1.2), as for *Sophora tonkinensis*. The xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 2.2). The shape of the vessel elements was roundish, having various angles at the edges (Figure 2.2). The vessels were arranged as single, double, or multiple layers interspersed with fibers (Figure 2.2). In the case of xylem parenchyma tissues, single or double layer of rays were observed (Figure 2.2). Inside the parenchyma cells, starch grains had accumulated (Figure 3.2). The cortex was fundamentally composed of parenchyma cells and fibers (Figure 3.2). Fibers were found dispersed throughout the cortex although they occur less frequently than those in xylem tissues (Figure 3.2). Crystals were found in the cortex area, usually in the boundary between cortex and cork layers.

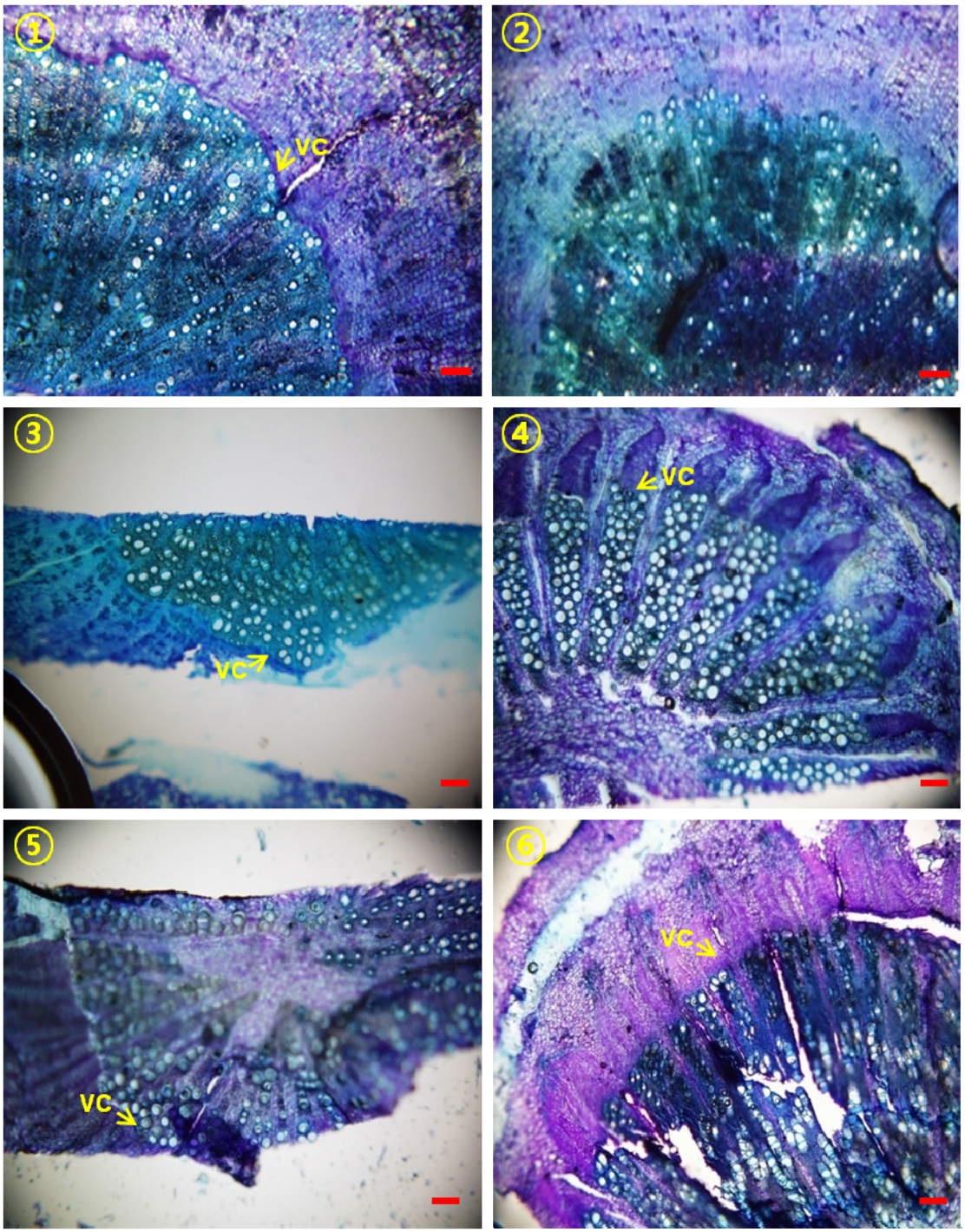

Figure 1. Cross-sections of herbal drug *Sophora* samples, showing root wood characteristics. 1. *Sophora tonkinensis*, 2. *Sophora tonkinensis* var. *tonkinensis*, 3. *Sophora tonkinensis* var. *polyphylla*, 4. *Sophora alopecuroides,* 5. *Sophora flavescens*, 6. *Sophora glauca* var. *albescens*. The scale bars (in red) indicate 50 μm; vc, vascular cambium

3.3 Sophora tonkinensis var. polyphylla S. Z. Huang & Z. C. Zhou

Tissues such as xylem, phloem, cortex, and cork cambium were distinctly developed (Figure 1.3). The xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 2.3). The shape of the vessel elements was roundish or having various angles at the edges (Figure 2.3). The vessels were also

arranged alternatively as a single, 2-layers, or groups of more than 2-layers to fibers (Figure 3.3). In case of xylem parenchyma tissues, a single or two-layers of rays were developed (Figure 3.3). Inside the parenchyma cells, starch grains were accumulated (Figure 3.3). The cortex was fundamentally composed of parenchyma cells and fibers (Figure 3.3). Fibers were found dispersed throughout the cortex although they occur less frequently than those in xylem tissues (Figure 3.3). Crystals were found in the cortex area, usually in the boundary between cortex and cork layers (Figure 3.3).

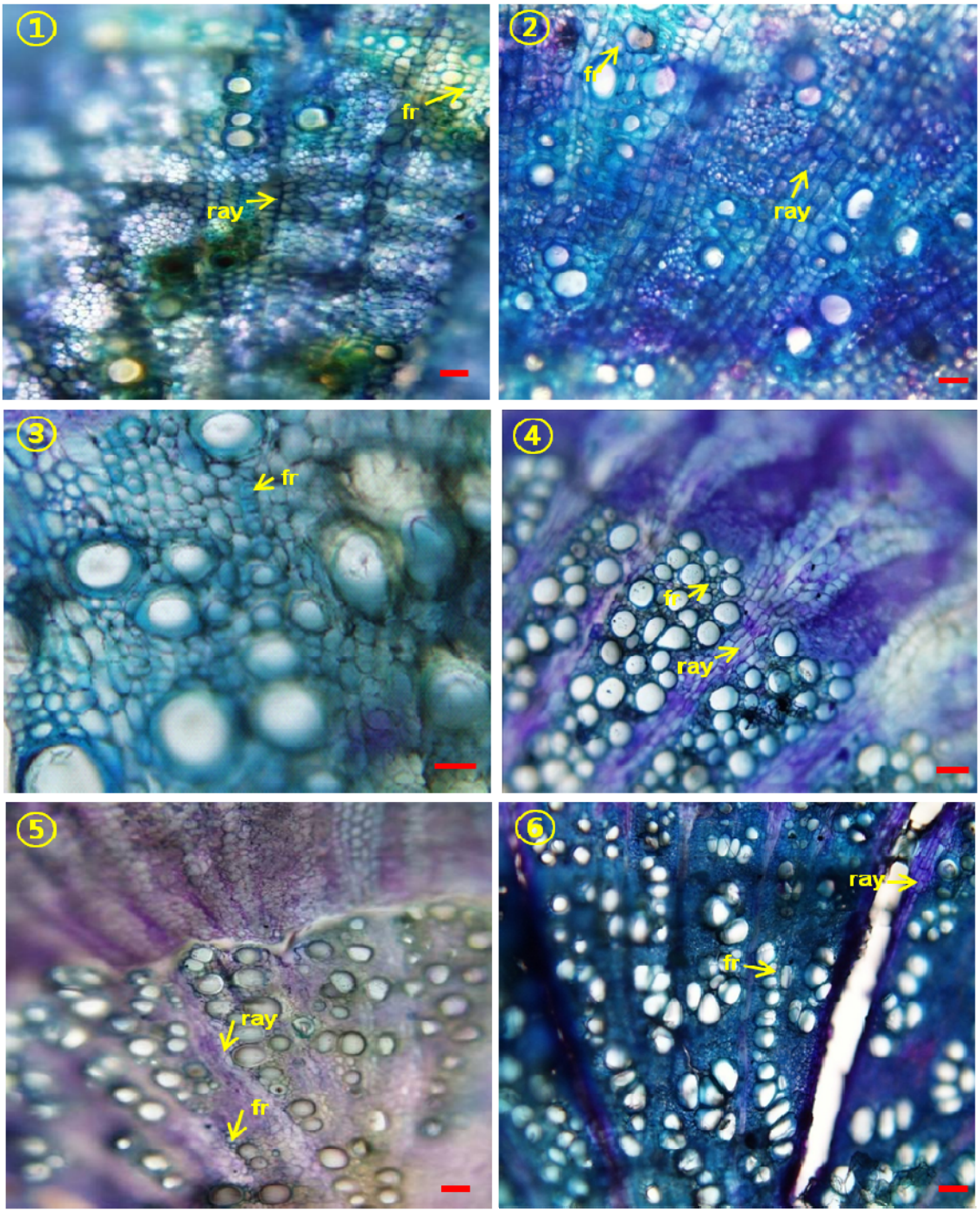

Figure 2. Xylem characteristics are shown with cross-sectioned root samples. 1. *Sophora tonkinensis*, 2. *Sophora tonkinensis* var. *tonkinensis*, 3. *Sophora tonkinensis* var. *polyphylla*, 4. *Sophora alopecuroides*, 5. *Sophora flavescens*, 6. *Sophora glauca* var. *albescens*. The scale bars (in red) indicate 25 µm (1, 2, 4, 5, 6) and 12.5 µm (3); fr, fiber; ray, ray tissue

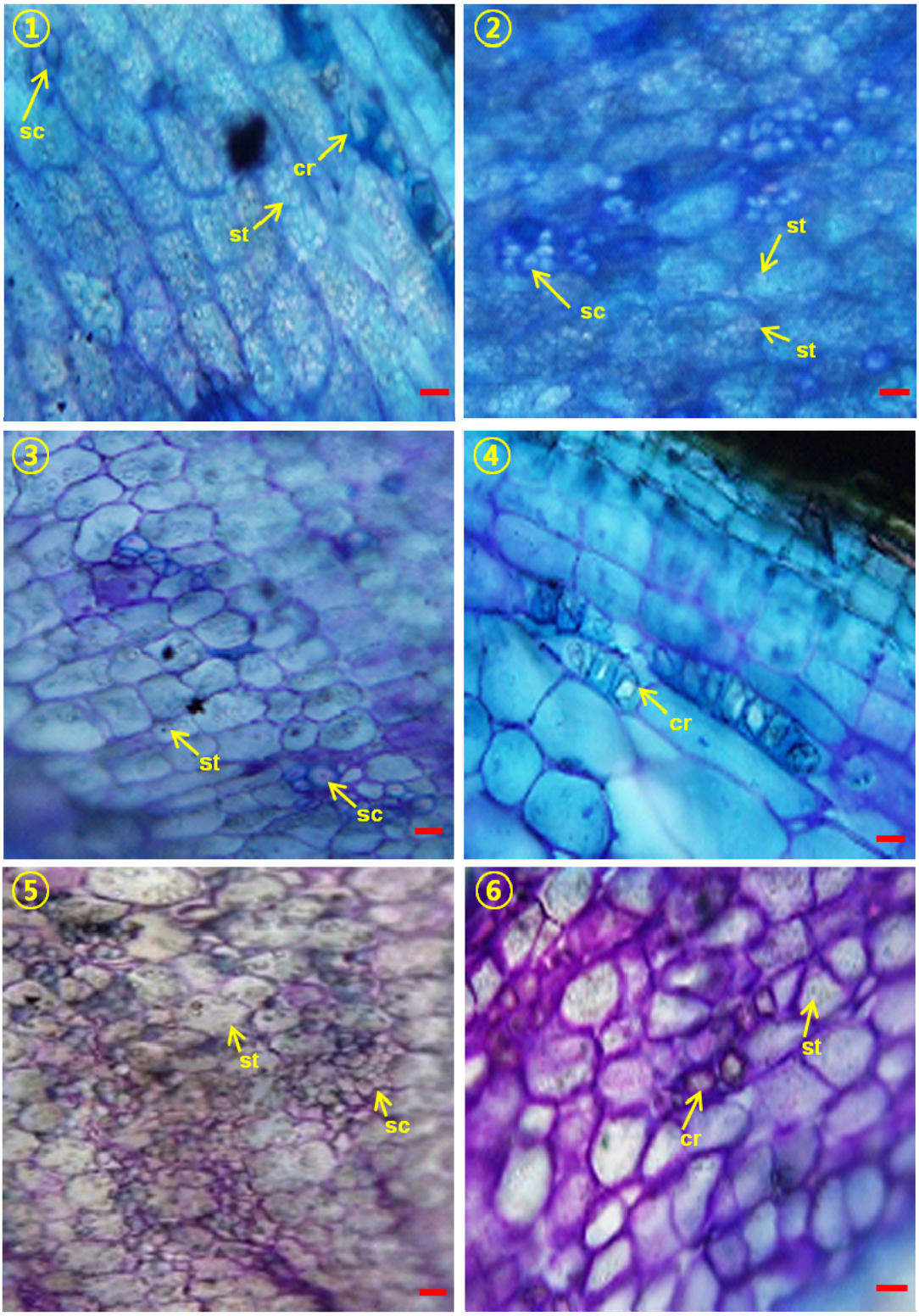

Figure 3. Accumulated starch grains in parenchyma in the cortex and crystal cells in the borderlines between the cortex and cork layers are shown. 1. *Sophora tonkinensis*, 2. *Sophora tonkinensis* var. *tonkinensis*, 3. *Sophora tonkinensis* var. *polyphylla*, 4. *Sophora tonkinensis* var. *polyphylla,* 5. *Sophora flavescens*, 6. *Sophora glauca* var. *albescens*. The scale bars (in red) indicate 12.5 μm (2, 4) and 25 μm (1, 3, 5, 6, 7); cr, crystal cells; sc, sclerenchyma cell; st, starch grain

3.4 Sophora alopecuroides L.

Tissues such as xylem, phloem, cortex, and cork cambium were distinctly developed (Figure 1.4, Figure 2.4). The xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 1.4, Figure 2.4). The shape of the vessel elements was roundish, oval or flattened oval (Figure 1.4, Figure 2.4). The fibers were relatively less developed comparing to *Sophora tonkinensis*, *S. tonkinensis* var. *tonkinensis*, and *S. tonkinensis* var. *polyphylla* (Figure 1.4, Figure 2.4). In the case of xylem parenchyma tissues, 3-4 layers of rays were developed (Figure 1.4, Figure 2.4). The presence of pith was a distinctive feature. Characteristic phloem wedges were seen (Figure 1.4, Figure 2.4), and the tips pointed towards the cork layers. In the terminal regions of the phloem wedges, fibers formed groups and surrounded the tips like caps (Figure 1.4, Figure 2.4).

3.5 Sophora flavescens Ait.

Tissues such as xylem, phloem, cortex, and cork cambium were well-developed as multiple layers (Figure 1.5). Xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 1.5, Figure 2.5). The shape of the vessel elements was roundish without angles (Figure 2.5). In xylem parenchyma tissues, 3 layers of rays were developed (Figure 2.5). The presence of pith was a distinctive feature (Figure 1.5). As in the case of *S. alopecuroides*, characteristic phloem wedges were found (Figure 2.5).

3.6 Sophora glauca Lesch. var. albescens Rehd. Et. Wils.

Tissues such as xylem, phloem, cortex, and cork cambium were distinctly developed (Figure 1.6). The xylem tissues were composed of typical vessel elements, tracheids, parenchyma, and fibers (Figure 1.6, Figure 2.6). The shape of the vessel elements was roundish, oblong without angles (Figure 2.6). In xylem parenchyma tissues, 2-3 layers of rays were developed (Figure 2.6). The presence of pith was a distinctive feature. As in *S. alopecuroides* and *S. flavescens*, characteristic phloem wedges were found (Figure 1.6).

4. Discussion

The roots of investigated *Sophora* herbal samples showed characteristic features at intra- and infra-specific levels in the current study in terms of xylem arrangement, the thickness of rays and the condition of xylem parenchyma. The root xylem characters of *Sophora* may be related to plant habit as reported previously (Chock, 1956). For instance, *S. alopecuroides* and *S. flavescens* are herbal or subshrub, while *S. tonkinensis*, *S. tonkinensis* var. *tonkinensis*, *S. tonkinensis* var. *polyphylla* and *S. glauca var. albescens* are shrubs. The differences in habit are confirmed in this study to be another helpful indicator for identification of commercial specimens of *Sophora* herbal drugs.

According to Cumbie and Mertz (1962), depending on habit differences the pith area forms distinctive multiple layers in trees with less distinctive layers in shrubs, while in herbs layers are absent. In the genus *Sophora,* multiple layer formation is terminated at the early developmental stages of secondary xylem and this agrees with the previous report by Cumbie and Mertz (1962) in relation to the appearance or non-appearance of rays. In the case of plants which belong to the family Fabaceae (Leguminosae), the vessel elements show limited morphological variations; for instance, all the genera of the Legume family have simple perforation plates, alternate arrangement, and vesturing pitting (Cumbie and Mertz, 1962). In order to observe in detail the lengths of vessel elements of *Sophora* herbal drugs, a maceration technique will be used in work planned for the near future.

In the current study, the cross-sections of the herbal materials showed characteristic variations in the sizes of vessel elements of growth rings, and this can be useful for identification of *Sophora* herbal medicines (Figures 1-2). As was reported by other workers (Cumbie and Mertz, 1962), the distribution patterns of vessel elements differ between *Sophora* species. In this study, single, double and multiple patterns were found (Figures 1-2). Previously, Cumbie and Mertz (1962) reported that *S. alopecuroides* forms characteristic multiple layers of vessels, and this was confirmed for the root woods studied here.

Crystal cells were observed in the drug root samples in this study, and they may be related to the presence of fungi (Scurfield, Michell, & Silva, 1973). High levels of crystals were found in the hyphae of fungal plant pathogens (Scurfield et al., 1973), and it was considered that the crystals may be metabolic products of fungi (Scurfield et al., 1973). Most plants of Legume family members are known to be symbiotic with fungi. Therefore, the presence of crystals in the *Sophora* roots may be related to symbiosis or possibly to fungal attack. Even though various crystal shapes and components are known, the relevance to taxonomy and genetics has been controversial (Scurfield et al., 1973). Further studies are planned using the same *Sophora* root drugs, in order to gather quantitative as well as qualitative data at specific and infraspecific levels. Numerical taxonomic analyses such as principal components analysis should extend our knowledge and be very helpful for identification of

herbal medicines. Even though DNA barcoding (Chen et al., 2010) can be a very modern technique for authentic identification of herbal drugs, anatomy can be a strong supportive tool for comparative studies with DNA works. The resin embedding of herbal drugs is also superior to wax embedding by its efficiency and time - saving.

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