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RESEARCH ARTICLE

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## MICROMORPHOLOGICAL ANALYSIS OF *ORIGANUM VULGARE* L. LEAVES CULTIVATED IN TWO DIFFERENT LOCATION IN ALBANIA

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### ABSTRACT

The article provides data on the micromorphological evaluation of sp. *O. vulgare* L. subsp *hirtum*, known as White Oregano cultivated in Durrës and Konispol, Albania. The study of the micromorphological parameters of the leaves of *O. vulgare* L. subsp *hirtum* includes the characterization of epidermis (stomata and trichomes). The study of epidermal structures was performed with the techniques of stereomicroscopy and optical microscopy. The material in the study was prepared by the following main methods: a) fresh leaves for stereomicroscope observation, b) nail polish method and c) epidermal excision techniques. During the preliminary study of the epidermal structures it was found, the presence of stomata on both leaf surfaces which classifies the leaves of *O. vulgare* subsp *hirtum* as amphistomatic. It is observed an unequal distribution of stomata. The data obtained classify the stomata as diacytic. Trichomes were classified as non-glandular and glandular of two types, capitate and peltate.

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## INTRODUCTION

*Origanum vulgare* L. belongs to the genus *Origanum* L. with 43 species, 6 subspecies and 18 hybrids included in the family Lamiaceae. Species of this genus are widespread in Eurasia and North Africa (Ietswaart, 1980). The eco-geographical characteristics of Albania, especially the soil and the climate, present favorable conditions for the spontaneous growth of oregano in some areas of the country, especially in the Lauretum-Kastanetum plant association (Xhuveli & Lipe, 1996). In our country *O. vulgare* L. known as common oregano grows in various environments, in forests and bushes up to rocks and grassy places. It is known as a medicinal and aromatic plant; used as a spice, in the treatment of cough, asthma, bronchitis, in stomach diseases and as appetite stimulant (Demiri, 1983). There are two subspecies, known in Albania *O. vulgare* L. ssp. *vulgare* and *O. vulgare* L. ssp. *hirtum*, respectively "red oregano" and "white oregano". White oregano is common in southern areas, not found in eastern or northern areas. It is densely located on the rocky heights of Saranda-Konispol, Vrina-Vlora.

With lower density is encountered in the areas of Durrës-Krujë, Tirana-Kavaja, etc. (Kongjika et al., 2005). White oregano is an aromatic perennial herbaceous plant and grows in areas with warm climates. It grows in hilly areas with an altitude of 800-900m above sea level. This species is found in sandy-limestone or clayey-sandy loam, stony soils, in dry places (Kongjika et al., 2005). In the Red Book (Vangjeli et al., 1995), this species is described with the status as an endangered species. Since the subspecies of *O. vulgare* L. ssp *hirtum* and *O. vulgare* L. ssp *viridis* are difficult to distinguish morphologically (De Martino et al., 2009), they have been the subject of discussion among botanists. By Rakaj et al., (2013). in the catalog of new reports, this species is defined as *Origanum vulgare* L. subsp. *hirtum* (Ietswaart, 1980; Meyer, 2011). Comparative anatomical, histo-cytological studies have been used by different authors to distinguish oregano subspecies. Some anatomical features described in these studies highlight the leaves as amphistomatic and stomata as diacytic, anomocytic and anisocytic. The stomata located on both surfaces are almost at the same level of the epidermis, so they are of the mesomorphic type. The anticlinal walls of epidermal cells on both surfaces are wavy. (Temel & Tokur, 2014; Dinç & Doğu, 2013). The pharmaceutical, economic, value of oregano is related to the production of essential oils, which is also carried out in the secretory

glands, which are found both in the vegetative air organs and in some reproductive organs. For *Origanum* species and oregano populations (*Origanum vulgare* L.), the presence of peltate trichomes in the leaves and their ontogeny has been described by (Bosabalidi, 1983). The study of the type of trichomes in white oregano in our country in two habitats (Llogara-Gjirokastra) has been realized by defining three types of trichomes (non-glandular, peltate glandular and capitate glandular) and the change of their number in different stages of development of plants (Vegetation, flowering and fruiting) have been studied by Zekaj (2005). Studies on the content of the amount of essential oils have been carried out for some localizations of Albania for the species *Origanum vulgare* L. ssp. *vulgare* and ssp. *hirtum*. It has been found that oregano populations in the southern parts of Albania have a higher content of essential oils compared to those of the northern areas. (Elezi et al., 2013; Gixhari et al., 2014).

*Origanum vulgare* L. has been cultivated in some limited areas in Albania. Recent years due to high export demand of sp. *Origanum vulgare* L. are increased the cultivated areas of this species. In this context, the micromorphological evaluation of habitats with cultivated *Origanum vulgare* L. was seen as important. The study of white oregano cultivated in Durrës and Konispol, at the cytological level, will serve also for the study and detection of secondary metabolites at the histochemical level.

## MATERIALS AND METHODS

The plants of *Origanum vulgare* L. ssp. *hirtum* used for this study are cultivated in Durrës (41.332278 ° N, 19.451007 ° E), and Konispol (39.6590233 ° N, 20.1820494 ° E) in Albania. Plants planted and grown in these two places were taken in the vegetative phase and studied in the Cytology-Cytogenetics laboratory of the Department of Biotechnology at the Faculty of Natural Science, Tirana, Albania.

For the analysis of epidermal structures we have analyzed five leaves from five plants for each area. The plants are studied during their vegetative phase. In this study we are based on microscopic observation of the samples with stereomicroscope and light optical microscope.

### Micromorphological study with stereomicroscope

It was performed by preparing live preparations for study by stereomicroscope.

### Micromorphological study with optical light microscope

It was realized through the processing of leaves samples by means of three techniques:

- Technique with detachment of epidermis from both leaf surfaces.
- The technique used is the simple technique of detaching the lower and upper epidermis of the leaf. The leaves (third or fourth from above) of the studied plants are left for 60-90 minutes in running water. The epidermis (upper and lower) is then detached. These preparations were stained with iodine (wagner), then observed under an Olympus microscope and photographed with a C200 digital camera and several times from the side tube with a camera from a Samsung mobile phone (Raole, & Desai 2009).
- Nail polish technique.
- This technique is based on observing the traces left by the leaves on a substance such as nail polish. On one leaf apply nail polish in an area where there is no leaf vein as it would hinder the process. Leave to dry for a few moments and apply an adhesive to the area where the nail polish was painted. Carefully pull the glue and we will take the trace of the leaf surface. The trace

adhesive is then placed on the blade to be observed under an optical microscope (Fig. 1). (Brewer, 1992).

- By means of fixation technique, enzymatic hydrolysis, staining with orcein and cutting or pressing the leaves in 1/3 of their middle (Zekaj, 2005).

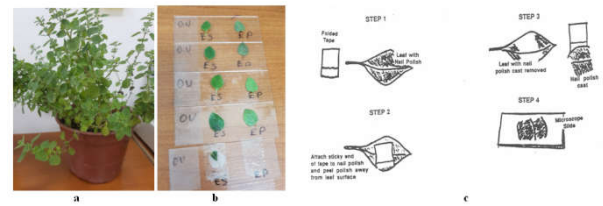


Fig. 1. *Origanum vulgare* L. subsp. *hirtum*. Cultivated plant in vase (a) , nail polish technique (b,c)

- Microscopic observation of the stomata, trichomes and epidermal cells was performed with the Olympus optical microscope with 10x and 40x magnification objective. The photos were taken with the Paralux Camera. In some cases, Samsung phone photography from the side tube has been done.

## RESULTS AND DISCUSSION

**Macromorphology of *O. vulgare* L. subsp. *hirtum* cultivated in Durrës and Konispol:** Stereomicroscopic examination of samples prepared with live leaf material of both the upper epidermis (adaxial leaf surface) and the lower epidermis (abaxial leaf surface) of the leaves of *O. vulgare* L subsp. *hirtum* of both areas, demonstrates the presence of non-glandular trichomes, and glandular trichomes of peltate type. The number of these peltate trichomes in the upper epidermis is greater than in the lower epidermis (Fig. 2), which is also encountered in the estimates of (Werker et al., 1985). Their number ranges from an average of 8 per mm<sup>2</sup> in the upper epidermis, to no more than 6 per mm<sup>2</sup> in the lower epidermis for *O. vulgare* L. subsp. *hirtum* of the Konispol area.

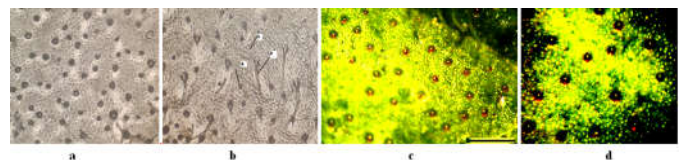


Fig. 2. *O. vulgare* L subsp. *hirtum*. Durrës: Upper epidermis (adaxial) (a,b). *O. vulgare* L subsp. *hirtum* Konispol: Upper epidermis (adaxial) (c), and lower epidermis (d)

**Micromorphology of *O. vulgare* L. subsp. *hirtum* cultivated in Durrës and Konispol:** The micromorphological study of the samples prepared from the leaf samples of both areas performed with the Olympus microscope gives us details at the cellular level for both surfaces of the leaf epidermis of both varieties of *O. vulgare* L. studied. The epidermal structures studied include: analysis of epidermal cells and epidermal structures (stomata and trichomes).

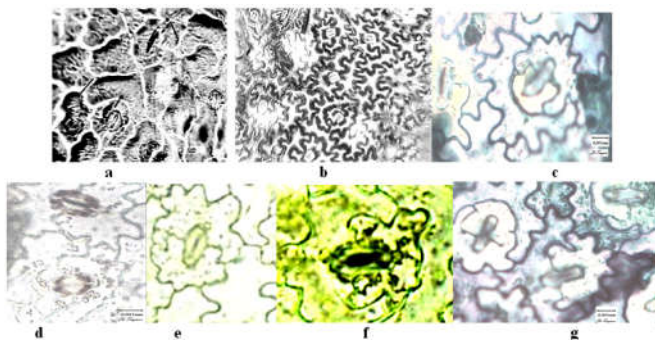
**Epidermal cell:** The leaf epidermis in *O. vulgare* L. both on the upper and lower side of the leaf consists of wavy epidermal cells, relatively thick walls, containing a mucilaginous layer Fig.3 a, b.

**Stomata:** Stomata are present in both epidermis which characterizes the leaves of *O. vulgare* L subsp. *hirtum* as amphistomatic the same as the definitions of the authors above (Temel & Tokur, 2014; Dinç & Doğu, 2013). These stomata are mostly of diacetic-type, which is characterized by the presence of two epidermal cells located perpendicular to the guard cell of the stoma. Rarely, anisocytic-type stomata surrounded by four cells of different dimensions also appear.



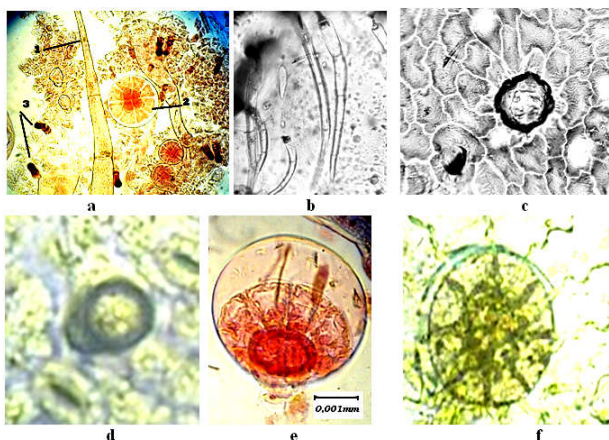
The frequency of this last type is very rare (Fig. 3). However, these data are consistent with the data of Dinç & Doğu, (2013). Somata of the anomocytic type have not been encountered which contradicts the fact brought by Dinç & Doğu, (2013). A phenomenon not uncommonly observed in preparations of *O. vulgare* L subsp. *hirtum* of the Konispol area is also the observation of stomatal clusters as in Figs. 3.g. It is thought that these structures when presented at high frequency are a significant parameter for the assessment of environmental pollution. In the case of our observations they do not occur frequently.

**Trichome type:** We observed three types of trichomes on the leaf surfaces of *O. vulgare* L subsp. *hirtum* both in Durrës and Konispol areas. Non-glandular trichomes, peltate glandular trichomes and capitate glandular trichomes. Non-glandular trichomes appear in filiform shape which appear as unicellular at first, but later appear composed of several cells (4-5) as in Fig. 4.



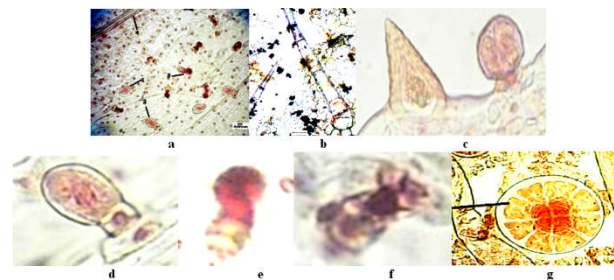
**Fig. 3. *O. vulgare* L subsp. *hirtum*. Durrës: epidermal cell, stomata on upper epidermis (a) and lower epidermis (b). Konispol: Stoma lower epidermis (c,d), upper epidermis (e,f), stomatal cluster (g). Olympus. Photo Paralux**

**Peltate trichomes;** Peltate trichomes consist of a basal cell a stalk cell and a secretory head with four-eight, randomly ten peripheral cells according to the stage of development as in Fig. 4. Fig. 5.e shows a peltate trichome in lateral view with the two-cell stalk and funnel-shaped head. Fig. 4.f and Fig. 5.g shows from above the peltate trichome head structure which is composed of four cells in the center and eight cells in the periphery. Distribution of peltate trichomes occurs more frequently in the upper epidermis compared to the lower epidermis, which is demonstrated both in stereomicroscope observations (Fig. 2) and in microscope observations (Fig. 4a).



**Figura 4. *O. vulgare* L subsp. *hirtum*. Durrës: Upper epidermis, 3 type of trichomes (non-glandular -1, peltate glandular -2 and capitate glandular -3) (a), non-glandular trichomes (b), peltate trichomes (c). Konispol: capitate trichomes (d), peltate (e, f). Olympus light microscope, magnification 40X**

**Capitate trichomes:** Trichomes that are composed of one basal cell, one stalk cell and a single-celled head are classified as capitate glandular trichomes I. (Fig. 5.d,e).



**Fig. 5. *O. vulgare* subsp. *hirtum* Konispol: three type of trichomes (a), non-glandular trichomes with three-cells (b), non-glandular one-cell type trichome and glandular trichome(c). capitate glandular trichome type I (d,e), capitate type II (f) and peltate (g).**

Trichomes with one basal cell, up to three stalk cells and one pear-shaped head cell are classified as capitate glandular trichomes II. (Fig. 5.f). Our observations are consistent with the study data of Werker et al., (1985). The presence of capitate and peltate trichomes is more common on the surface of the leaf epidermis that is not permeated by the leaf nerves.

## CONCLUSION

From the analysis of the leaf samples of two ecotype of *O. vulgare* L. subsp. *hirtum* we can conclude that:

There are no significant variations of oregano grown in these two areas of Albania. The study of micromorphology gave us the opportunity that for the first time in *O. vulgare* L. subsp. *hirtum* to determine histo-cytological parameters for the stomata and three types of trichomes. There is a difference between the adaxial and abaxial leaf surfaces in the number of peltate trichomes which are more frequent in the upper epidermis. The stomata are diacytic type and the leaves are amphistomatic. In both ecotypes stomata are significantly more frequent on the lower leaf surface and extremely rare on the upper. Knowledge of these leaf epidermis structures creates a basis for deeper study of these ecotypes in terms of detecting secondary metabolites to recognize and variations in the chemotypes of *Origanum vulgare* L.

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