Scientia Pharmaceutica (Sci. Pharm.) **69**, 237–247 (2001)

Anatomy of the underground parts of four *Echinacea*-species and of *Parthenium integrifolium*

R. Länger

Institute of Pharmacognosy, University of Vienna

Center of Pharmacy, Althanstrasse 14, A – 1090 Vienna, Austria

Improved descriptions and detailed drawings of the most important anatomical characters of the roots of *Echinacea purpurea* (L.) MOENCH, *E. angustifolia* DC., *E. pallida* (NUTT.) NUTT., and of *Parthenium integrifolium* L. are presented. The anatomy of the rhizome of *E. purpurea*, which was detected in commercial samples, and of the root of *E. atrorubens* NUTT., another known adulteration for pharmaceutically used *Echinacea*-species, is documented for the first time. The possibilities and limitations of the identification by means of microscopy are discussed. The anatomical differences between the roots of *E. angustifolia*, *E. pallida* and *E. atrorubens* are not sufficient for differentiation, however, root and rhizome of *E. purpurea* and the root of *Parthenium integrifolium* appear well characterized. Because of the highly similar anatomy the microscopic proof of identity and purity of crude drugs of *Echinacea* must be done with uncomminuted material and the examination of cross sections.

(Keywords: Echinacea angustifolia, Echinacea atrorubens, Echinacea pallida, Echinacea purpurea, Parthenium integrifolium, Asteraceae, microscopy, anatomy, identification)

1. Introduction

The first, and for a long period only, detailed anatomical descriptions of the underground parts of *Echinacea* were published at the beginning of the last century[1, 2]. Due to later changes in the taxonomy within the genus *Echinacea*, unfortunately the plant sources for these descriptions remain unclear. The increasing interest in *Echinacea* and the adulterations that had been observed frequently caused Heubl et al.[3] in the late eighties to examine the roots of *E. purpurea* (L.) MOENCH, *E. angustifolia* DC., *E. pallida* (NUTT.) NUTT., and of *Parthenium integrifolium* L. Their key for the identification of *Echinacea*-powders and the detection of *Parthenium* became standard [4, 5].

In the course of the elaboration of microscopical descriptions for the American Herbal Pharmacopoeia ™ we obtained authentic material of the three mentioned *Echinacea*-species, of *Parthenium integrifolium* and of *E. atrorubens* NUTT., a further adulteration detected in the United States. The application of the key of Heubl failed, mainly due to a confusing terminology and the

lack of detailed drawings. In addition, our samples of *E. purpurea* consisted to a great extent of the rhizome, the anatomy of which has not been published yet.

The objectives of this study are an improvement of the anatomical descriptions including detailed drawings and the estimation of the value of microscopy for the proof of identity and purity of underground parts of *Echinacea*, considering additionally the anatomy of *E. atrorubens*, which is described for the first time.

2. Experimental

PLANT MATERIAL:

A limiting fact when studying Echinacea is the availability of authenticated material, commercial samples are inappropriate. This circumstance is the reason for the small number of examined samples.

E. purpurea (L.) MOENCH, *E. angustifolia* DC., *E. pallida* (NUTT.) NUTT.: from each species: 1 sample from the American Herbal Pharmacopoeia™, USA; 2 samples from the Medicinal Plants Garden of the Institute of Pharmacognosy, Vienna (flowering plants authenticated by the author); 1 sample provided by Arzneipflanzen Sandfort GmbH, Olfen, BRD.

E. atrorubens NUTT.: wild collected sample (D. Hurlburt, USA)

Parthenium integrifolium L.: wild collected sample (R. Upton, USA)

MICROSCOPICAL TECHNIQUES:

From all samples cross sections and longitudinal sections of the rhizome and the root were examined and compared with the characters of the powdered drug. The cuttings were done by hand and for microscopic observation cleared by boiling with a 60% solution of chloral hydrate in water. The drawings were prepared using a drawing tube, measurements were taken with a calibrated measuring ocular.

3. Results

3.1. ECHINACEA ANGUSTIFOLIA

The underground parts consist of a short rhizom and thickened tapering roots bearing few thickened lateral roots.

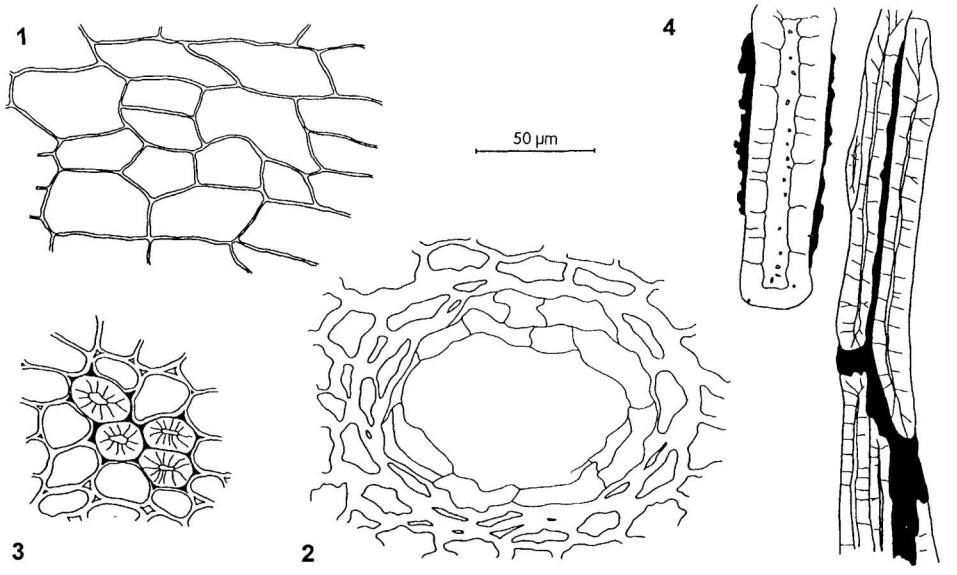
Rhizome: Apart from the different arrangement of the primary and secondary elements cell structures identical with those of the root.

Root: A brown pigmented exodermis (fig. 1) or, in older roots a brown cork, represents the outermost layer of the root. Secretory cavities (up to 200 µm in diameter, fig. 2) are located in the cortical parenchyma. Sclereids with phytomelanin are found alone or in groups of two or three, or sometimes up to ten. These sclereids are up to 50 µm in diameter and up to 300 µm in length. Phytomelanin fills in the triangular intercellular spaces around the

sclereids, making them appear star-shaped in cross section (fig. 3). In longitudinal view these sclereids are characterized by numerous pit channels and a rather small lumen (fig. 4).

The secondary xylem consists of radial rows of tracheas, alternating with broad medullary rays. Within the radial rows the vessels are arranged in small groups separated by parenchyma. The vessels are up to 60 µm in diameter and have secondary walls with reticulate or scalariform thickenings or bordered pits. Frequently fibres, usually without phytomelanin, are associated with the vessel members. In longitudinal section, a thinner cell wall and a slender shape with pointed ends distinguishes these fibres from sclereids. The length of the fibres is not detectable. Within the secondary xylem, sclereids are located in the medullary rays only, whereas secretory cavities are scattered throughout the parenchyma tissue. Surrounding the small pith, sparse groups of primary xylem with narrow vessels are situated at the inner end of the medullary rays.

Powder: Frequently sclereids with phytomelanin, mostly in elongated multiseriate groups; occasional bundles of fibres without phytomelanin; fragments of exodermis; colourless parenchyma; tracheas with reticulate thickening or bordered pits.



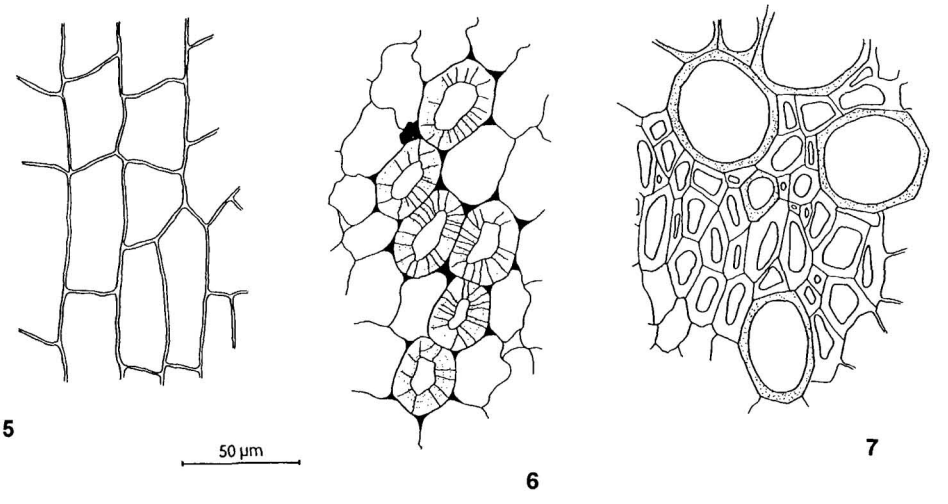
Figures 1—4: *Echinacea angustifolia*, root. 1: Exodermis (surface view); 2: Secretory cavity in the cortical parenchyma (cross section); 3: Group of sclereids in the cortical parenchyma (cross section); 4: Sclereids in the powdered drug (longitudinal view).

3.2. E. PALLIDA

Rhizome and root: the main structure and the cellular elements are very similar to *E. angustifolia*, but, possibly due to the doubled chromosome number, the size of the exodermis cells (fig. 5), sclereids and secretory cavities on average are larger in *E. pallida*:

Sclereids coated with phytomelanin are solitary or in groups. Solitary sclereids are more or less isodiametric with a diameter up to 80 µm, whereas those in groups (fig. 6) are slender and elongated with a diameter up to 50 µm and a length of approximately 500 µm. The secretory cavities, often in cross section tangentially elongated, reach dimensions up to 600 µm. The structure of the xylary fibres (fig. 7) is like in *E. angustifolia*.

Powder: Frequently sclereids with phytomelanin, mostly in elongated and multiseriate groups; only few solitary sclereids; few bundles of fibres without phytomelanin; fragments of exodermis; colourless parenchyma; tracheas with reticulate thickening or bordered pits.



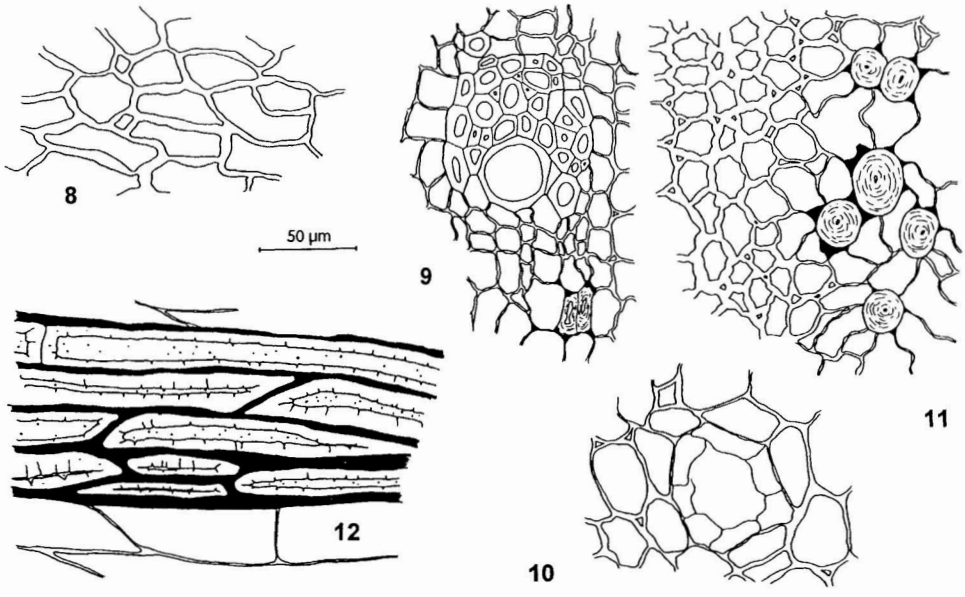
Figures 5—7: ***Echinacea pallida***, root: 5: Exodermis (surface view); 6: Group of sclereids in the cortical parenchyma (cross section); 7: Vessels with fibres (cross section).

3.3. E. ATRORUBENS

The sample consisted of the taproot only. The cylindrical root is considerably thickened (diameter about 2 cm), the cork is dark brown with longitudinal furrows. In cross section a radiate structure of light tracheas and dark grey medullary rays is obvious.

Root: Under a brown cork layer (fig. 8) a small primary cortex with tangentially elongated, ovate cells is located. Within the secondary phloem, broad areas of crushed conducting tissue and small cells alternate with medullary rays of considerably larger and often ruptured cells. The seondary xylem consists of narrow radial rows of vessels, alternating with narrow medullary rays. Within the radial rows the vessels are arranged in small groups separated by parenchyma. The space between the vessel elements often is filled with fibres lacking phytomelanin covering (fig. 9). The tracheas reach diameters up to 70 µm. Secretory cavities with a diameter up to 160 µm (fig. 10) are present in all parenchymatic tissues. Sclereids with phytomelanin are situated in the cortex, the secondary phloem (fig. 11) and the medullary rays of the secondary xylem. Their diameter is approximately 40 µm, their length up to 300 µm. The majority of the sclereids is arranged in small groups, forming long strands in longitudinal view (fig. 12).

Powder: from all Echinacea roots the powder with the highest amount of phytomelanin-coated sclereids, most of them in multiseriate groups; secretory cavities can be seen in their entirety; colourless parenchyma; brown fragments of cork; vessels with some fibres attached.



Figures 8—12: *Echinacea atrorubens*, root: 8: Exodermis (surface view); 9: Secondary xylem: vessels with attached fibres and sclereids with phytomelanin (cross section); 10: Secretory cavity in the secondary phloem (cross section); 11: Phytomelanin coated sclereids in the secondary phloem (cross section); 12: Sclereids of the secondary xylem (longitudinal view).

3.4. E. PURPUREA

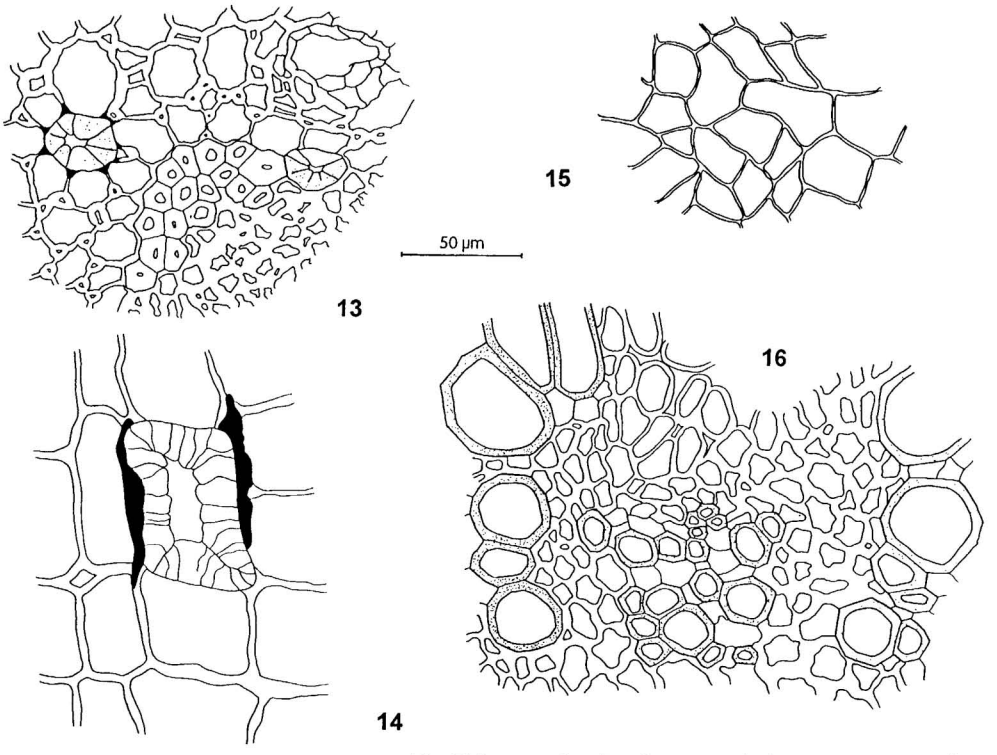
The underground parts consist of a large rhizome and numerous thin lateral roots.

Rhizome: The pigmented exodermis consists of polygonal cells. The cortex is characterized by slightly thickened parenchyma cells with small triangular intercellular spaces. Sclereids with a diameter of approximately 60 µm and a length up to 300 µm occur singly or in small groups. Phytomelanin usually covers the cell wall of these sclereids and fills in the triangular intercellular spaces around them, making them appear star-shaped in cross section. Groups of fibres are placed like a cap outside of the phloem areas (fig. 13). Within the cambial line the vascular bundles are separated and arranged circumferentially. In the secondary xylem, the parenchyma between and within the cambial bundles is replaced by fibres and thickened isodiametric cells, creating a solid ring of xylem tissue without medullary rays. Most vessel members have bordered pits. The sizeable pith contains sclereids (fig. 14) with phytomelanin. Secretory cavities occur in the cortex, the secondary xylem, and the pith with diameters in the range of 80 – 180 µm; the largest ones occur in the pith. In the longitudinal section the differences in the shapes of the more rectangular sclereids and the small and pointed fibres are evident.

Root: The anatomical structure of the examined samples in most points corresponds with the published data: the root is covered with a dark brown exodermis consisting of polygonal cells (fig. 15). The cortical parenchyma has thinner walls than those found in the secondary phloem. At the border between cortex and secondary phloem secretory cavities occur, mostly in groups of 3 or 4 at radial positions of the groups of tracheas within the xylem part. Their lumen is ovate and up to 80 mm in tangential section and is filled with orange to brown matter.

The vessels and fibres of the secondary xylem are grouped into cuneiform bundles tapering towards the center of the root. In younger roots more parenchyma is located within these areas, whereas in older ones the secondary xylem becomes a completely solid structure. Small groups of primary xylem are located at the inner end of the medullary rays outside of the central pith (fig. 16). In longitudinal section, pitted and scalariform vessels are found associated with elongated and pointed fibres. Sclereids and phytomelanin are absent in the root.

Powder: long bundles of fibres; few isodiametric sclereids with phytomelanin coating; vessels with reticulate or pitted secondary walls; secretory cavities with orange to brown content.



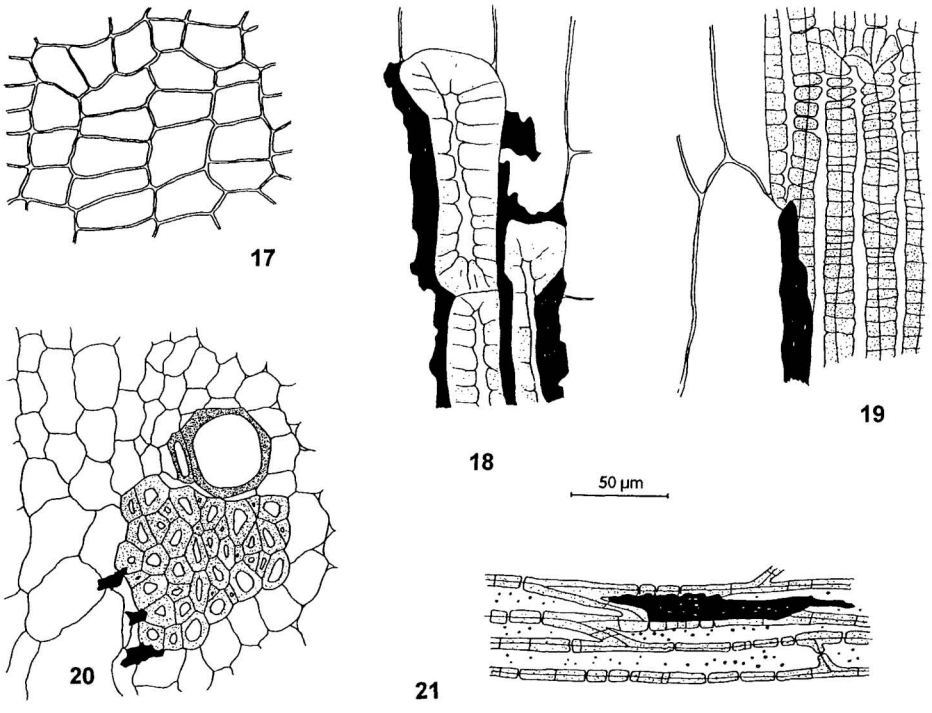
Figures 13-16: ***Echinacea purpurea***: 13: Rhizome showing inner cortical parenchyma with sclereids, a secretory cavity, and a small group of phloem fibres (cross section); 14: Sclereid in the rhizome (longitudinal view), 15: Root, exodermis (surface view); 16: Root showing a small bundle of primary xylem outside of the pith and located between two strands of secondary xylem (cross section).

3.5. PARTHENIUM INTEGRIFOLIUM

Root: the main structure of the root of Parthenium integrifolium is very similar to that of Echinacea spp. The root is covered by a dark brown exodermis (fig. 17). Secretory cavities occur in cortex, secondary phloem, and secondary xylem with a diameter up to 200 µm. Phytomelanin-coated sclereids with a diameter of approximately 40 to 60 µm occur in the cortical parenchyma (fig. 18) and the medullary rays (fig. 19). In contrast to the fibres, most sclereids are yellow. The secondary xylem has a radiate structure dominated by more or less rectangular groups of phytomelanin-coated fibres (fig. 20, 21). These fibres are generally associated with vessels and are arranged in radial circumferential rows, interrupted by

parenchyma. The circumferential rows of parenchyma, as well as the radial medullary rays, are narrow. The medullary rays contain very large, often ruptured cells. Within the secondary xylem, sclereids only occur in the medullary rays. Compared to the roots of Echinacea spp., the root of Parthenium integrifolium has the smallest amount of parenchyma in the secondary xylem. Elongated narrow fibres free of phytomelanin deposits may be found within the region of the primary xylem.

Powder: large multiseriate fragments of phytomelanin-coated fibres with associated pitted vessels; sclereids with phytomelanin-coating; solitary sclereids; colourless parenchyma; infrequent bundles of fibres, brown fragments of the cork.



Figures 17-21: ***Parthenium integrifolium***, root: 17: Exodermis (surface view); 18: Sclereids of the cortex (longitudinal view); 19: Sclereids of the secondary xylem (longitudinal view); 20: Vessels, fibres with phytomelanin, and a medullary ray (cross section); 21: Xylary fibres (longitudinal view).

4. Discussion

The examination of samples of the underground portions of *Echinacea*-species and *Parthenium integrifolium* showed, that the published data of the anatomy are a good basis for the identification, but they must be reformulated more precise. Some of the proposed differential characters did not stand the verification, like the size and shape of the exodermal cells and the different localization of the secretory cavities. The implementation of the data from *E. atrorubens* and the rhizome of *E. purpurea* lets the borders between the anatomy of all these species merge, especially ranges of the size of sclereids, fibres and secretory cavities overlap. Furthermore the declared lack of phytomelanin in *E. purpurea* cannot be kept as diagnostic character of powdered samples because of the presence of phytomelanin-coated sclereids in the rhizome of this species.

A major problem in the application of the published key is the correct determination of fibres and sclereids. In contrast to literature in all our samples of *Echinacea* spp. only sclereids are coated with phytomelanin, whereas fibres are coated solely in *Parthenium integrifolium*. A certain differentiation between fibres and sclereids is only possible using cross sections because of their localization within the tissues: while fibres are mostly associated with the vessels (additionally outside of the phloem parts in the rhizome of *E. purpurea* only), the sclereids occur in the cortical parenchyma, the medullary rays, and in the rhizome of *E. purpurea* in the pith. Although in the powdered drugs typical sclereids and typical fibres may be seen, continuous transitions between their shapes are discernible.

In table 1 the main characters are presented, the structures of the cross sections are illustrated in figure 22: the roots of *E. angustifolia*, *E. pallida*, and *E. atrorubens* are very similar. Due to the small number of examined samples the higher amount of phytomelanin-coated sclereids and of fibres associated with vessels in *E. atrorubens* can only be rated as indication. Both root and rhizome of *E. purpurea* differ considerably from the other *Echinacea*-species, the identification by means of cross sections seems to be possible. The root of *Parthenium integrifolium* is characterized by the highly regular network of groups of phytomelanin-coated fibres associated with vessels, and by radial and tangential rows of parenchyma.

Because of the highly similar anatomy the microscopic proof of identity and purity of crude drugs of *Echinacea* must be done with uncomminuted material and the examination of cross sections.

By now no data of the chemical constituents of the root of *E. atrorubens* are published. Because of the resulting uncertainty of the declared chemical differences between the *Echinacea* – species (absence of echinacosid in *E. purpurea*, presence of cynarin *in E. angustifolia*, presence of ketoalkenes in *E. pallida*[5]) the proof of identity and purity by means of microscopy is unavoidable.

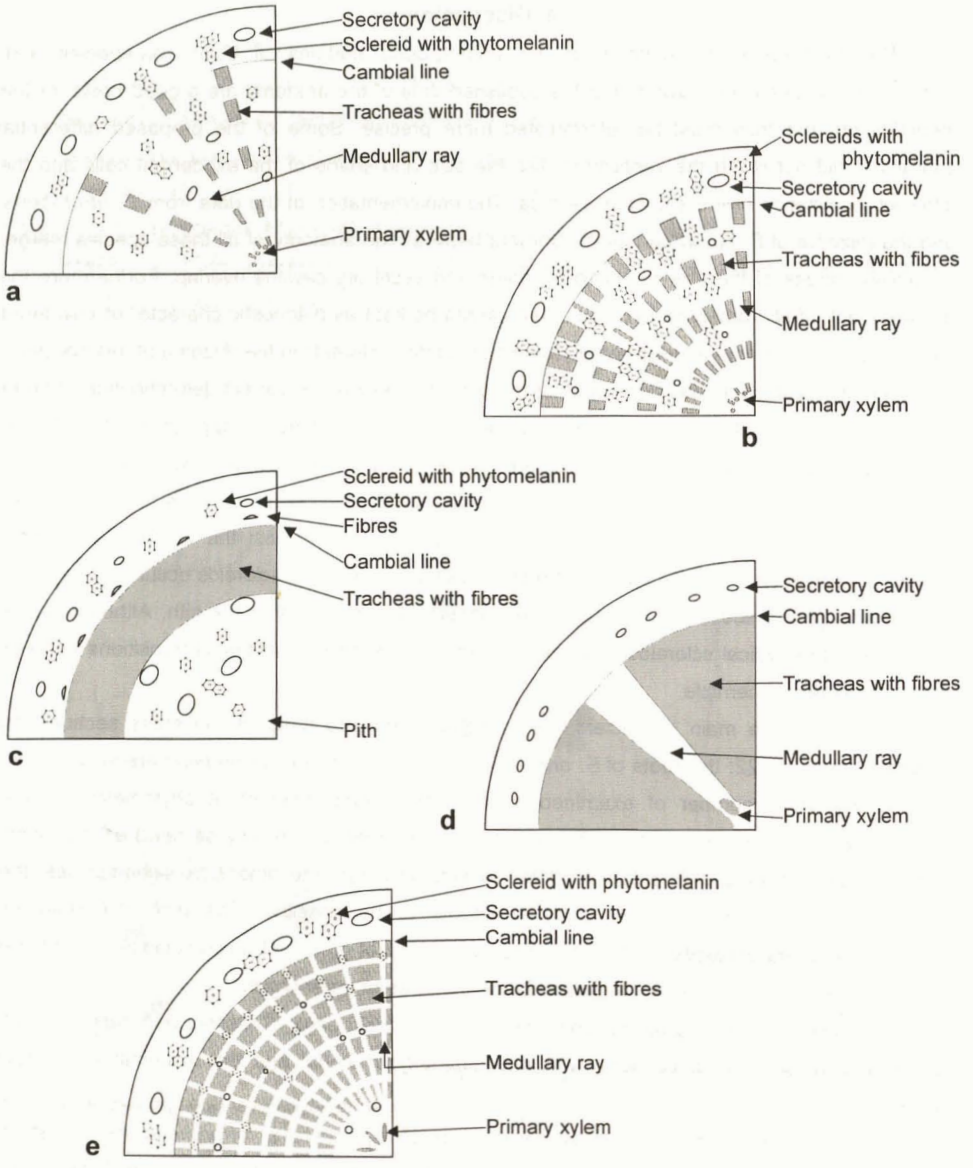


Figure 22: Schemes of the cross sections of the underground parts of *Echinacea angustifolia* (a), *E. pallida* (a), *E. atrorubens* (b), *E. purpurea* (rhizome, c), *E. purpurea* (root, d), and *Parthenium integrifolium* (e).

	Structure of cross section	Sclereids diameter	Secretory cavities	Phytomelanin deposition
***E. purpurea* (rhizome)**	Vascular bundles with fibres in a circumferential line, cells between bundles thickened, together forming a solid ring	- 60 µm	Cortex, secondary phloem, secondary xylem, pith; ∅ - 180 µm	Sclereids
***E. purpurea* (root)**	Large cuneiform areas of vessels and fibres	-	In circumferential line in the cortical parenchyma; ∅ - 80 µm	-
***E. angustifolia* (root)**	Small radial rays of vessels and fibres, broad medullary rays	- 40 µm	Cortex, secondary phloem, secondary xylem; ∅ - 140 µm	Sclereids
***E. pallida* (root)**	Small radial rays of vessels and fibres, broad medullary rays	- 80 µm	Cortex, secondary phloem, secondary xylem; ∅ - 600 µm	Sclereids
***E. atrorubens* (root)**	Small radial rays of vessels and fibres, small medullary rays	- 40 µm	Cortex, secondary phloem, secondary xylem; ∅ - 160 µm	Sclereids
***Parthenium integrifolium* (root)**	Radial rays of vessels and fibres, small medullary rays, and small circumferential parenchymatic lines forming a regular network	- 60 µm	Cortex, secondary phloem, secondary xylem; ∅ - 250 µm	Sclereids and fibres

Table 1: Microscopic diagnostic characters of the underground parts of *Echinacea*-species and *Parthenium integrifolium*.

5. Acknowledgements

The author is thankful to Mr. Roy Upton, American Herbal Pharmacopoeia, Santa Cruz, USA, and to F. Graf vom Hagen-Plettenberg, Heilpflanzen Sandfort GesmbH & CoKG, Olfen, BRD, for providing authenticated plant material.

6. References

1. Moser J. (1910), Am. J. Pharm. **82**: 224
2. Kraemer H., Sollenberger M. (1911), Am. J. Pharm. **83**: 315
3. Heubl G. R., Bauer R., Wagner H (1988), Sci. pharm. **56**: 145
4. Heubl G. R., Bauer R. (1989), Dtsch. Apoth. Ztg. **129**: 2497
5. Bauer R., Wagner H. (1990), Echinacea – Handbuch für Ärzte, Apotheker und Naturwissenschaftler, Wissenschaftl. Verlagsgesellschaft mbH, Stuttgart

Received May 3rd, 2001
Accepted August 10th, 2001