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BOTANICAL PROPERTIES OF *SPINACIA OLERACEA* COLLECTED IN HUE, VIETNAM

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Background: Increased consumption of fresh vegetables that are high in polyphenols has been associated with a reduced risk of oxidative stress-induced disease. *Spinacia oleracea* is a good source of the bioflavonoid quercetin with many other flavonoids which exhibit anti-oxidant activity. With the purpose of creating a database to precisely identify this species to avoid confusion in the process of collecting samples of medicinal plants as well as to gradually build indicators as a basis for later research, we studied the characteristics of the leaves of *Spinacia oleracea* including: Microsurgery of leaf, powder characteristics of leaf, in order to standardized herbal *Spinacia oleracea*. **Materials and Methods:** Leaves of *Spinacia oleracea*. Samples were collected in Hue city in March of 2016. **Results:** Powder characteristics of leaf: fiber bundles, starch, colored pieces, pieces of vascular, pieces of epidermis carrying stomata. **Microsurgery of leaf:** Section of leaves nerve has protruding upper and lower nerve. From outside to inside: epidermis, collenchyma cells, parenchyma cells. Next is phloem and xylem. The center of leaves nerve is usually empty. Leaves blade is characterized by palisade mesophyll cells and spongy mesophyll cells. **Conclusion:** These findings contribute to the testing and identifying standards of herbal *Spinacia oleracea*, in order to standardize this herbal.

Keywords: *Spinacia oleracea*, microsurgery, powder characteristics.

1. INTRODUCTION

Overproduction of oxidants (reactive oxygen species and reactive nitrogen species) in human body is responsible for the level of pathogenesis of some diseases [9]. The scavenging of these oxidants is thought to be an effective measure to depress the... of oxidative stress of organisms. Overwhelming evidence indicates that diets rich in fruits and vegetables are protective against common chronic diseases, such as cancer, obesity and cardiovascular diseases. Leafy green vegetables, in particular, are recognized as having substantial health-promoting activities that are attributed to the functional properties of their nutrients and non-essential chemical compounds. Spinach (*Spinacia oleracea* L.) is a leafy green vegetable that came originally from south-western Asia and is now grown in most parts of the world. Spinach is widely regarded as a functional food due to its diversified nutritional composition, which includes vitamins such as vitamin C, vitamin A and vitamin E and minerals like magnesium, manganese, iron, calcium and folic acid. Spinach is also rich in carotenoids beta-carotene and lutein. It

is a good source of the bioflavonoid quercetin with many other flavonoids which exhibit anti-oxidant activity [7]. Spinach-derived phytochemicals are able to (i) scavenge reactive oxygen species and prevent macromolecular oxidative damage, (ii) modulate expression and activity of genes involved in metabolism, proliferation, inflammation, and antioxidant defence, and (iii) curb food intake by inducing secretion of satiety hormones [8]. These biological activities contribute to the anti-cancer, anti-obesity, hypoglycemic, and hypolipidemic properties of spinach.

Currently in Vietnam the study of Spinach is still very limited. With the purpose of creating a database to precisely identify this species to avoid confusion in the process of collecting samples of medicinal plants as well as to gradually build indicators as a basis for later research, we studied the characteristics of the leaves of Spinach.

2. MATERIALS AND METHODS

2.1. Materials

Spinacia oleracea collected in Hue on March, 2016

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Figure 1. *Spinacia oleracea*

2.2. Methods

2.2.1. Method for determining the scientific name for herbal research [6]

- Sampling all of necessary parts such as stems, leaves, flowers and fruit. Making dry specimen. Recording all information of the species studied, such as morphological description, color of flowers, fruits, distribution and habitat, condition of the plants in the wild; size, growth of natural development. Photographing habitat, environment plants, branches bearing flowers/fruit; Imaging used parts.

- Description of plant characteristics, comparative morphology, with reference to the key morphological characteristics of plant classification, compared with the specimens stored in the herbarium - Department of Botany- Hanoi university of Pharmacy in conjunction with the support of experts on plants for the scientific name.

2.2.2. Method for identifying plant characteristics [1], [2], [5]

2.2.2.1. Powder characteristics of leaf

After collecting, medicinal herbs are washed, dried, pulverized to fine powder. Making leaf powder. Take a picture and describe the characteristics of the powder under a microscope.

2.2.2.2. Microsurgery of leaf

-Cutting, bleaching, dual staining, using electron microscope, microscope photography to observe, photograph and describe the characteristics of microsurgery.

- Description of dual staining method for temporary template:

+ Fresh or dried medicinal herbs are softened, cut with a razor or with microsurgical instruments, choose thin slices.

+ Soak the slices in water Javen or chloramine solution to remove substances contained in the cells, soaking time depends on the type of material, time can be shortened by heating.

+ Wash slices with water: if it has more starch that needs bleaching, then after washing, soaking or boiling in chloral hydrate water solution and wash again.

+ Wash slices with acetic acid.

+ Wash slices with water.

+ Dye slices with a solution of methylene blue, depending on the time course of dye staining of the object (usually about 10 seconds to 1 minute).

+ Wash slices with water.

+ Dye slices with red vermilion alum or carmine, dyeing time depending on the object (probably from about 1 minute to 1 hour).

+ Wash slices with water.

+ Place the slices on a drop of glycerin on glass slides, cover glass leaves, shed on the microscope.

3. RESULTS AND DISCUSSION

3.1. Botanical descriptions

It is an annual plant (rarely biennial) growing from 30 to 60 cm tall. Stem is round, smooth, piped, succulent, sometimes reddish. The leaves are alternate, simple, ovate to triangular, and very variable in size about 2–30 cm long and 1–15 cm width, with larger leaves at the base of the plant and smaller leaves higher on the flowering stem, smooth at both sides. The flowers are yellow-green, 3–4 mm in diameter.

3.2. Identify the scientific name

Based on identification key and comparison with the published data, the scientific name was identified as *Spinacia oleracea*, a member of the

family Amaranthaceae.

3.3. Microscopic characteristics

3.3.1. Microscopic characteristics of the leaf powder

Dark green powder. Observing under a microscope (40×objective) found: epidermis bring

stomata (1) (4), fragment of vessel reticulate (2), fibers arranged in bundles (3) or stand alone (5): Long narrow cells, thin-walled, color very pale yellow, fragment of vessel spiral and scalariform (8), colored blocks (6), the starch granules (7), Collenchyma cell (9).

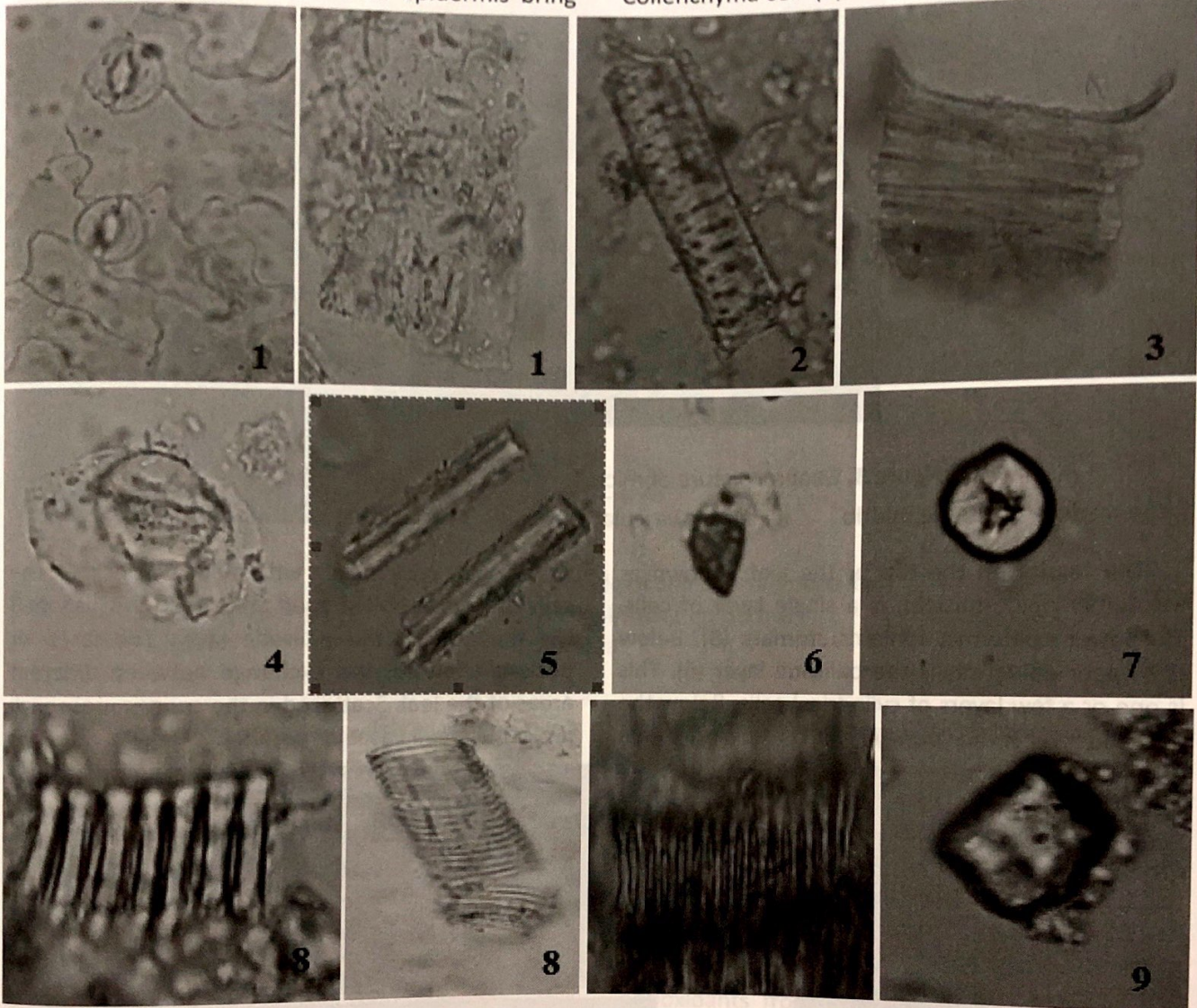


Figure 2. Powder microscopic details characteristics of leaf

- | | | |
|--|-------------------|---|
| 1. fragment of epidermis bring stomata | 4. Stomata | 7. the starch grains |
| 2. fragment of vessel reticulate | 5. fiber | 8. fragment of vessel (spiral, scalariform) |
| 3. Fiber bundles | 6. colored blocks | 9. Collenchyma cell |

3.3.1. Microsurgery characteristics of leaf

Leaf of *Spinacia oleracea* consists of two parts: the blade (the lamina) and the midrib.

The midrib: Midrib upper and lower surfaces are convex. The epidermis surrounds the leaf and is therefore visible on the abaxial (lower) and adaxial (upper) side of the leaf. The epidermis consists

of a rectangular cell layer (1). Upper and lower collenchyma cells (2) are thick tissues at the corner. Upper collenchyma has 3-4 layers of cells, lower collenchyma has 5-6 layers of cells. They are polygonal cells, whose size is not uniform, are arranged clutter forming discontinuous ring. Parenchyma (3) consists of thin-walled cells, polygons, irregular size. Vascular

system has 3-5 vascular bundles. The center of the leaf is empty. The structure of vascular bundles: The xylem (5) is in the center of the vascular bundles

with the phloem distributing on the abaxial side of the xylem. The phloem (4) is composed of compact, thin-walled sieve tissue.

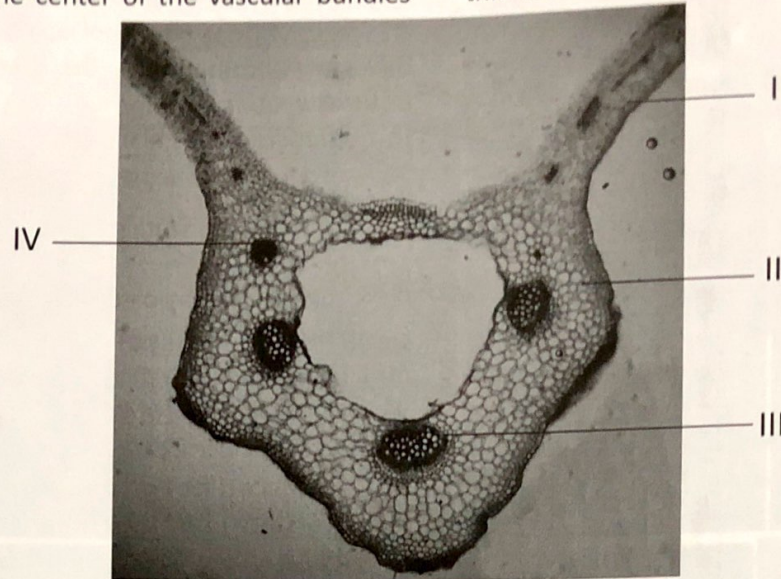


Figure 3. General picture of microsurgey leaf of *Spinacia olerace*

I: the blade II: the midrib III: the big vascular bundles IV: the small vascular bundles

The blade: on the top of the leaf, known as the upper epidermis (1), is a single layer of cells. The lower epidermis contains stomata (8). Below the upper epidermis is the palisade layer (6). This is one or a few layers of cylindrical cells. Below the palisade layer is the spongy layer (7). This is an area

with loosely packed cells with many air pockets. The cells are more ball-shaped than cylindrical-shaped like the cells in the palisade layer. The large air pockets allow for gas exchange between different areas of the leaf. Scattered in the spongy mesophyll are some small vascular bundles.

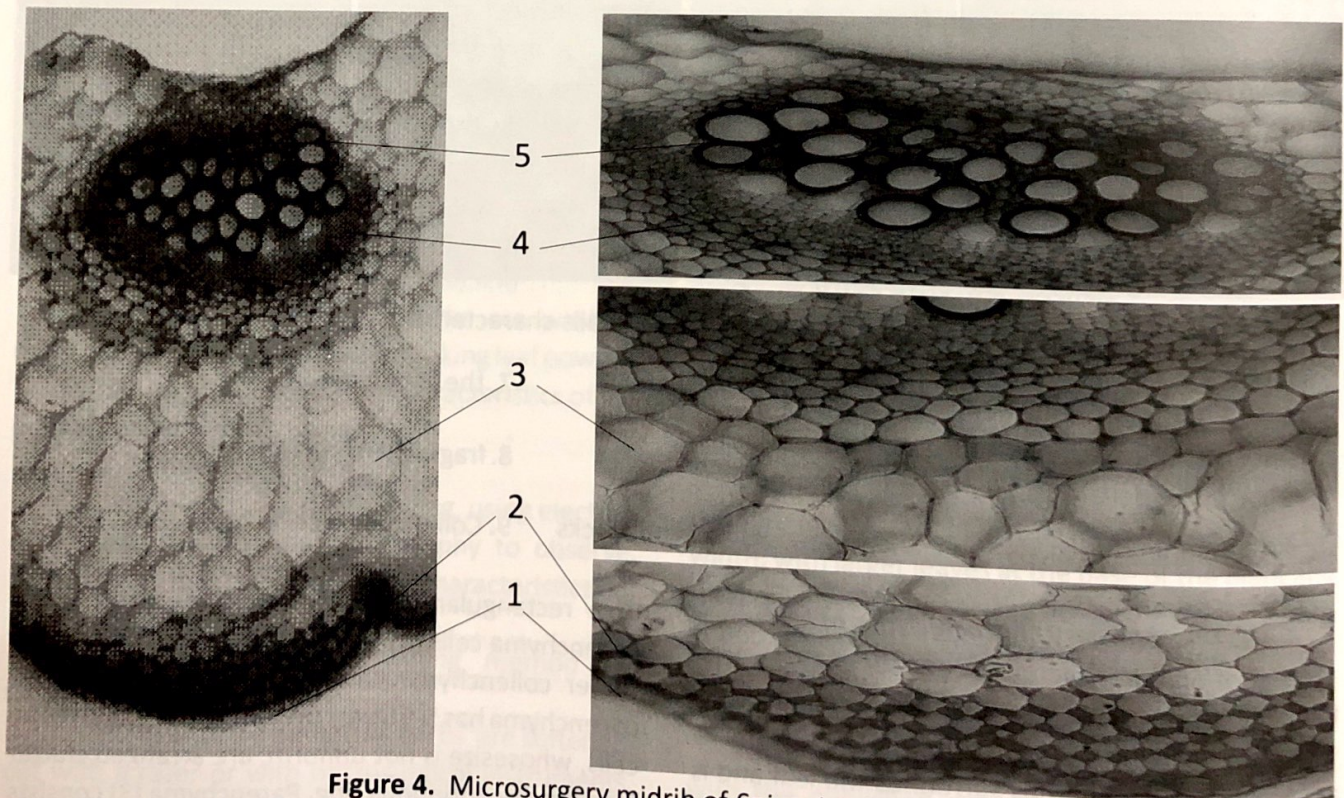


Figure 4. Microsurgey midrib of *Spinacia olerace* leaf

1: epidermis cell 2: collenchyma cells 3: parenchyma cells 4: phloem 5: xylem

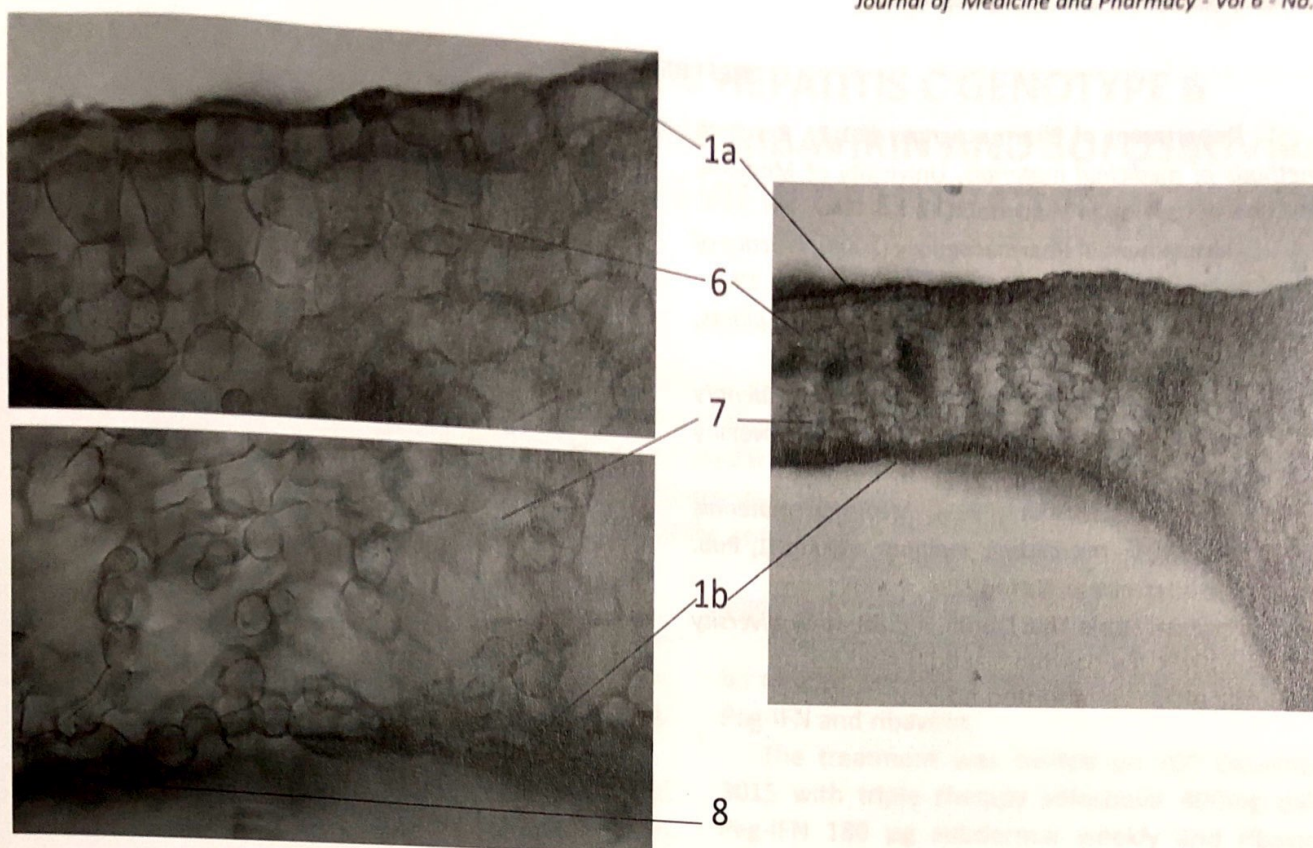


Figure 5. Microsurgery blade of *Spinacia oleracea* leaf

1a. Adaxial/upper epidermis

1b. Abaxial/lower epidermis

6. Palisade mesophyll

7. Spongy mesophyll

8. Stomata

The research sample was identified the scientific name of *Spinacia oleracea*, belonging to Amaranthaceae.

This study used botanical analysis and morphological anatomy methods which are classical methods, commonly used in the classification of plants.

Spinacia oleracea was the only species in the genus *Spinacia* [10], so far no study has yet been published on microscopical characteristics. This study shows that *Spinacia oleracea* species has some outstanding characteristics: the midrib has many vascular bundles, the blade has some small vascular bundles, collenchyma cells are concentrated in the lower epidermis. The powder of *Spinacia oleracea* leaf has many fragments of vessels and fibers. They stand alone or gather into bundles.

The morphological characteristics of the sample are similar to the published documents on plant characteristics of Spinach [3]. Also in this study, we have built a preliminary data on anatomical characteristics of leaves and microscopic characteristics of the powder of *Spinach*. Currently, there is no monograph in Vietnam Pharmacopoeia IV about *Spinacia oleracea*.

We first study the morphological and microscopical characteristics of *Spinacia oleracea*, contributing to provide preliminary data for the construction of Vietnam Pharmacopoeia monograph in the future.

Currently, there is few research on the chemical composition and biological effects of this species in Vietnam, with known biological effects, *Spinach* is a potential research subjects in order to create antioxidants from nature. To be the object of a study, correct identification of medicinal plants is an important work in the collection, testing, and pharmaceutical research. Determining the correct scientific name of the plant is vital in the initial research and the development of drugs derived from plants [4].

4. CONCLUSION

The analytical phytotomy of leaves was described in detail. This is the first report on the phytotomy of the plant *Spinacia oleracea*. The results provided the morphological and microscopical characteristics for identification and standardization of *Spinacia oleracea*.

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