Open Access Journal
International Journal of Medical Research and Pharmaceutical Sciences
Volume 4 (Issue 6): June 2017 ISSN: 2394-9414
DOI- 10.5281/zenodo.810782 Impact Factor- 3.109

# COMPARATIVE MORPHOLOGICAL AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF PLANTAGO MEDIA L. LEAVES WITH P. MAJOR L. AND P. LANCEOLATA L. LEAVES

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***Keywords:*** *Plantago major* L., *Plantago lanceolata* L., *Plantago media* L., *Morphological study, Qualitative phytochemical analysis.*

***Abstract***

*Plantago major* L. and *Plantago lanceolata* L. are medicinal plants with remarkable variety of curative properties: expectorant, diuretic, antispasmodic, antibacterial, antioxidant, antiinflammatory and immunomodulatory. *Plantago media* L. is a meagerly studied herbaceous plant from *Plantago* genus. This paper presents a comparative morphological and qualitative phytochemical investigation of *P. media* L. leaves with *P. major* L. and *P. lanceolata* L. leaves in order to ensure their proper identification and avoid adulteration. The qualitative phytochemical analysis revealed that *P. media* leaves, similarly to *P. major* and *P. lanceolata,* contain important groups of bioactive constituents as carbohydrates, flavonoids, tannins and alkaloids. These results define *P. media* leaves as a promising natural source of biologically active compounds and would be beneficial in future pharmacological studies. The obtained data could serve as a reference material in the preparation of herbal monograph for *Plantago media* L. leaves.

## Introduction

*Plantago* genus belongs to *Plantaginaceae* family and it is represented by perennial annual weeds widespread in temperate climate areas.[1,2] *Plantago major* L., *Plantago lanceolata* L. and *Plantago media* L. are the most commonly used *Plantago* species in Bulgaria. They have been applied by local people as a cure for many diseases including inflammations of respiratory tract, digestive problems and skin irritations.[1-4] *Plantago major* and *Plantago lanceolata* leaves are described in standard documents as European Pharmacopoeia, World Health Organization Monographs and European Medicines Agency.[5-7] They have been known as a rich source of bioactive compounds like flavonoids, hydroxicinnamic acids, terpenoids, polysaccharides, unsaturated fatty acids and vitamins (vitamin C, vitamin K, β-carotenes).[1-6] *Plantago media* leaves are meagerly studied both in terms of their pharmacognostic characteristics and their pharmacological activities.

Our study presents morphological and qualitative phytochemical evaluation of *P. media* leaves and their comparison with *P. major* and *P. lanceolata* leaves. Such investigation would be beneficial in order to ensure the proper identification of the studied *Plantago* species and may serve as a basis for further biological examinations.

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## Materials and methods

### Collection of plant material

*Plantago major* L. and *Plantago lanceolata* L. leaves were collected in June 2015 from Thracian valley floristic region (Bulgaria), located in range of 160 and 270 meters above sea level. *Plantago media* L. leaves were collected from an altitude of 1250-1300 meters above sea level from Rhodope Mountains floristic region (Bulgaria) in June 2015. The plants were taxonomically identified up to species, subspecies and forma level according to Tutin *et al*.[8] and Delipavlov *et al*. [9]

### Morphological study

Morphological and organoleptic parameters such as taste, odor, shape, size and color of the fresh leaves were studied according to the procedures described in the World Health Organization (WHO) guidelines on quality control methods for medicinal plant material.[10]

### Microscopic histochemical analysis

Thin transverse sections of *P. major*, *P. lanceolata* and *P. media* leaves were stained with 10 % aqueous solution of Chinese ink[11] and observed under light microscope (Leica DM 2000 LED, Leica Microsystems, Germany). Photomicrographs were taken using digital camera Leica DMC 2900 and processed using imaging software Leica Application Suite.

### Extraction process

Aqueous, ethanol, acetone and chlorophorm extracts were obtained using cold maceration technique according to WHO guidelines.[10] Four grams of powdered dried material were macerated with 100 ml of each solvent. The first 6 hours the macerated plant material was shaken frequently, then allowed to stand for 18 hours and filtered.

### Qualitative phytochemical analysis

The obtained aqueous, ethanol, acetone and chlorophorm extracts of the investigated *Plantago* species were subjected to qualitative phytochemical test analyses using standard methods.[12-14]

***1. Tests for carbohydrates***

*1.1. Molish's test*. Few drops of alcoholic α-naphthol solution were added to 3 ml of each test extract. In the presence of carbohydrates, when a concentrated sulfuric acid is added to the mixture, a violet ring at the junction of two liquids is observed.

*1.2. Fehling's test.* One milliliter of Fehling's A and Fehling's B solutions were mixed and boiled for one minute. To obtained mixture was added 1 ml extract and boiled for 10 min in water bath. Brick red precipitates of cuprous oxide indicate the presence of reducing sugars.

***2. Test for phenolic compounds***

*2.1. Ferric chloride test*. To the extracts were added few drops of neutral ferric chloride solution. Change of color in dark green or blue shows the presence of phenolic compounds.

***3. Tests for flavonoids***

*3.1. Shinoda's test.* Few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings were added to 1 ml of each extract. Change of color in pink-red indicates the presence of flavonoids.

*3.2. Lead acetate solution test*. Few drops of 10% aqueous lead acetate solution were added to 1 ml of each extract. Reddish brown precipitates indicate the presence of flavonoids.

*3.3. Alkaline reagent test.* To 1 ml of each extract were added 4-5 drops of 5% sodium hydroxide solution, followed by addition of 2 ml 10% hydrochloric acid. A yellow solution that turns colorless upon addition of hydrochloric acid shows the presence of flavonoids.

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***4. Tests for tannins***

*4.1. Gelatin test*. To 1 ml extract were added few drops of 1% aqueous solution of gelatin. In the presence of tannins white buff-colored precipitates are observed.

***5. Tests for alkaloids***

*5.1. Wagner's test.* One milliliter of Wagner's reagent (iodine in potassium iodide solution) was mixed to 1 ml of each extract. Reddish brown precipitates show the presence of alkaloids.

***6. Tests for proteins and amino acids***

*6.1. Biuret test*. To 3 ml of each extract were added few drops 4% sodium hydroxide solution and 1% copper sulfate solution. Change of color in violet or pink indicates the presence of proteins.

*6.2. Xanthoprotein test*. One milliliter concentrated sulfuric acid was added to 3 ml extract. Formation of white precipitates shows the presence of proteins.

## Results and discussion

### Morphological study

The leaves of *P. major*, *P. media* and *P. lanceolata* [Figure 1], their subspecies and forms occurring in Bulgaria, were observed for morphological and organoleptic characteristics. The noted traits were presented in Table 1. The main distinctive features for macroscopic identification of plantain leaves were the shape and trichomes of lamina. *P. major* had the widest, broadly elliptic to ovate lamina, which was glabrous or sparsely pubescent. *P. media* had ovate-elliptic to lanceolate-elliptic lamina, with abundant or sparsely scattered curly trichomes. *P. lanceolata* was with the narrowest, lanceolate-elliptic to linear-lanceolate lamina, with or without trichomes.

***Table 1. Morphological and organoleptic characterisation of P. major, P. media and P. lanceolata leaves.***

| Morphological traits | *Plantago major* | | | *Plantago media* | | *Plantago lanceolata* | |
|---|---|---|---|---|---|---|---|
| | *P. major ssp. major* | *P. major ssp. intermedia* | | *P. media ssp. media* | *P. media ssp. urvilleana* | *P. lanceolata ssp. lanceolata* | *P. lanceolata ssp. eriophylla* |
| | | *f. intermedia* | *f. minor* | | | | |
| Leaf length (cm) | (5) 8 - 40 | 4 - 15 | 1 - 4 | (2) 5 - 16 (30) | | 2 - 30 | |
| Leaf width (cm) | (3) 5 - 10 (15) | 2 - 5 (10) | 0.5 - 3 | (1.5) 2.5 - 8 | | 0.5 - 3(4) | |
| Leaf shape | broadly elliptic to ovate heart-shaped at the base | narrowing at the base into a channeled petiole | | ovate to elliptic | lanceolate-elliptic | lanceolate to lanceolate-elliptic or linear-lanceolate | |
| Leaf margin | irregularly toothed | | | irregularly toothed | | toothed, undulate | |
| Trichomes of lamina | absent | sparsely scattered | | curly, abundant or sparsely scattered | | absent | abundant |
| Venation | parallel, 3 - 9 prominent veins | | | parallel, 7 - 9 veins | parallel, 5 veins | parallel, 5 - 7 veins | parallel, 3 - 5 veins |
| Petiole length | equalling or slightly longer/rarely shorter than lamina | | | 3 - 6 x shorter than lamina | 1.5 - 3 x shorter than lamina | 1/3 or equaling the length of lamina | |
| Taste | slightly bitter | | | salty to slightly bitter | | slightly salty to bitter | |
| Color | green to brownish-green | | | light-green to dark-green | | yellowish-green to brownish-green | |
| Odor | slight, unspecific | | | slight, unspecific | | unspecific, similar to hay | |

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*Figure 1. Plantago lanceolata L., Plantago media L., Plantago major L. leaves.*

**Histochemical analysis**

The histochemical staining with Chinese ink is a specific qualitative test for identification of mucilage in plant tissues.[11] The Chinese ink cannot penetrate in the mucilage tissue and therefore the mucilage shows up as transparent, spherically dilated fragments on a black background. The histochemical analysis indicated that the mucilage was localized mostly in the lying beneath the leaf epidermis collenchyma and around the vascular bundles sclerenchyma tissue. The cell walls of parenchyma sheath contained significantly lower amounts of mucilage [Figure 2].

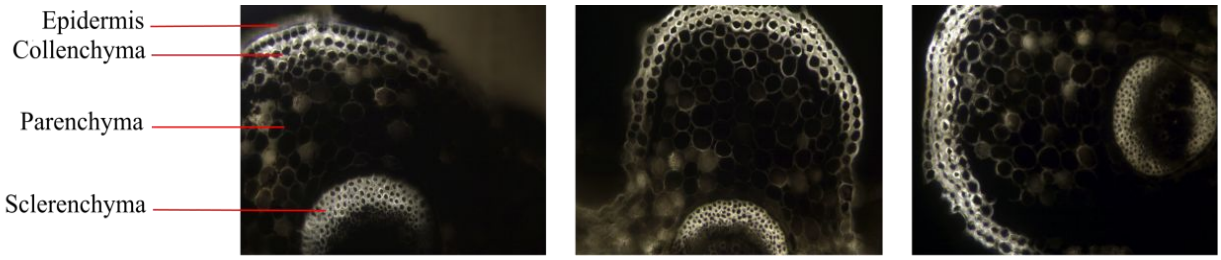


*Figure 2. Transverse sections of Plantago major L., Plantago lanceolata L. and Plantago media L. leaf vein after staining with Chinese ink.*

**Qualitative phytochemical analysis**

Qualitative phytochemical analysis was carried out in order to investigate the active constituents of the plant material. The results from tests of major phytoconstituents of aqueous, ethanol, acetone and chlorophorm extracts of *P. major*, *P. lanceolata* and *P. media* leaves were presented in Table 2.

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The aqueous plant extracts of the three investigated *Plantago* species showed the presence of carbohydrates and phenolic compounds, including flavonoids and tannins. *P. media* and *P. lanceolata* aqueous extracts were the richest source of carbohydrates. The ethanol and acetone extracts resulted to have the highest amount of phenolic compounds, among which flavonoid content was significantly higher compared to the content of tannins. Although alkaloid content in all extracts was low, chlorophorm as an organic solvent had extracted highest amount of alkaloids. These results supposed that alkaloids in *Plantago* leaves existed primarily in the form of basis. The conducted tests for protein and amino acids resulted negative for all extracts. The negative results may be due to the lower content of proteins in *Plantago* leaves or their linkage with sugars in form of glycoproteins.

***Table 2. Qualitative phytochemical analysis of P. major, P. media and P. lanceolata leaves.***

| Group of chemical compounds | Test | Extragents | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Water | | | Ethanol | | | Acetone | | | Chlorophorm | | |
| | | *P.maj* | *P.med* | *P.lanc* | *P.maj* | *P.med* | *P.lanc* | *P.maj* | *P.med* | *P.lanc* | *P.maj* | *P.med* | *P.lanc* |
| Carbohydrates | Molish's test | ++ | +++ | +++ | ++ | ++ | ++ | + | ++ | + | + | ++ | ++ |
| | Fehling's test | + | +++ | +++ | ++ | ++ | ++ | - | + | + | - | +++ | +++ |
| Phenols | $FeCl_3$ test | + | + | + | +++ | +++ | +++ | ++ | ++ | ++ | + | + | + |
| Flavonoids | Shinoda's test | ++ | + | + | ++ | ++ | ++ | ++ | + | + | + | + | + |
| | Lead acetate test | + | + | + | +++ | ++ | ++ | ++ | ++ | ++ | + | + | + |
| | Alkaline reagent | ++ | + | + | + | + | + | +++ | +++ | +++ | + | + | + |
| Tannins | Gelatine test | - | - | - | + | + | + | + | + | + | - | - | - |
| Alkaloids | Wagner's test | - | - | - | + | + | + | + | + | + | ++ | ++ | ++ |
| Proteins | Biuret's test | - | - | - | - | - | - | - | - | - | - | - | - |
| | Xantho-protein test | - | - | - | - | - | - | - | - | - | - | - | - |

- No presence; + Low concentration; ++ Moderate concentration; +++ High concentration.

## Conclusion

The presented morphological standards can be considered as identifying parameters to authenticate the studied *Plantago* species. The conducted qualitative phytochemical analysis revealed that *P. media* leaves, similarly to the well-known medicinal plants *P. major* and *P. lanceolata,* contain important groups of bioactive constituents as carbohydrates, flavonoids, tannins and minor amounts of alkaloids. These results define *P. media* leaves as a promising natural source of biologically active compounds with potential applications in pharmaceutical and food industries. The obtained data from the present study could also serve as a reference material in the preparation of herbal monograph for *Plantago media* L. leaves.

## Acknowledgements

The authors are grateful to the Medical University – Plovdiv for the financial support through University Project SDP – 03/2015.

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DOI- 10.5281/zenodo.810782 Impact Factor- 3.109

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