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MORPHO-ANATOMICAL CHARACTERISTICS OF THE RAW MATERIAL OF THE HERBAL DRUG *OLIVAE FOLIUM* AND ITS COUNTERFEITS

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Abstract – The olive tree leaf is a very significant plant raw material from the medical and economic points of view (Ph. Eur. 5, PDR). In the region of Southeast Europe, olive leaves are most commonly adulterated with oleander leaves and the leaves of *Pittosporum tobira*. This paper deals with the morphological and anatomical features of leaves of the following species: *Olea europaea, Nerium oleander* and *Pittosporum tobira*. The aim of this research was to define concrete diagnostic parameters permitting detection of adulterants in commercial samples of the herbal drug *Olivae folium*.

Key words: Olivae folium, Nerium oleander, Pittosporum tobira, counterfeits, leaves, anatomy, morphology

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INTRODUCTION

Olea europaea L. (*Oleaceae*) is an evergreen tree or shrub that is widespread throughout the whole Mediterranean region in the form of wild or cultivated populations (A m a r a l and R o c h a, 1972). The olive tree is an important biological source of pharmaceutical raw material (*Olivae folium, Olivae oleum*) and food products (*Olivae fructus, Olivae oleum*).

Nerium oleander L. (*Apocynaceae*) is an evergreen shrub or tree widely distributed in Mediterranean region as an important horticultural species (M a r k g r a f, 1972). Oleander contains components that induce strong pharmacological activity – production of cardiotonic glycosides - and uncontrolled application may therefore produce some undesirable reactions, as well as serious poisoning.

Pittosporum tobira (Thunb.) Aiton (*Pittosporaceae*) is an evergreen shrub or small tree originating from Northern Japan and Eastern China, but also occuring widely in Europe, above all in the Mediterranean region, as a cultivated species owing to its beautiful and very fragrant flowers (A m a r a l, 1964).

In practice, depending on the form of olive leaves (whole or minced), they can be intentionally or unintentionally adulterated with *Nerium oleander* or *Pittosporum tobira* leaves. However, based on morphological and anatomical features of the leaves of these three species, the purity of plant raw material obtained from them can be reliably established, i.e., adulterants can be detected and undesirable substitutions can consequently be avoided.

This paper deals with the macroscopic (morphological) and microscopic (anatomical) characteristics of the olive, oleander and pitosporum leaves. Elements important for their differentiation are established, thereby making possible an easier and more accurate control of commercial samples of olive leaves and detection of the presence of prohibited adulterants and falsifications.

MATERIALS AND METHODS

Macroscopic and microscopic analyses were done on specimens of the species *Olea europaea, Nerium oleander*, and *Pittosporum tobira* from the Adriatic Coast (Montenegro). The collected plant material was either placed in the Herbarium of the Institute of Botany, Faculty of Pharmacy, University of Belgrade (HFF), or fixed in 50% alcohol.

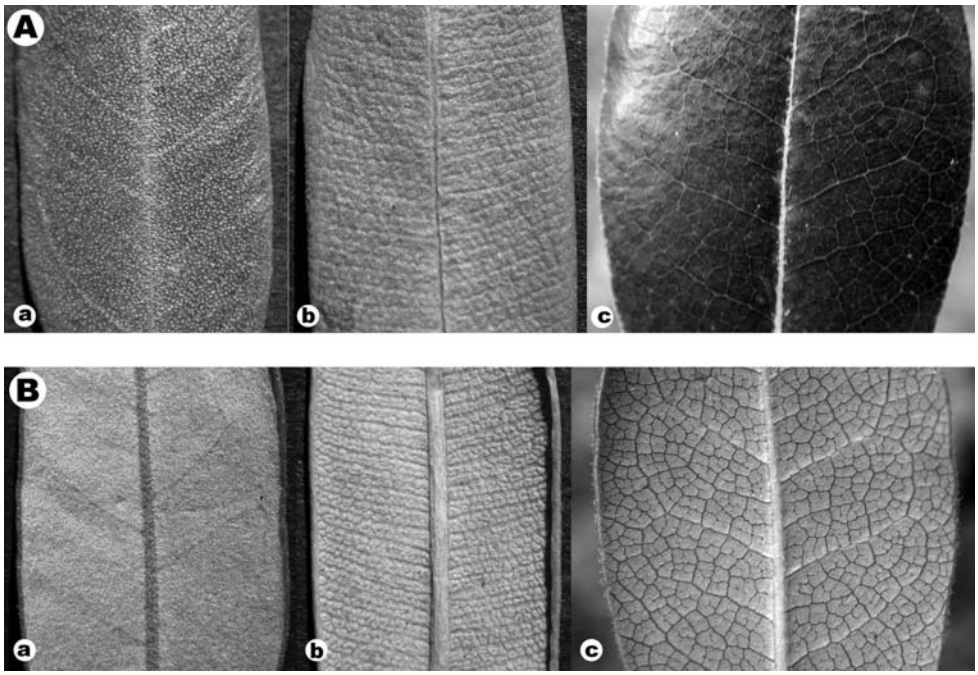

Fig. 1. **A.** Adaxial leaf surface **B.** Abaxial leaf surface **a.** *Olea europaea* **b.** *Nerium oleander* **c.** *Pittosporum tobira***.**

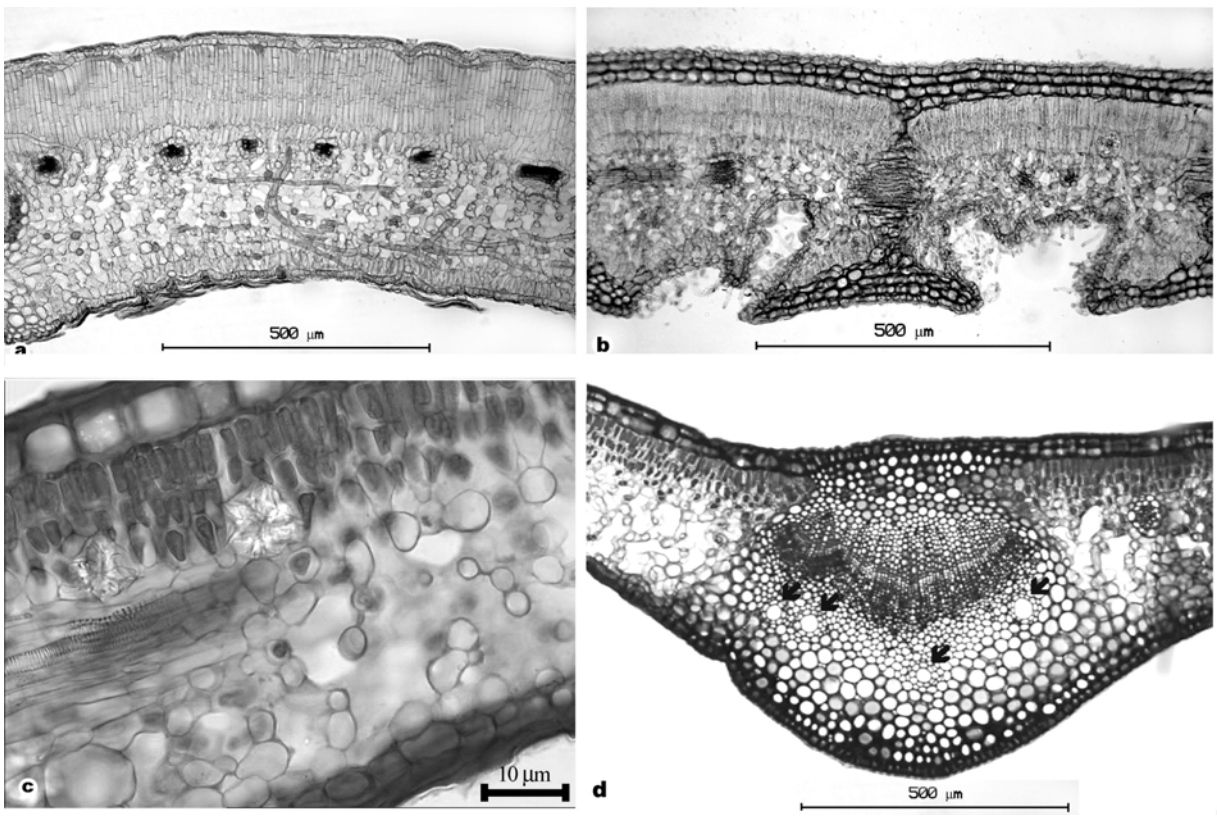

Fig. 2. Cross section of the leaf. **a.** *Olea europaea* **b.** *Nerium oleander* **c, d.** *Pittosporum tobira*..

Anatomical analyses of the leaves were done on permanent slides prepared by the standard method for light microscopy. Cross-sections (up to 10 µm thick) of leaves were cut on a Reichert sliding microtome. The sections were cleared in Parazone and thoroughly washed before staining in safranin (1 % w/v in 50 % ethanol) and alcian blue (1 % w/v, aqueous). Epidermal peels for surface structure and stomata analyses were prepared using Jeffrey's solution (10 % nitric acid and 10 % chromic acid, 1:1) and stained in safranin and alcian blue. All slides were mounted in Canada balsam after dehydration. The density and type of leaf hairs, as well as the paradermal aspect of epidermal cells, were also studied by scanning electron microscopy (with a JOEL JSM-6460 instrument), for which the samples sputtered with gold.

RESULTS

Macroscopic (morphological) leaf features

Olea europaea - The leaf is simple, subsessile, thick, coriaceus, lanceolate to obovate, more or less pointed at the apex. The margins are entire and folded abaxially. Nervation of the leaf is reticular (Fig. 1Ba). The upper surface is dark-green, glabrous and shiny (Fig. 1Aa), the lower surface shimmering silver, and tomentous (densely pilous) (Fig. 1Ba).

Nerium oleander - The leaf is simple, sessile, thick, coriaceous, opposite or grouped with three or four in one whorl, entire, linear – lanceolate, acute. The margins are entire and folded abaxially. Nervation of the leaf is reticular (Fig. 1Bb). The adaxial surface is gray-light green (Fig. 1Ab) and glabrous, the abaxial one is a bit darker, pilous and furrowed in parallel lines (Fig. 1Bb).

Pittosporum tobira - The leaf is simple, alternately arranged on short leaf blades, very coriaceous, entire, obovate-oblong to oblong, obtuse. Nervation is reticular (Fig. 1Bc). Both the adaxial and abaxial surfaces are shimmering, dark green, and glabrous (Fig. 1Ac, 1Bc).

Microscopic (anatomical) leaf features

Olivae folium - The leaf has iso-bilateral structure (Fig. 2a). Both the adaxial epidermis and the abaxial epidermis are simple, with thick cuticle. The anticlinal walls of both adaxial and abaxial epidermal cells are straight (Fig. 3a). The adaxial epidermis has sporadic and the abaxial epidermis numerous densely distributed, non-glandular, very large scutiform trichomes (Fig. 4). The leaves are hypostomatic and their numerous stomata possess highly cutinized guard cells.

The mesophyll is constituted of three layers of condensed long palisade cells located under the adaxial epidermis and one or two layers of short palisade cells on the abaxial epidermis. The central part of the leaf contains tiny cells of spongy parenchyma with large intercellulars (Fig. 2a). Present in the mesophyll are filiform sclereids,

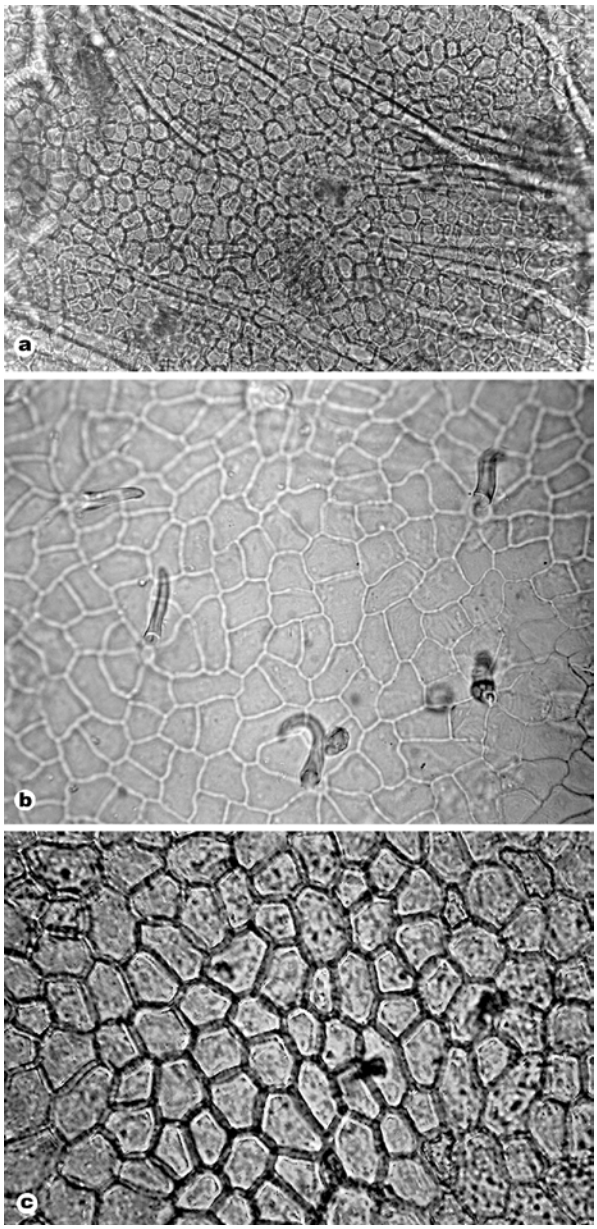

Fig. 3. Paradermal view, straight anticlinal walls. **a.** *Olea europaea* **b.** *Nerium oleander* **c.** *Pittosporum tobira.*

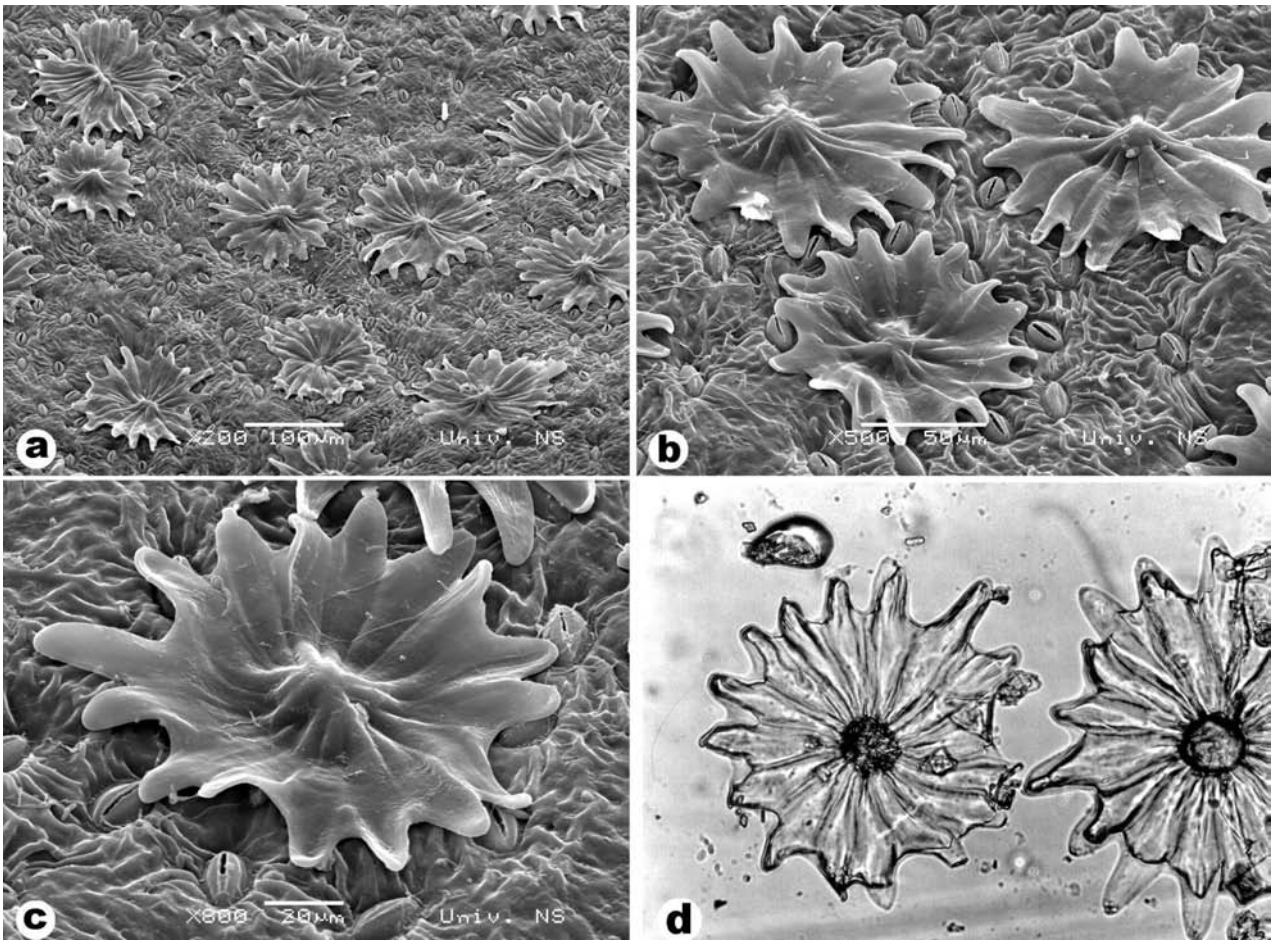

Fig. 4. *Olea europaea*, scutiform trichomes on the abaxial side of leaf **a,b,c.** SEM; **d.** light microscopy.

which are long, fiber-like, and sometimes branched (Figs. 2a, 3a).

Oleandri folium - The leaf has isobilateral structure (Fig. 2b). The adaxial and abaxial epidermis are covered by a thick layer of cuticle. Anticlinal walls of cells of both the adaxial (Fig. 3b) and abaxial (Fig. 5c) epidermis are almost straight. The adaxial epidermis has scattered unicellular trichomes, which are also present on the abaxial epidermis, where they are located in deep crypts (Fig. 5). The leaf is hypostomatic.

The mesophyll is composed of two or three layers of dense palisade cells on both leaf surfaces. Cells of the spongy parenchyma are located in the narrow central part of the leaf (Fig. 2b).

Present in the abaxial part of the leaf are numerous crypts (Figs. 2b, 5). The epidermis is simple with non-glandular, unicellular, long trichomes and stomata. On the peeled-off epidermis, these crypts have on oval or enlogated cylindrical shape with clearly expressed trichomes (Fig. 5c).

Pittospori folium - The leaf has dorsiventral structure (Fig. 2c). The surface of the simple adaxial and abaxial epidermis is covered by a thick layer of cuticle. Anticlinal walls of cells in both the adaxial (Fig. 3c) and the abaxial epidermis are straight.

The mesophyll consists of three layers of condensed short palisade cells located under the adaxial epidermis. The rest of the mesophyll is constructed of tiny cells of spongy parenchyma, among which there are relatively large intercellular spaces. Dominant in the mesophyll, particularly at the interface of palisade and spongy parenchyma, are cells with large cluster crystals (Fig. 2c). Se-

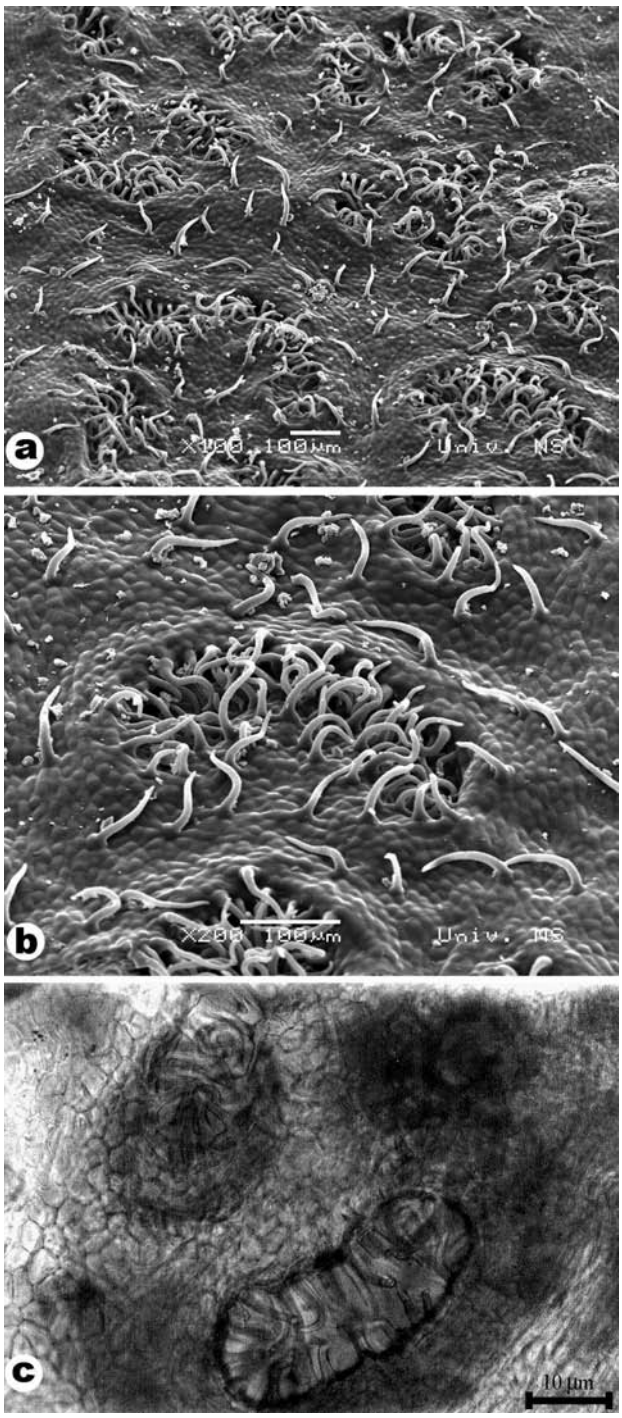

Fig. 5. *Nerium oleander*, specific crypts on the abaxial side of leaf **a.b.** SEM; **c.** light microsopy.

cretory channels are developed in the leaf phloem. Several of them (3-4) are located in the phloem of the main nerve (Fig. 2d); the side bundles contain one of these secretory channels.

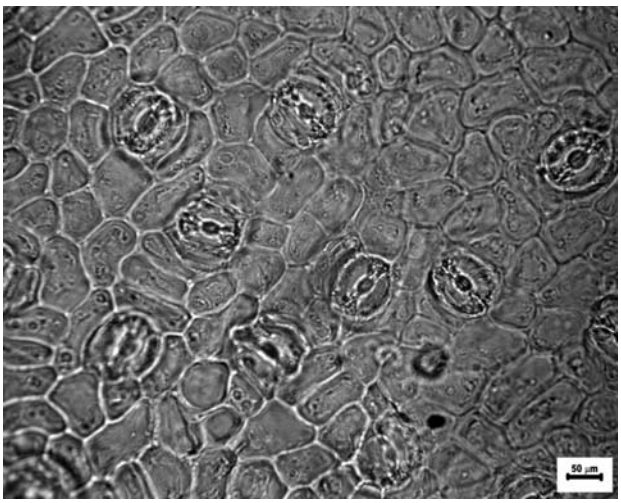

Fig. 6. *Pittosporum tobira,* anomocytic stomata on the abaxial side of leaf.

The leaf is hypostomatic, the stomata anomocytic (Fig. 6).

Pulvis

After commercial samples had been powdered, the presence of adulterants in counterfeits was discovered by microscopic analysis.

The pulvis of olive leaves is characterized by fragments of long filiform sclereids (Fig. 7b) and large scutiform trichomes (Fig. 7a). That of oleander leaves is characterized by fragments of epidermis with oval forms, so-called crypts, with unicellular trichomes.

Pittosporum leaf pulvis is characterized by epidermis having numerous anomocytic stomata and large cluster crystals.

DISCUSSION AND CONCLUSIONS

For macroscopic identification of olive leaves and detection of adulterants in counterfeits, leaf nervation and the nature of the leaf surface are the most important things to consider.

The olive leaf is characterized by reticular nervation with arched secondary nerves and a white abaxial surface consisting of a dense indumentum. The oleander leaf has reticular nervation with parallel secondary nerves, which,

together with crypts, form a rather specific macroscopic appearance of the leaf abaxial surface. The pittosporum leaf clearly expressed reticular nervation, on both the adaxial and abaxial surfaces.

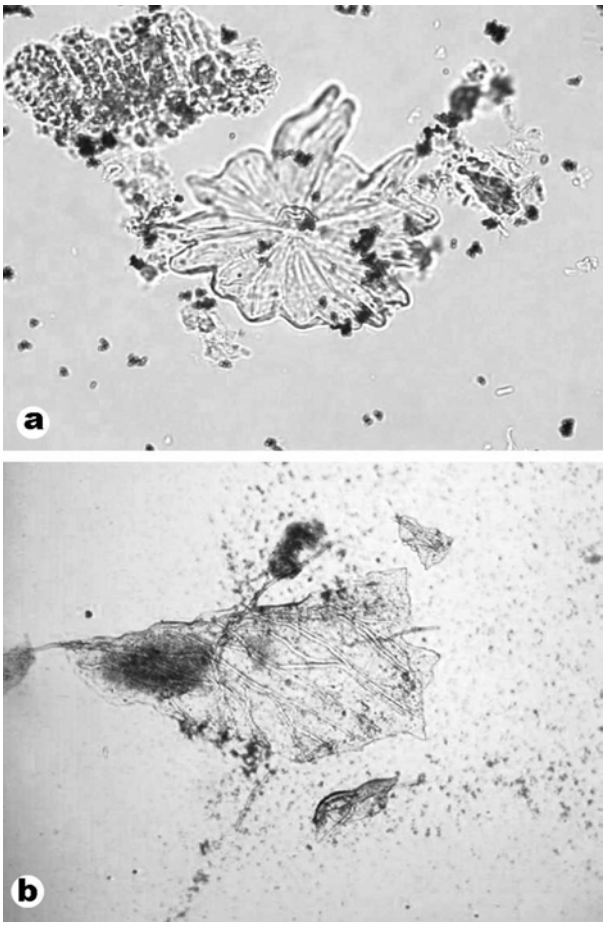

Fig. 7. *Olea europaea*, pulvis: **a.** scutiform trichome; **b.** fragment of epidermis with long filiform sclereids.

Microscopically, the olive leaf can be clearly identified from fragments of epidermis with long filiform sclereids and characteristic very large scutiform trichomes. The presence of fragments of epidermis with crypts indicates that olive leaves were adulterated with oleander leaves, while the presence of numerous anomocytic stomata and large cluster crystals indicates adulteration with pitosporum leaves.

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МОРФО–АНАТОМСКЕ КАРАКТЕРИСТИКЕ БИЉНЕ СИРОВИНЕ *OLIVAE FOLIUM* И ЊЕНИХ ФАЛСИФИКАТА

БРАНИСЛАВА ЛАКУШИЋ[1] ВИОЛЕТА ПОПОВ [2] И ДУШАНКА РУЊАЈИЋ-АНТИЋ[2]

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Лист маслине (*Olea europaea*) представља значајну сировину у фармацеутској индустрији. У југоисточној Европи, као фалсификат ове сировине појављују се листови лијандера (*Nerium oleander*) и питоспорума (*Pittosporum tobira*). Циљ овог рада је да се утврде морфо-анатомска својства као дијагностички параметри, значајни за откривање могућих фалсификата у комерцијалном узорку листова маслине (*Olivae folium*).