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Morphological and anatomical study of the bark, leaves and seeds of *Euonymus europaeus* L

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Abstract

Morphological and microscopic examination of the bark, leaves and seeds of *Euonymus europaeus* L. is presented in the study. The plant has an antioxidant, insecticidal, antimicrobial action. *Euonymus europaeus* L. can be a potential source of biologically active compounds. The purpose of the work was to determine the diagnostic features for the correct identification of plant materials. Light microscopy was used in the study. As a result, the morphological analysis of the raw material was carried out, the anatomical structure of the leaf, the leaves upper and lower epidermis, type of stomata, cross sections of the bark and seeds were investigated.

Keywords: *Euonymus europaeus* L., microscopy, leaves, bark, seeds

Introduction

European spindle (*Euonymus europaeus* L.) belongs to the *Celastraceae* family. *Euonymus europaeus* L. is native to much of Europe, including the surrounding Atlantic and Mediterranean islands. In the north it extends all the way to the southern part of Sweden, in the east to the Caucasus Mountains and Asia Minor^[1, 2]. In Ukraine it most often occurs in forest and forest-steppe zones. In all countries, populations of this species are numerous and do not need protection^[3].

Usually it is a deciduous shrub 1.5-2 m tall, rarely a small tree 5-10 m tall^[1, 2].

The old branches are grayish-brown, cross-linked by cortical growths, the young stems are green, in section round or indistinctly quadrangular^[1]. With age four corky edges, which are grow opposite each other, are formed giving a square stem. Later on the corky edges grow together and the stems turn grey^[1]. Leaves opposite, petiolate, elongated to oblong-ovate in shape, tapered at the base, serrate on the edge. Leaf petioles grooved, 2-15 mm long, leaf blades 3-11.5 cm long and 1-6.5 cm wide. The leaves are slightly leathery to the touch, their color is rich green, the upper side is naked, the veins are on the underside are short-pubescent. Autumn leaves turn yellow-green, crimson or wine colors.

Capsular fruit are drooping, four-sided, 0.7-1.3 cm long, 1.5 cm wide. Its surface is bare and smooth, the end is depressed, and the blades are curved. At the beginning of ripening they are green, with full ripeness of pink-crimson. Each nest of the fruit contains one seed of 0.5-1 cm in length. The seed itself is white, but above it is covered with an orange aril^[1-4].

According to the literature, extracts of *Euonymus europaeus* L. have antimicrobial, insecticidal, antioxidant effects and can be used in the treatment of dermatological diseases^[5].^{6]} *Euonymus europaeus* L. can be a source of biologically active substances such as di-, tri-, and sesquiterpenes, alkaloids, cardiac glycosides, lectins, squalene etc.^[6, 7].

The aim is to establish macroscopic and microscopic diagnostic features of *Euonymus europaeus* L. bark, leaves and seeds as a potential medical raw material.

Materials and methods

Bark, seeds and leaves of European spindle (*Euonymus europaeus* L.) were selected as the most promising sources of biologically active substances. The bark was harvested in the period February-March from young, green stems, leaves in April-May, fruits in September-October 2019 in the Lviv region, Ukraine. For softening and lighting the raw materials were boiled with 3% KOH solution for 3-5 minutes (leaves) and 5% KOH for 15-20 minutes (bark) and the dried seeds were soaked in water. Surface preparations and cross sections were made with a blade. Recorded using gelatin-glycerol solution, which was prepared by the known method. To 1 g of gelatin was added 50 ml of water for swelling. Excess water was filtered off. 6 ml of purified water was added and heated to dissolve the gelatin. Then added 7 grams of glycerol^[8]. For micro-structured researches we used an Olympus bx51 microscope (Olympus, Japan) equipped with high-resolution 1.5x, 5x, 10x, 40x lenses and a LUMC-B11 / Sony camera (Labtron, WB).

The obtained images were processed using computer programs.

Results and Discussion

The main morphological features that make it possible to differentiate *Euonymus europaeus* L. from other species of

genus *Euonymus* are four-sided stem, wedge-shaped base of leaf blade, greenish small flowers with yellow anthers and fully covered with arils seeds. The data of comparative morphological analysis, according to the analysis of the literature data [3, 4, 9] with the *Euonymus* species common in the territory of Ukraine is presented in Table 1.

Table 1: Comparison of diagnostic features of wild species of genus *Euonymus* in Ukraine

	Flowers	Leaves	Stems	Fruits
<i>Euonymus europaeus</i> L.	Green-yellow Stamens with yellow anthers	elongated ovate, elliptical-lanceolate or inverted ovoid, fine-toothed with cuneate base	Branches glabrous green, square in cross section with brownish tinged ribs, which later turn grayish	capsular fruit deeply lobed, arils cover the whole seed
<i>Euonymus verrucosus</i> Scop.	Brownish petals	Smaller	Round stems with verrucas	arils cover half seed; seed are orange with verrucas
<i>Euonymus Maaki</i>	Stamens with purple anthers	Leaf with rounded base	Flat, green	capsular fruit are not deeply lobed

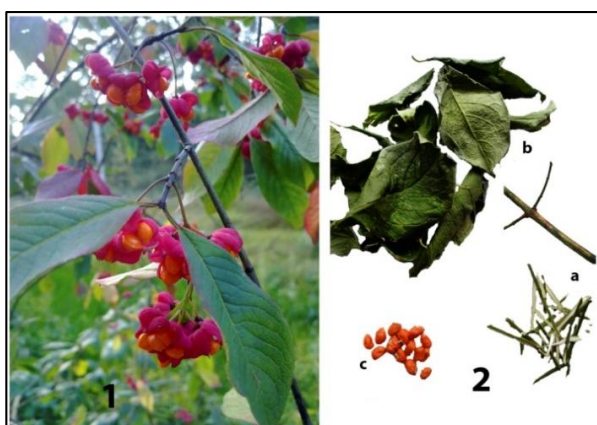


Fig 1: *Euonymus europaeus* L. (1), raw material (2): quadrangular stem, bark (a), leaves (c), seeds (c)

Microscopic analysis

The cells of the both epidermises of leaves are isodiametric, arranged chaotically with straight thick walls. The stomatas arranged complexly of the anomocytic type in lower epidermis side. Above the veins the cells are narrow and elongated, without stomatas. The trihoms are simple, rarely found and mainly on the veins on the side of the lower epidermis.

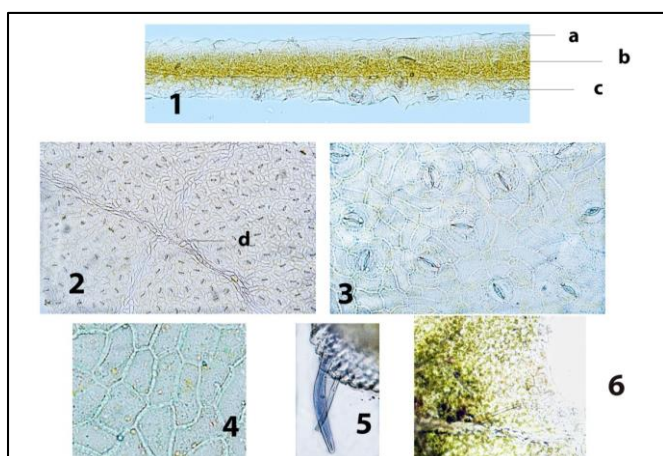


Fig 2: Elements of the leaf anatomy. Cross-section of leaf blade (1), (upper epidermis-a, parenchyma-b, lower epidermis-c), lower epidermis (2) (elongated cells over the vascular system-d), the lower epidermis with stomatas (3), cells of the upper epidermis (4), trichoms (5,6)

The margin of the leaf is presented in Fig. 3

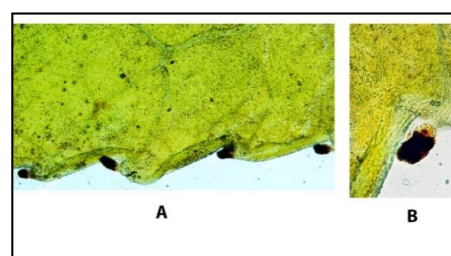


Fig 3: Leaf margin serrated, ending with a multicellular dark formation (Fig. 3. B)

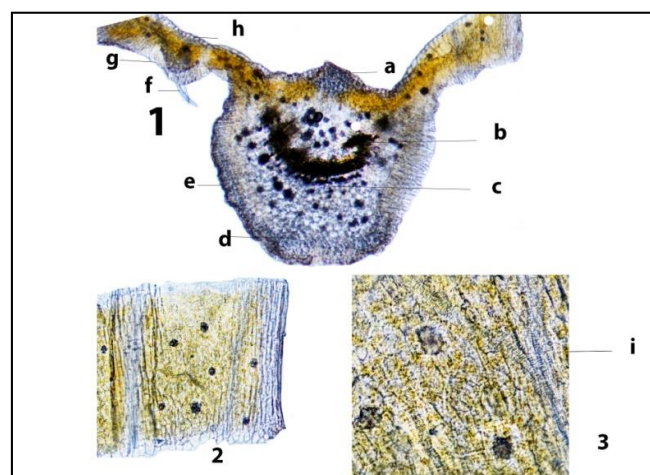


Fig 4: Cross section of the mildrib (1), collenchima –a,d, xylem-b, phloem-c, upper epidermis-h, lower epidermis-g, trichoms-f, stellate druses of calcium oxalate – (2,3) fragments of the vascular system - 3i.

Barks anatomy

Under the multilayered peridermis primary cortex is characterized by the presents of extremely large dilatations with crystal druses in their cells. This feature is of diagnostic value, because according to Schweingruber F *et al.* [10] the dilatations in the bark of *Euonymus verrucosus* Scop. differed. The wings at the corners of the stem are covered with cork. Primary cortex under this area is without dilatations. Sieve tubes of phloem angular, accompanied by round to oval parenchyma cells. Fibers and sclereids absent, it is one more diagnostic feature, compared to *Euonymus verrucosus* Scop., where they are present [10].

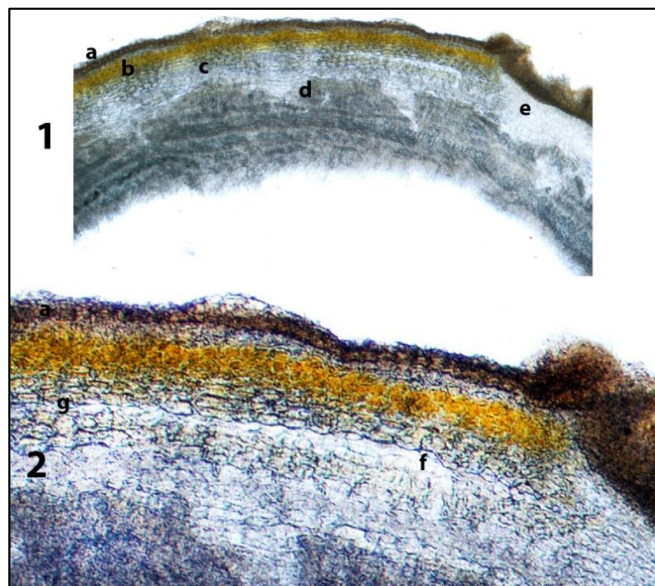


Fig 5: Cross section of *Euonymus europaeus* L. bark (1,2), cork (a), periderm (b), cortex parenchyma (c), phloem (d), dilatations, crystal druses in dilatations (g,f), cortex under the wings(e)

Seeds microscopy

Seeds are covered with the bright orange aril. The results of our studies of seeds anatomy are consistent with those in the paper of Vandyshev *et al.* [11] According to this data aril is two-layer, the layers are separated by a cuticle with orange fat inclusions of different sizes.

Cells of endosperm are oval, it is white. Endosperm cells and cotyledons also contain different sizes of fatty inclusions.

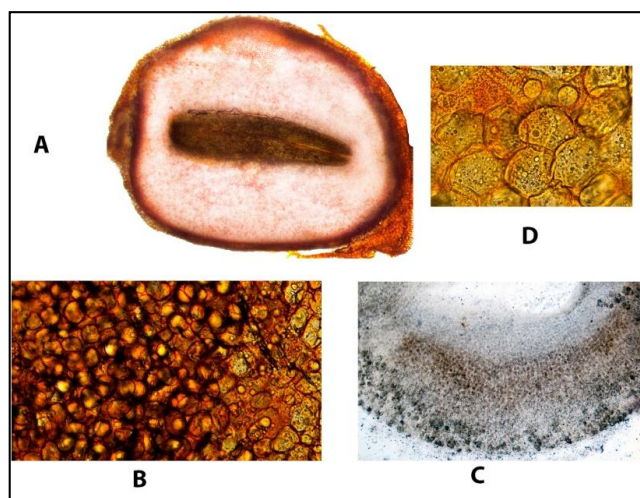


Fig 6: Cross-section of the seed (A), cells of the aril (B), endosperm without aril (C), aril cells with aleurone grains (D)

Conclusion

The present study established macroscopical and microscopical diagnostic features of leaves, bark and seeds of *Euonymus europaeus* L. for the correct identification of plant material.

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