ejpmr, 2018,5(2), 580-586

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CONTRIBUTION TO MICROSCOPIC STUDY OF *LAURUS* NOBILIS L. WIDE SPREAD IN SYRIA

Mohammad Said Chmit[1], Hussein Moussa Kanaan[3*] and Isam Hasan Agha[2]

[1]Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Damascus University, Damascus, Syria.
[2]Department of Pharmacognosy and Medicinal Plants, Faculty of Pharmacy, Syrian Private University, Damascus countryside, Syria.
[3]Laboratory of Chemical Synthesis and Extraction of Polysaccharides from Seaweed, Faculty of Pharmacy, Lebanese University.

***Corresponding Author: Prof. Hussein Moussa Kanaan**

Laboratory of Chemical Synthesis and Extraction of Polysaccharides from Seaweed, Faculty of Pharmacy, Lebanese University.

Article Received on 20/12/2017 **Article Revised on 10/01/2018** **Article Accepted on 31/01/2018**

ABSTRACT

The microscopic study is not less important than the Phytochemical study, and it is considered as the first step in the diagnostically determination of the spices studies. Microscopic Sections of *Laurus nobilis* leaves and fruits were done by using paraffin molds, and a hand Microtome in order to conducting a light microscopical study to determine the histological tissue of this plant parts. Also, light microscopic study was done on the crushed leaves and fruits of *Laurus nobilis* in order to determine the specific diagnostic elements of this plant parts. The study revealed the existence of microscopic diagnostic elements characteristic of *Laurus* leaves, such as the oil cells, the pore shape, and the elongated epidermis cells, while the fruits powder are characterized by the stone cells which formed the endocarp.

KEYWORDS: *Laurus nobilis*, Microscopic section, Microscopic of *Laurus.*

INTRODUCTION

Laurus is an aromatic, evergreen (Vardapetyan et al. 2013) and perennial (Zeković et al. 2009), shrub or small tree (Nurba et al. 2005), usually growing up to height of from 3 to 15 m high (9 to 50 feet) at a slow rate (Moghtader et al. 2013). It belongs to Lauraceae family, which comprises numerous aromatic and medicinal plants (Hogg et al. 1974). The genus Laurus is consists of the two species *Laurus azorica* and *Laurus nobilis* (Basak et al. 2013). *Laurus nobilis* is a very important medicinal plant and there is a comprehensive monograph about this plant in many Herbal Pharmacopoeia (Ghannadi et al. 2002), and it has a long history of folk use in the treatment of many ailments (Moghtader et al. 2013). Laurus Leaves appear in January (Moghtader et al. 2013), alternate, they are 5–12 cm long and 1.8–5 cm wide, oblong, leathery, on both surfaces, lateral veins 10–12 pairs, margin slightly undulate, apex acute or acuminate, with a dark green color (Peter et al. 2010). The leaves do not fall during winter (Basak et al. 2013), they have a strong pungent odor because they contain essential oil up to 3%. The leaves can be used either dried or fresh but the flavour is stronger in dried and grinded leaves, but the leaves which are stored longer than a year they will lose their flavour (Bown., 2001). Laurus fruits: are small and olive-like, fleshy, dark purple when mature, ovoid, and it contains essential oil up to 1% (Baytop., 2000).

Laurus nobilis is distributed in western Syria from the northwest to the southwest. There are also individual numbers scattered here and there in the middle region near the coast. Kesab is a town in Syria famous of Laurel Soap manufacture, and it is characterized by its extended forests on Mount Al-Aqraa, such as Fouroloq forests. Kesab is 800 meters above sea level, and overlooking at the Mediterranean Sea. Laurus samples were harvested from the Al-Sajra area with coordinates (35°55′30″N 35°59′19″E). Slinfah is a Syrian city with a height of 1130 meters above sea level and it is famous with Oak forests (Quercus) and has many natural plant growing. Laurus samples were harvested from an area with coordinates (35°36′1″N 36°10′43″E). The ridge of the mount Nabi Yunis is one of the highest top of Middle Forest Mountains in Syria. It is rich with forests, and many nature reserves, such as "AL-Shouh wa AL-Arz" (Abies cilicica and Cedrus libani), which allocated since 22 July 1996 for the scientific research. Laurus samples were harvested from an area with coordinates (35°38′29″N 36°13′0.9″E).

The need of the medicinal plants to use in treatment and food industries is increasing, due to increasing of population and to spread new types of diseases, also due to the resistance caused by pathogens such as bacteria which become stubborn against some types of antibiotics. The increasing demand for plants caused to

wanton harvesting of plants, which led to decrease the enumeration of these plants, and the spread of commercial cheat, and this led to a sharp decline in the population and natural spread, which led to the spread of fraud in the trade of these herbs, which called for the need to develop diagnostic rules microscopic and analytical chemical to ensure the purity of these samples and free of fraud. The Normal microscopic examine is the first and the most important step in these investigations, for example, the large spherical aromatic cells with the paracytic type stomata are the most microscopic diagnostic elements of the leaves of Lauraceae (Metcalfe et al. 1950). Also, Chromatography techniques are the most important to determine the purity of the essential and fatty oil, which are extracted from Laurus leaves and fruits.

Given the scarcity of the microscopic studies on Laurus in Syria, which has not been reported to date, the aim of the current study was to form comprehensive diagnostic methods for Laurus leaves and fruits.

MATERIALS AND METHODS

Plant samples

The samples of Laurus leaves and fruits were collected from three different Areas in Syria, the first from Kesab which located at 800 m above the sea level with the Coordinates (35°55′30″N 35°59′19″E), the second from Slinfah which located at 1130 m above the sea level with the Coordinates (35°36′1″N 36°10′43″E), and the last one from Mount Nabi Yunis which located at 1562 m above the sea level with the Coordinates (35°38′29″N 36°13′0.9″E).

The Samples were air-dried at room temperature in the shade for some weeks. They had a final moisture content of 10.0 %. Before using them, the dried samples were grinded in a blender, at the end of the milling process, the particle sizes were in the range of 0.1–0.4 mm, in order to use as a powder for the microscopic study.

On the other hand, fresh samples of Laurus leaves were stored separately by putting drying papers between each it, and then keep it in the refrigerator at 8°C. The samples were started to used it for preparation of microscopical sections within one or two weeks.

The same processes were done for Laurus fruits samples.

Preparation of Microscopical Sections

The newly harvested plants parts need to pass through multiple stages to be ready as clear microscopic sections. Thus, the microscopic sections have undergone the following stages:

1- **Fixation of plant parts:** Is a process that is intended to stabilize living cells and prevent them from destruction. Plant parts (leaves and fruits) were placed in CRAF III solution 24h (30 ml Chromic acid 1%, 20 ml Acetic acid 10%, 100 ml Formaldehyde, and 40 ml water) in order to be stable.
2- **Dehydration:** This process is carried out by increasing concentrations of Ethanol as prelude to molding them in paraffin molds. At first the plant parts are washed with water to remove traces of the fixing solution, then washed with Ethanol 60% for 24 hours and then with Ethanol 90% for one hour, at end with absolute Ethanol twice time, to be ensure that plant parts have become completely free of water.
3- **Pouring of Paraffin:** The plant parts are placed in three consecutive baths of hot pure paraffin. the first bath last for five hours, and the second for 24 hours and the third for 7 hours, then the paraffin will be poured in molds contain the plant parts.
4- **Sectioning the Paraffin molds:** hand Microtome was used model (Microtec, Cut 4050), and the thickness of the section was equal to 8 micrometers. The sections are received on clean glass plates loaded with gelatin water (0.5 g of gelatin in 100 ml distilled water), in order to stick the plant sections to the glass plate. The glass plates are dried at 40 ° C for three days.
5- **Eclaircissement of plant sections:** the glass plates are immersed in 3 baths (xylol for 15 minutes, absolute alcohol and xylol 1:1 for 5 minutes, and absolute alcohol with Formaldehyde 8:2 for 5 minutes), the plates are then lifted and placed in hot water until the water from the plates becomes colorless.
6- **Staining of plant sections:** double staining method was carried on by using picro-carmine and iodine-green.

RESULTS AND DISCUSSION

The Microscopic Study of the Section of Laurus leaves

The study of transverse sections of Laurus leaves at the central nerve area (Midrib) showed the following microscopic layers (fig. 1).

1- **Upper Cuticle:** Thick with green color.
2- **Superior epidermis:** Consists of a single row of compacted cellulose cells, and free of Chloroplast.
3- **Collenchyma:** a type of angular collagen.
4- **Sclerenchyma:** is located under the Collenchyma, and is composed of cells located along the primary vascular tissue.
5- **Xylem:** consists of primary Xylem which formed of a few small vascular cells, and secondary Xylem which formed up of wooden vascular of various diameter that tends to be larger from the top to the bottom in a radial form, and separated by sclerenchyma cells.
6- **Phloem:** composed of a secondary Phloem which consisting of regular shaped cellulose cells, follows by the primary Phloem which consisting of number of irregularly shaped cellulose cells.
7- **Sclerenchyma:** appears in an arc shape, follows the primary Phloem. Its cells consist of woody fibers

with thick walls, so that sometimes nothing remains from the cell only a small and narrow gap.

8- **Collenchyma:** consists of a group of circular cells with thin walls tends to be thicker as they approach to the lower epidermis.

9- **Inferior epidermis:** consists of a single layer of cellulose cells.

10- **Lower Cuticle:** Thick with green color.

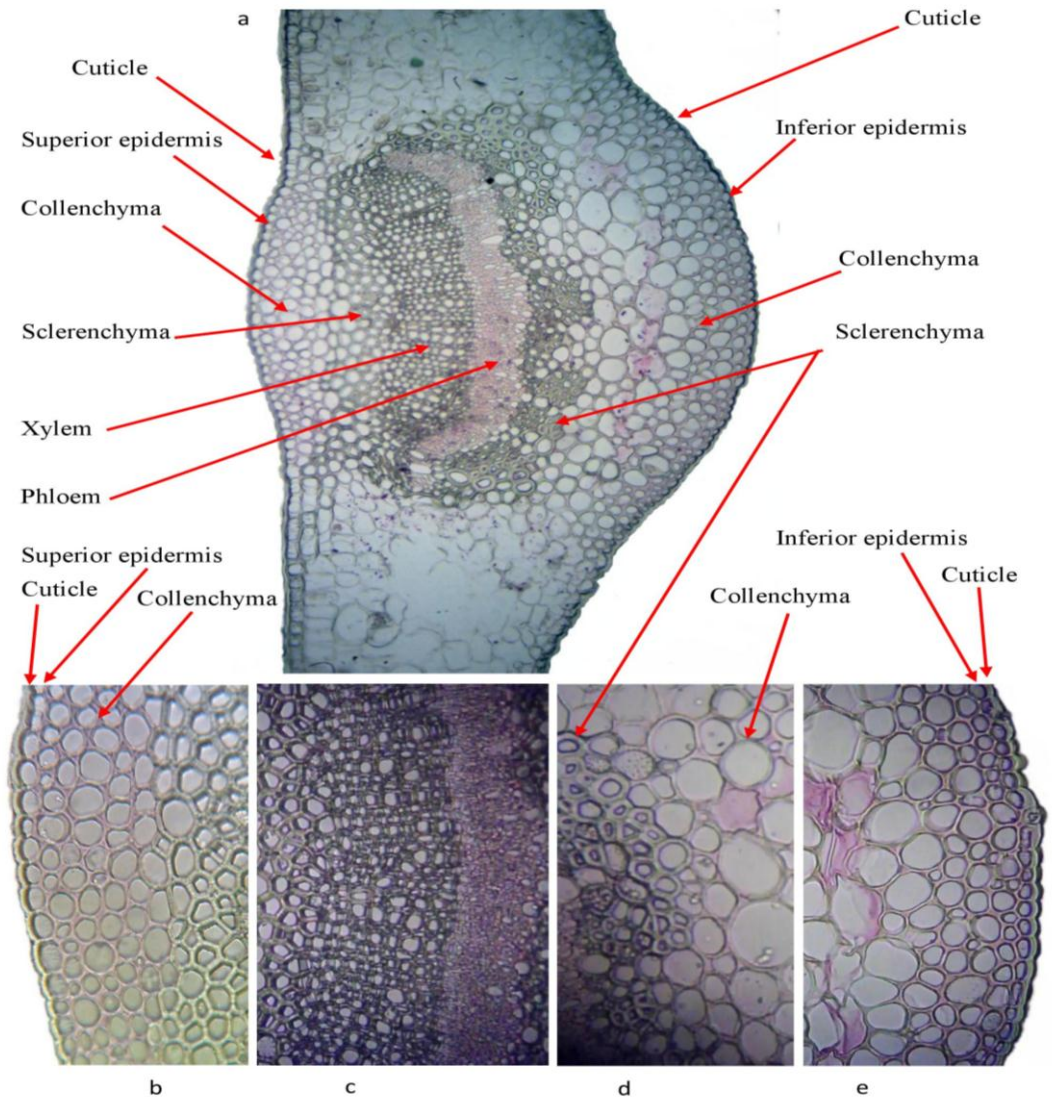


Fig. 1: The Microscopic study of the transverse sections of Laurus leaves at the central nerve area (a; X10), (b+c+d+e; X40).

The study of transverse sections of Laurus leaves at the lamina area (blade) showed the following microscopic layers (fig. 2).

1- **Cuticle:** Thick with green color.

2- **Superior epidermis:** Consists of a single row of compacted cellulose cells, and free of Chloroplast.

3- **Parenchyma:** ground tissue of non-woody structures and located between the upper and lower Epidermis, and divided into two parts: **Palisade Parenchyma** which located under the upper Cuticles, and consists of rectangular cells rich with Chloroplast. **Spongy parenchyma** located above the lower Cuticles, consists of irregular cells with large interspaces between them, and poor in Chloroplast.

4- **Oil cell:** Large thin-walled circular cells contain droplets of essential oil and spread with large numbers in the lamina.

5- **Vessels:** located between Palisade and Spongy parenchyma.

6- **Inferior Epidermis:** consists of a single layer of compacted cells, interspersed with many partially sunken stomata, which is free of Chloroplast, and is covered with a thick layer called Cuticle.

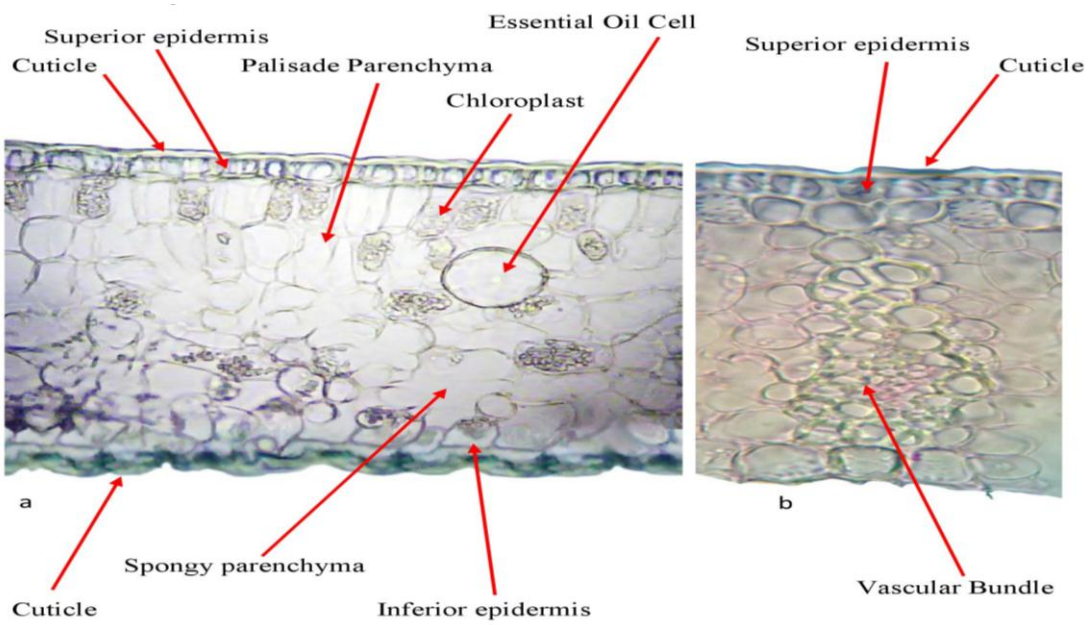


Fig. 2: a; The Microscopic study of the transverse sections of Laurus leaves at the lamina area (blade), b; The Microscopic study of the transverse sections of Laurus leaves at the lamina area (Vascular Bundle).

The Microscopic Study of Laurus leaves Powder

The study of Laurus leaf Powder showed the following diagnostic elements (Fig. 3):

1- The Superior Epidermis in a surface view.
2- Cuticle with the Superior Epidermis in a lateral view, and under them appears the Palisade Parenchyma and the Oil Cells.
3- Oil cells.
4- Spotted sclereide due to holes and oblique crevices.
5- Inferior Epidermis (upper view), consist of elongated cells, and showed the paracytic type stomata.

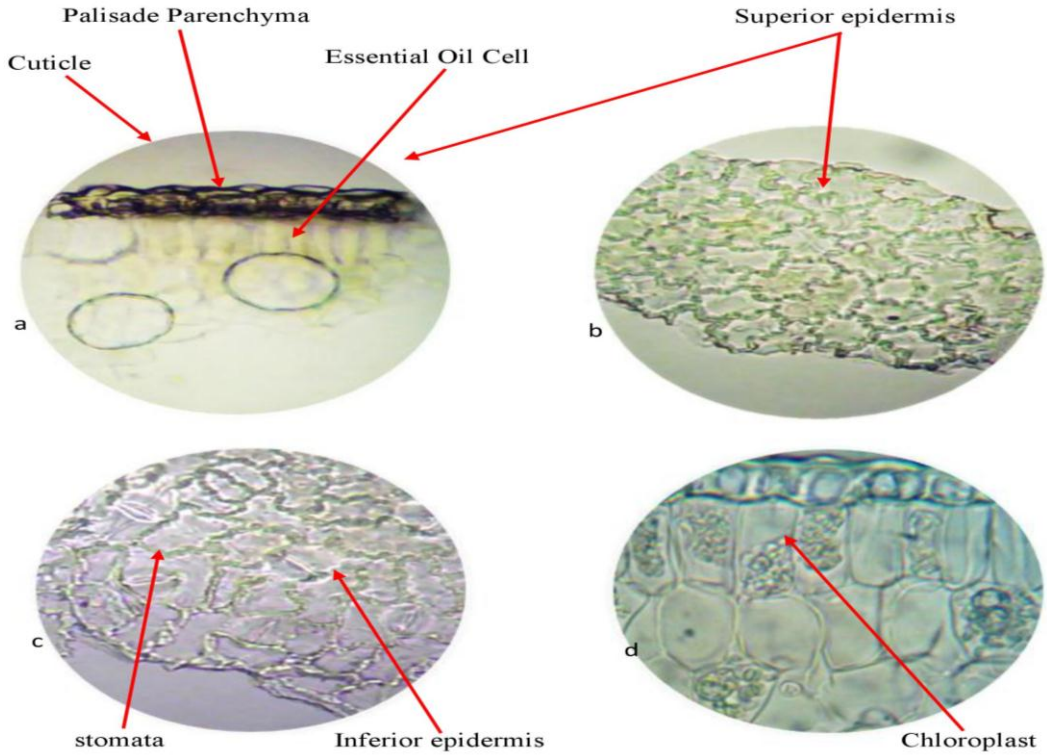


Fig. 3: a; The Microscopic study of Laurus leaves Powder, a; Cuticle with the Superior Epidermis in a lateral view, and under them appears the Palisade Parenchyma and the Oil Cells, b; The Superior Epidermis in a surface view, c; Inferior Epidermis (upper view) and paracytic type stomata, d; the rectangular cells of Palisade Parenchyma rich with Chloroplast.

The Microscopic Study of the Section of Laurus fruits
The study of transverse sections of Laurus fruits at the central level showed the following microscopic layers (fig. 4).

1- **Epicarpe:** consists of two layers of cells, and containing anthocyanic dyes give it the brownish blue color.
2- **Mesocarpe:** the middle coating of the fruit, contains the aromatic cells, and forms the thickest layer of Laurus fruit.
3- **Endocarpe:** consisting of one layer of stone cells.
4- **Tegument:** consisting of several layers of endosperm tissue which contain starch and fatty oil.

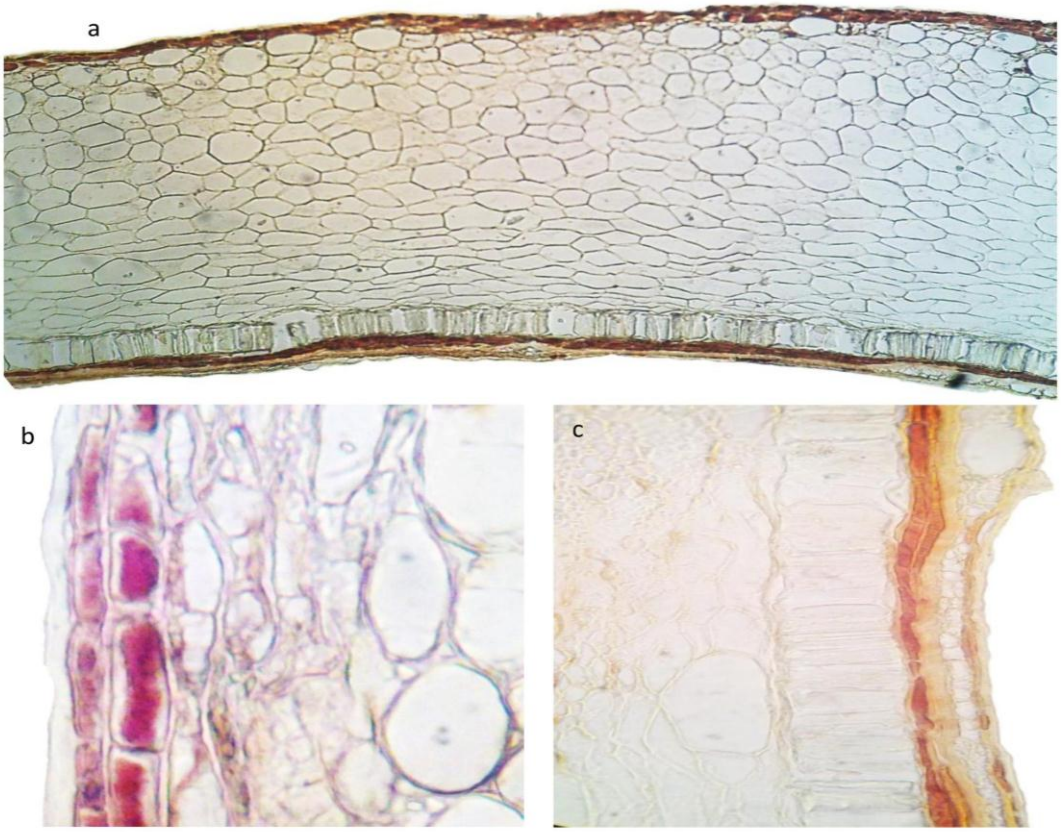
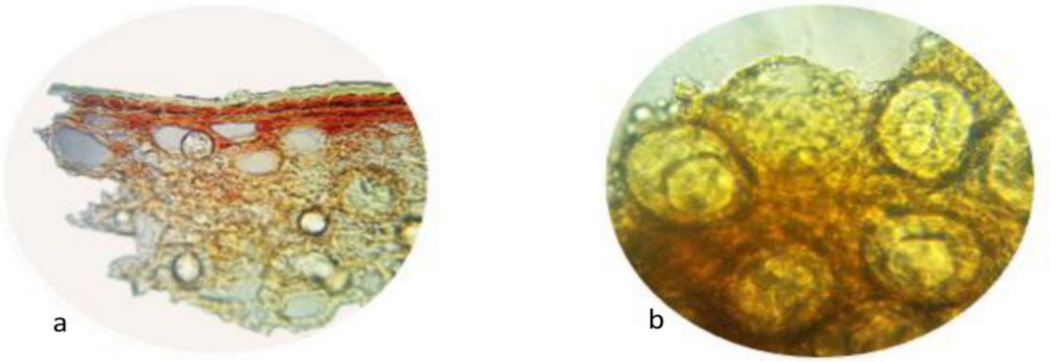


Fig. 4: The Microscopic Study of the Section of Laurus fruits. a; Complete Section of Laurus fruits (X10). b; Cortex, two layers of epicarpe cells, many layers of mesocarpe cells with aromatic cells between them. c; many layers of mesocarpe cells with aromatic cells between them, one layer of endocarpe (stone cells), the seed cortex.

The Microscopic Study of Laurus fruits Powder
In the powder of Laurus fruits can be found the following diagnostic elements (fig. 5):

1- Lateral view showed the **cortex**, two layers of **epicarpic** cells, and **mesocarpic** cells with many **volatile oil cells**.
2- **Stone cells** in surface view.
3- **Stone cells** in lateral view.
4- Parts of **endosperm** tissue which contain **starch** and **fatty oil**.



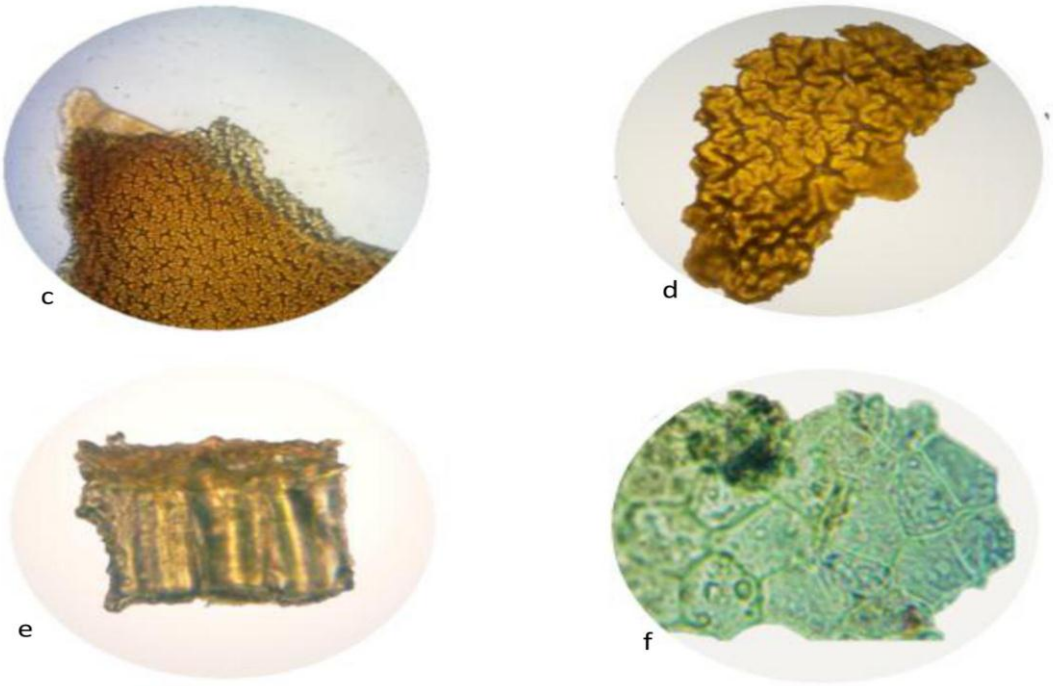


Fig. 5: The Microscopic Study of the powder of Laurus fruits. a; lateral view showed the cortex, two layers of epicarpic cells, and mesocarpic cells with many volatile oil cells. b; many volatile oil cells. c; Stone cells in surface view (X10). d; Stone cells in surface view (X40). e; Stone cells in lateral view. f; Parts of endosperm tissue which contain starch and fatty oil.

CONCLUSION AND DISCUSSION

There are no significant diagnostic differences between the three studied samples of the Syrian Laurus, and this corresponds to the field study which conducted by Mouterde P. who confirmed the existence of one species of Laurus in Syria which is *Laurus nobilis* (Mouterde P., 1978). The most important characteristics of the transverse section or the powder of Laurus leaves are: the existence of many large aromatic cells, which have a thin wall and inside it drops of essential oil. Also, the paracytic type stomata, which is the species that distinguishes all plants related to the family Lauraceae (Metcalfe et al. 1950). The upper and lower epidermis are made up of a single layer of chloroplast-free cells and externally coated with a thick cuticle, but the lower epidermis is characterized by the fact that its cells are longer and contain stomata. Chloroplasts are abundant in the Palisade Parenchyma but their numbers are decreasing in the Spongy parenchyma. The cells of Palisade Parenchyma are regular and located under the upper epidermis, while the cells of the Spongy parenchyma are irregular and located above the lower epidermis.

The most important characteristics of the transverse section or the powder of Laurus fruits are that Epicarpe, which consists of two layers of melanocytes cells, and that the Endocarpe consisting of one layer of stone cells. Also, the volatile oil cells are placed within the cells of the Mesocarpe, which is the most thicken layer between the three layers. The stone cells with a surface view are very distinctive, and they are similar to brain gyri.

REFERENCES

1. Basak Serap Sahin and Ferda Candan, Effect of *Laurus* nobilis L. Essential Oil and its Main Components on α-glucosidase and Reactive Oxygen Species Scavenging Activity, Iranian Journal of Pharmaceutical Research, 2013; 12(2): 367-379.
2. Baytop T. Theraphy with Medicinal Plants in Turkey (Past and Present), Nobel Tıp Press, İstanbul, 2000; 13-31.
3. Bown D., Encyclopaedia of Herbs and their Uses, 2001; 448.
4. Ghannadi A. Lauri Folium. In: "Iranian Herbal Pharmacopoeia". Tehran: Publications of Iranian Ministry of Health, 2002.
5. Hogg, J.W., S.J. Terhune and B.M. Lawrenc: Dehydro-1.8-cineole: A new monoterpene oxide in *Laurus* noblis oil. Phytochem, 1974; 13: 868-869.
6. Metcalfe, C.R. and Chalk L., Anatomy of dicotyledons, 1950, Vol. I. Clarendon Press, Oxford.
7. Moghtader. M and A. Farahmand. Evaluation of the antibacterial effects of essential oil from the leaves of *Laurus* nobilis L. in Kerman Province. Journal of Microbiology and Antimicrobials, 5(2): 13-17, February 2013 Available online http://www.academicjournals.org/JMA DOI:10.5897/JMA2012.0233 ISSN 2141-2308 ©2013 Academic Journals.
8. Mouterde Paul., Nouvelle flore du Liban et de La Syrie., Edition de l'Impr, Catholique, 1978., 272145806X, 9782721458063.
9. Nurba Macid. [Nourbakhsh], Yeliz Bal. Recovery of fixed and volatile oils from *Laurus* nobilis L. Fruit

and Leaves by solvent extraction method. Eski.ehir Osmangazi Universitesi Müh. Mim. Fak. Dergisi C. XVIII, S.2, 2005, Eng. & Arch. Fac. Eski. ehir Osmangazi University, Vol. XVIII, No: 2, 2005.

10. Peter H. Raven, yue gui., Flora of China, *Laurus* nobilis, ISBN 7030043391, 9787030043399., 2010.
11. Vardapetyan. H, Tiratsuyan. S, Hovhannisyan. A, Rukhkyan. M, Hovhannisyan. D., Phytochemical Composition and Biological Activity of *Laurus* nobilis L. Leaves Collected from Two Regions of South Caucasus. Journal of Experimental Biology and Agricultural Sciences, May - 2013; 1(2).
12. Zekovid. Zoran P, Žika D. Lepojevid1, Ibrahim O. Mujid. Laurel Extracts Obtained by Steam Distillation, Supercritical Fluid and Solvent Extraction. Journal of Natural Products, 2009; 2: 104-109.