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HISTO-ANATOMICAL STUDY OF THE ANNUAL OFFSPRING AND LEAF'S LIMB OF *VITEX NEGUNDO* L. (*VERBENACEAE*) SPECIES

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ABSTRACT

This paper presents the histo-anatomical study of the annual offspring and leaf's limb of Vitex negundo species, as an essential stage for the pharmacognostic expertise advancement. The pharmacological importance of the medicinal products (roots, leaves, seeds) obtained from V. negundo was also emphasized.

INTRODUCTION

Vitex negundo L., Chinese chaste tree, five-leaved chaste tree, *Verbenaceae* family, is a shrub or small tree, 2–8 m tall, native to tropical Africa (Kenya, Tanzania, Madagascar) and Asia (India, Indonesia, Vietnam, Malaysia, Philippines, China, Japan), widely naturalized and cultivated as an ornamental plant (*Vitex*, 2013).

For therapeutic purposes, roots, leaves and seeds are used because of their content of active principles such as iridoid glycosides, *e.g.* negundoside and derivatives (Tasduq *et al.*, 2008; Sharma *et al.*, 2009; Huang *et al.*, 2013), essential oil (Khokra *et al.*, 2008; Nagarsekar *et al.*, 2010), flavonoids (Sathiamoorthy *et al.*, 2007), sesquiterpenoids and diterpenoids (Zheng *et al.*, 2010; Zheng *et al.*, 2012), lignans (Xin *et al.*, 2013), fatty acids (Kannathasan *et al.*, 2008), ecdysteroids, sterols, catechic tannin, simple carbohydrates, proteins, enzymes, organic acids, vitamins, mineral salts (Vishwanathan & Basavaraju, 2010).

The medicinal products harvested from *V. negundo* have some important properties: hepatoprotective (Tasduq *et al.*, 2008; Kadir *et al.*, 2013), antibacterial (Khokra *et al.*, 2008; Nagarsekar *et al.*, 2010; Kamruzzaman *et al.*, 2013), antifungal (Sathiamoorthy *et al.*, 2007), anti-inflammatory (Zheng *et al.*, 2010; Chattopadhyay *et al.*, 2012), apoptosis-inductive and antitumoral (Xin *et al.*, 2013), larvicidal (Kannathasan *et al.*, 2008), vermicide (Sahare & Singh, 2013), antiandrogenic (Das *et al.*, 2004), anticonvulsant (Tandon & Gupta, 2005), antitussive (Haq *et al.*, 2012), anti-hyperglycemic (Sundaram *et al.*, 2013), antioxidant and anti-lipid peroxidation (Nagarsekar *et al.*, 2011), prevention of selenite-induced oxidative stress and cataractogenesis (Rooban *et al.*, 2012), inhibition of melanogenesis (Huang *et al.*, 2012). Extractive preparations obtained from the flowers of some *Vitex* sp. are used for their contraceptive effect in Southeast Asia, India and Africa (Das *et al.*, 2004).

Even considering its medicinal importance, in the specialty papers there are no data regarding *V. negundo* histo-anatomy. The aim of our paper was the histo-anatomical study of the annual offspring and leaf's limb of the above-mentioned species, as an important step for the pharmacognostic expertise.

MATERIAL AND METHODS

The vegetal material was collected from *V. negundo* plants in blossom, in May 2013, from "Alexandru Buia" Botanical Garden, University of Craiova, Dolj County, Romania.

Fixation and preservation of vegetal material (aboveground stems, leaves) were

achieved in 70% alcohol. The cross-sections were obtained by manually sectioning using a botanical razor.

After washing with distilled water, using 10% sodium hypochlorite (Javel water) the cross-sections were submitted to the clarification process. Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture was used for the cross-sections staining (Andrei & Paraschivoiu, 2003).

Depending on the chemical composition of cell membranes, the reactive induced various stains: pink to red for cellulose, yellow for suberin, brown for lignified membranes (Andrei & Paraschivoiu, 2003).

Stained and mounted cross-sections were analyzed on a Krüss binocular photon microscope at diverse objectives (×4, ×10, ×40) and then photographed on a Soligor SR 300 system adapted to the microscope.

Description of microscopic cross-sections was accomplished according to some classical authors (Toma & Rugină, 1998).

Using ×40 objective (corresponding on 0.037 mm^2 area), the analysis of stomatal index was made on a Nikon Eclipse 55i binocular photon microscope coupled with a Nikon DS–Fi1 high definition video camera.

Image acquisition and processing were performed by means of ImageProPlus ver. 6.0 software package.

For each bottom, middle and upper area of the examined leaf's limb, the average of 10 samples were taken into consideration.

RESULTS AND DISCUSSIONS

Structure of annual offspring

The annual offspring exhibits circular shape and secondary structure determined by libero-ligneous and subero-phellodermic cambium.

In cross-section, from the outside to the inside, in the lower third of annual offspring, the following histological sequence was observed:

Peridermis is made of 4–5 layers of suber, a single layer of subero-phellodermic cambium and 4–5 layers of cellulosic phelloderm.

Into the primary bark, numerous periphloemic sclerenchyma caps are observed.

The conducting tissues are predominant secondary being generated by the libero-ligneous cambium.

An external ring of secondary phloem tissue includes sieve tubes, annex cells and phloem parenchyma.

The libero-ligneous cambium has a circular-winding shape.

The secondary xylem tissue is well represented, consisting of secondary xylem vessels of different diameters spread into a mass of libriform tissue. Xylem vessels have curly and reticular thickenings highlighted in longitudinal radial sections. The secondary xylem tissue is organized in the form of annual rings.

Medullary rays are multicellular, uniseriate, cellulosic into the phloem tissue and lignified at the level of xylem tissue.

A well-represented meatus-type medullary parenchyma was noticed (Figures 1–5).

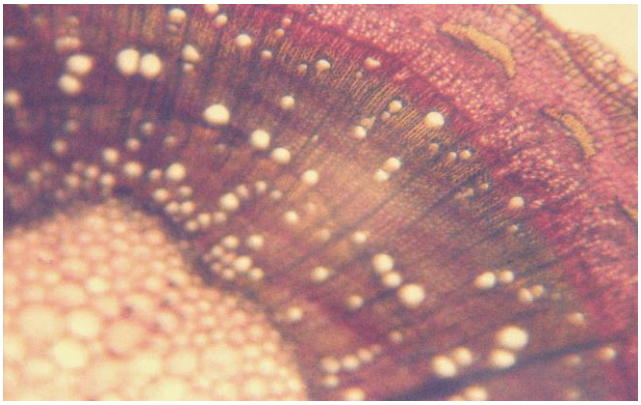

Figure 1. Cross-section through *V. negundo* annual offspring. Congo red–chrysoidine staining, ×100. Overview.

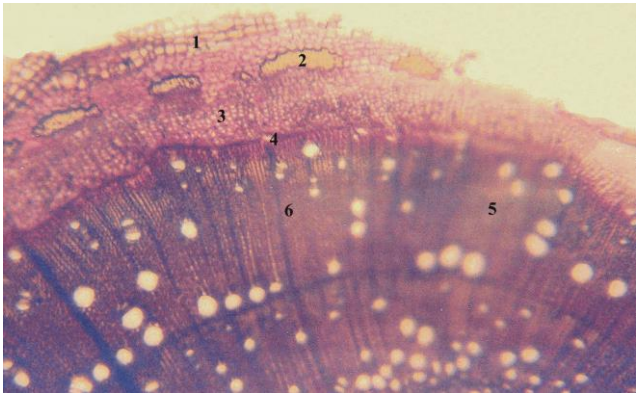

Figure 2. Cross-section through *V. negundo* annual offspring. Congo red–chrysoidine staining, ×100. 1 – Epidermis, 2 – Periphloemic sclerenchyma cap, 3 – Phloem tissue,
4 – Libero-ligneous cambium, 5 – Xylem tissue, 6 – Medullary ray.

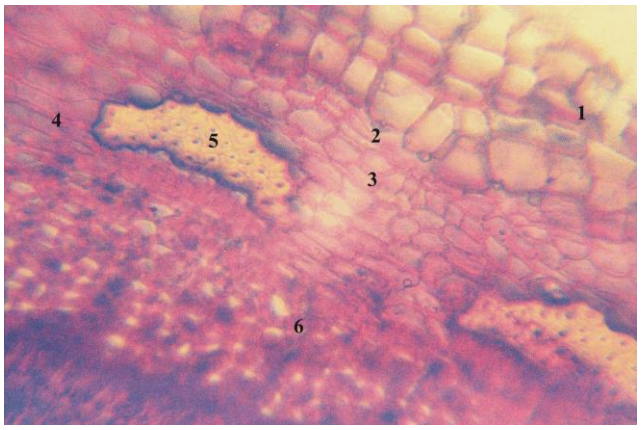

Figure 3. Cross-section through *V. negundo* annual offspring. Congo red–chrysoidine staining, ×400. 1 – Suber, 2 – Phellogen, 3 – Phelloderm, 4 – Cortical parenchyma,
5 – Periphloemic sclerenchyma cap, 6 – Phloem tissue.

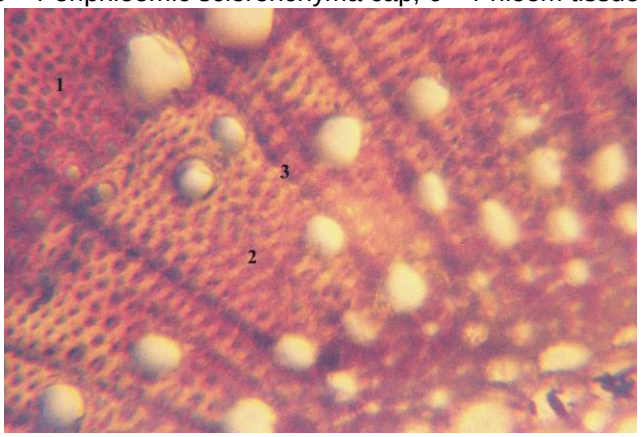

Figure 4. Cross-section through *V. negundo* annual offspring. Congo red–chrysoidine staining, ×400. 1 – Summer xylem, 2 – Autumn xylem, 3 – Medullary ray.

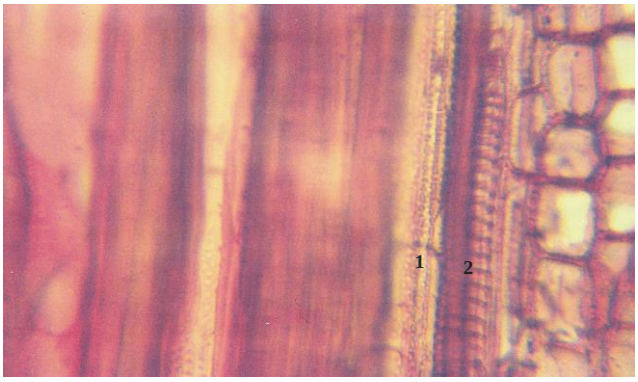

Figure 5. Longitudinal radial section through *V. negundo* annual offspring. Congo red–chrysoidine staining, ×400. 1 – Reticular xylem vessel, 2 – Curly xylem vessel.

Structure of leaf's limb

In cross-section, from the outside to the inside, the leaf's limb shows the following tissue sequence:

The upper epidermis is made of a single layer of flattened big cells, with thickened external and internal tangential walls and thin radial walls. Tangential external walls are covered by cutine. Unicellular sharp tector hairs are found from point to point. Stomata are missing at this level.

The homogenous mesophyll is made of three layers of palisade tissue with big prosenchymatous cells delineating small intercellular spaces and of 3–4 layers of smaller size prosenchymatous cells delineating much larger aeriferous spaces, which are also placed in palisade.

Numerous libero-ligneous conducting fascicles surrounded by an assimilatory sheath are found into the mesophyll. The plant is of C4 type. Some larger fascicles are attached to the epidermises through supporting tissue.

The lower epidermis is made of a single layer of small cells, closely linked together, with thin radial walls and thickened internal and external tangential walls. At this level, many flexed bicellular tector hairs and tetracellular glandular hairs are found. Into the lower epidermis, anisocytic stomata and cupuliform glandular hairs, in front of the mesophyll' conducting fascicles, are highlighted.

The leaf's limb has equifacial hypostomatic structure.

In cross-section, the median rib has circular shape. At this level, the epidermis is made of small heterodiametric cells with thickened external and internal tangential walls and thin radial walls. Here and there are found flexed bicellular tector hairs to the abaxial (dorsal) side, unicellular tector hairs to the adaxial (ventral) side, tetracellular glandular hairs and stomata.

Below the epidermis are found 2–3 layers of angular collenchyma and then the fundamental leaf's parenchyma.

At the level of the median rib, one big centrally placed libero-ligneous conducting fascicle is found (Figures 6–8).

Our analyses show that the stomatal index of *V. negundo* leaf (15.9–16.4) is different compared to *V. agnus-castus* (26.8–27.3, personal unpublished results). This fact is very important in the pharmacognostic expertise of medicinal products (leaves) harvested from these two species (Figures 9 and 10).

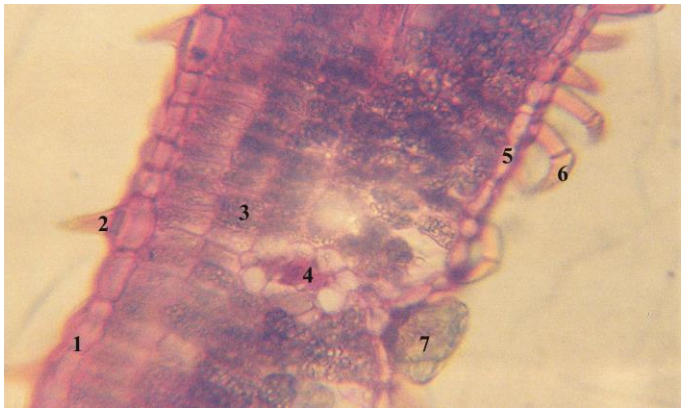

Figure 6. Cross-section through *V. negundo* leaf's limb. Congo red–chrysoidine staining, ×400. 1 – Upper epidermis, 2 – Unicellular tector hair, 3 – Palisade parenchyma, 4 – Libero-ligneous conducting fascicle, 5 – Lower epidermis, 6 – Flexed bicellular tector hair, 7 – Cupuliform glandular hair.

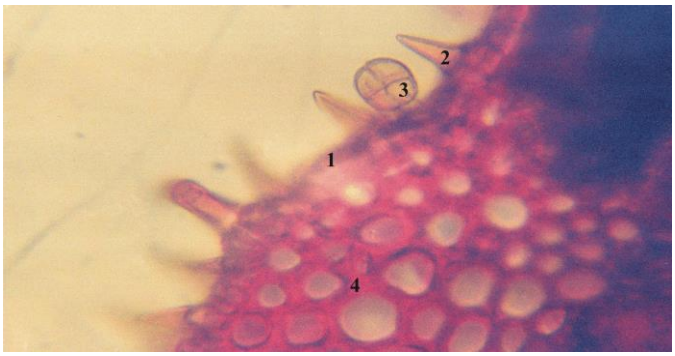

Figure 7. Cross-section through *V. negundo* leaf's median rib. Congo red–chrysoidine staining, ×400. 1 – Upper epidermis, 2 – Tector hair, 3 – Tetracellular glandular hair, 4 – Angular collenchyma.

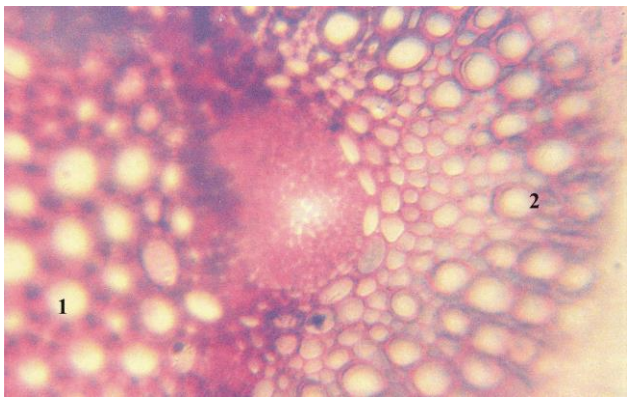

Figure 8. Cross-section through *V. negundo* leaf's median rib. Congo red–chrysoidine staining, ×400. 1 – Fundamental leaf's parenchyma, 2 – Xylem tissue.

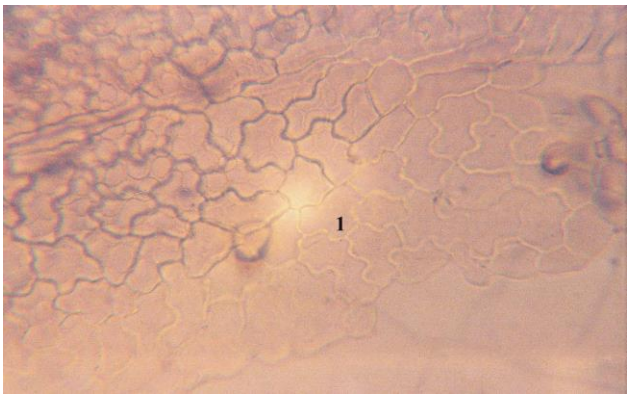

Figure 9. *V. negundo* upper epidermis. Congo red–chrysoidine staining, ×400.

1 – Epidermal cells.

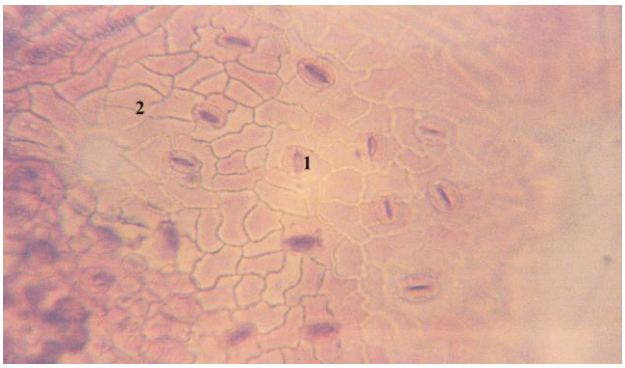

Figure 10. *V. negundo* lower epidermis. Congo red–chrysoidine staining, ×400.
1 – Anisocytic stomata, 2 – Epidermal cells.

CONCLUSIONS

The histo-anatomical study of the annual offspring and leaf's limb of *Vitex negundo* was achieved. The annual offspring exhibits circular shape and secondary structure. Many periphloemic sclerenchyma caps are observed into the primary bark. Produced by the libero-ligneous cambium, the conducting tissues are predominant secondary. The leaf's limb has equifacial hypostomatic structure with unicellular/flexed bicellular tector hairs, cupuliform or tetracellular glandular hairs and anisocytic stomata.

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