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Research Paper

Pharmacognosy and Phytochemical Screening of *Plantago Major* L. and *Plantago lenceolata* L. from Rawalakot, Azad Jammu and Kashmir

Safeena Tariq and Ansar Mehmood*

Department of Botany, University of the Poonch Rawalakot-12350, Azad Jammu and Kashmir.
Corresponding author's email: ansar.mehmood321@gmail.com

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Abstract

The evaluation of the quality and purity of crude drugs by means of various parameters is the most important aspect of pharmacognosy. Regardless of the modern techniques, identification of plant drugs by pharmacognostic study is more reliable. *Plantago* is one of the important genera of medicinal plants, having many traditional uses. The present study was aimed at establishing the pharmacognosy and phytochemical studies of *P. major* and *P. lanceolata*. Pharmacognosy includes macroscopic, microscopic, and physiochemical analysis. Both the plants were herbs, with an average height of 34.00 ± 7.30 and 44.80 ± 1.56 cm, respectively. Leaves were amphistomatic and stomata were anisocytic and diacytic. Moisture content was 78.43 ± 0.06 and 47.00 ± 0.06 % in *P. major* and *P. lanceolata* respectively. Total ash was 0.38 ± 0.01 and 0.33 ± 0.03 mg/g respectively. Water-soluble ash, acid insoluble ash, water-soluble extractives, chloroform-soluble extractives, ethanol soluble extractives, and petroleum soluble extractives were also determined. Higher water-soluble extractives were observed in *P. major* (42.00 ± 0.03 %) followed by *P. lanceolata* (37.00 ± 0.02 %). Other extractives were less in quantity. Phytochemical analysis showed the presence of alkaloids, flavonoids, phenols, saponins, and carbohydrates. Total phenolic contents were 0.95 ± 0.20 and 0.44 ± 0.12 mg/g in *P. major* and *P. lanceolata* respectively. Total flavonoid contents in *P. major* and *P. laneolata* were 0.69 ± 0.05 and 0.80 ± 0.15 mg/g respectively. This study can serve as an appreciated source of information and provide proper standards for the identification of *P. major* and *P. lanceolata* in future surveys and submissions.

Introduction

Medicinal plants have a time-honored history in many native societies and continue to provide useful treatments for handling various ailments (Kagithoju *et al*., 2013). The practices of old-fashioned medicine are based on hundreds of years of certainty and interpretations, which preceded the expansion and extent of modern medicine (Kadam *et al.*, 2012). In the early twentieth century, herbal remedies were as common in healthcare as antibiotics or painkillers. With the arrival of the allopathic system of treatment, herbal medicine has slightly misplaced its reputation amongst people, which is established on the firm satisfying activities of synthetic pills (Singh *et al*., 2007). It is not

surprising that one-fourth of the population, i.e., 1.42 billion people, are reliant on old-style medicines for the treatment of various disorders (Khan *et al.,* 2013). The demand for drugs derived from plants is increasing day-by-day, unluckily creating a massive burden on some nominated plants, which have high medicinal importance (Kagithoju *et al*., 2013). Herbal drugs or standardized extracts need analytical techniques to confirm the identity, quality, and purity, as well as the safety and efficiency of a plant. Herbal products cannot be considered scientifically valid if the drug tested has not been authenticated and characterized in order to ensure reproducibility in the manufacturing of products (Swapnil *et al*., 2013).

Pharmacognosy is the systematic study of the structural, physical, chemical, and sensory functions of medications. As late as the start of the 20th century, the theme had been established mainly on the botanical side, being concerned with antiquity, sympathy, assemblage, planning, and packing of botanic remedies (Divya, 2015). So, pharmacognosy can also be defined more broadly as the body of knowledge needed to understand all aspects of natural products and drug development, including pharmacological activity (Roy, 1805). Phytochemicals are the substances yielded by many parts of plants. These bioactive components of plants are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins, and glycosides. These combinations can be used as antimicrobial and uncontaminated commotion (Feroz *et al*., 1993).

The genus *Plantago* belongs to the family Plantaginaceae. It has 256 species, out of which two species *P. major* and *P. lenceolata* are commonly found in Rawalakot Azad Kashmir. *Plantago* is widely distributed in temperate regions of Asia, South Australia, North America, and North Africa. It grows from sea level to 3500 m altitude (Sagar and Harper, 1964). *Plantago* is rich in phenolic compounds, alkaloids, flavonoids, terpenoids, tannins, ethanol, vitamin C, antioxidant and anti-inflammatory agents. Greek physicians described the traditional use of *Plantago* in wound healing already in the first century A.D. (Samuelsen *et al.,* 1999). Leaves and seeds of the plant have been widely used in folk medicine for various purposes, including treatment of an extensive range of diseases and disorders such as respiratory complications and digestive system affections. It has been also used as an anti-inflammatory, antimicrobial and antitumor agent, skin diseases, infectious diseases, and problems concerning the digestive organs, respiratory organs, reproduction, and the circulation, against tumors, for pain relief and for reducing fever (Samuelsen, 2000).

Both these plants have important medicinal properties, but no proper guidelines are available to standardize these plants. This research work was emphasized on pharmacognostic screening, which is essential to creating the quality of plant material by executing macroscopic, microscopic, physiochemical, and phytochemical analysis of *P. major* and *P. lanceolata*.

Materials and Methods

Plant material

The whole plants of *P. major* and *P. lanceolata* were collected during May and June of 2017 from Rawalakot. The identification was made with the help of Flora of Pakistan and voucher specimens were submitted to the herbarium, Department of Botany, University of Poonch, Rawalakot. After washing with tap water, the plants were shade dried. The dried material was ground into fine powder and stored in an airtight container until further use.

Pharmacognostic characterization

Macroscopic description

Fresh samples were collected and macroscopic evaluation was made on 10 samples of each plant. The plants were described according to available literature (Kokate *et al*., 2006).

Anatomical description

For anatomical studies, a method of Akcin *et al*. (2010) was applied to study the microscopic characters on fresh samples. Transverse sections of leaf, stem, and root were made. All the sections were treated with 30, 50 70, 90 and 100 % alcohol and were stained with safranin and fast green. Permanent slides were prepared and analyzed under a compound microscope.

Physiochemical evaluation

The powder of both the plants was subjected to physiochemical analysis for their moister, ash (total ash, acid insoluble ash, water-soluble ash) and extractive (chloroform soluble, water-soluble, ethanol soluble, petroleum ether soluble) values (Evans, 2003). The color and taste of the powder were observed using the method of Siddiqui *et al.* (1995).

Phytochemical Analysis

Phytochemical screening was carried out by following the techniques of Harborne (1973), Sofowora (1984) and Evans (2003). The qualitative tests were performed for alkaloids, flavonoids, phenols, carbohydrate, and saponins. The quantitative tests were performed for total phenols and flavonoids. Gallic acid was used as a standard for phenols (Fig. 1) and quercetin for flavonoids (Fig. 2).

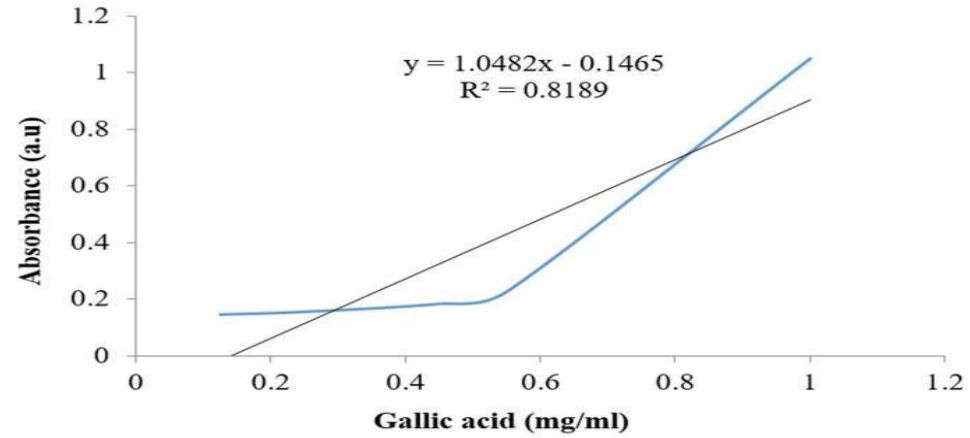

Fig. 1. Standard curve of gallic acid for phenols.

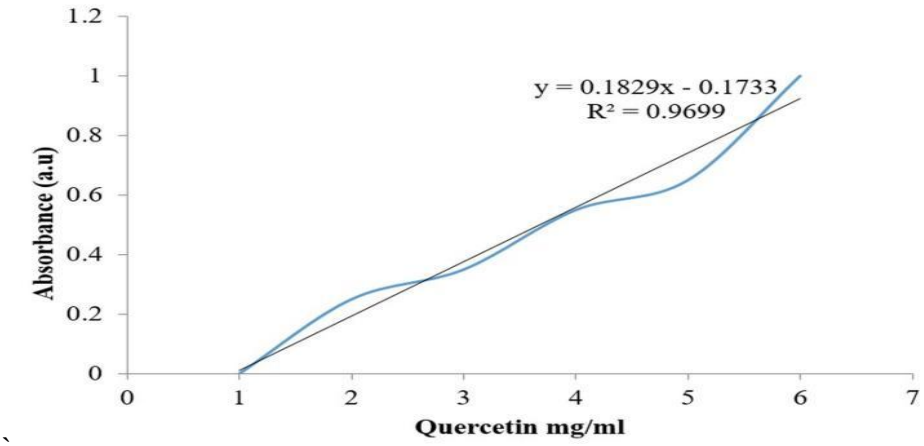

Fig. 2. Standard curve of quercetin for flavonoids.

Results

Plantago major

Morphological description

The average height of the plant was about 34 ± 7.31 cm. Leaves were oval-shaped with acute apex and smooth margins. The size of the leaves 19.6 ± 2.5 × 7.98 ± 0.88 cm. There were 3-9 parallel veins in leaves. Inflorescence was spike 1-30 cm in length; spikes bear flowers, which were yellowish white in color. The average length of flowers was 11 ± 0.66 cm and the average width was 3.16 ± 0.67 cm. the fruit was 3.32 ± 1.49 mm in length. Seeds were 0.6 ± 0.14 mm wide. The color of the plant was green, the taste was slightly bitter and the odor was unspecific (Table 1).

Anatomical features

Leaf anatomy

Leaves were amphistomatic, having stomata on upper and lower epidermis. On upper surface, 31 stomata were present per unit area with an average size of size 10 × 5 mm. On lower epidermis, the number of stomata was 40 with an average size of 10 × 5 mm. Stomata type was anisocytic along with diacytic (Fig. 3).

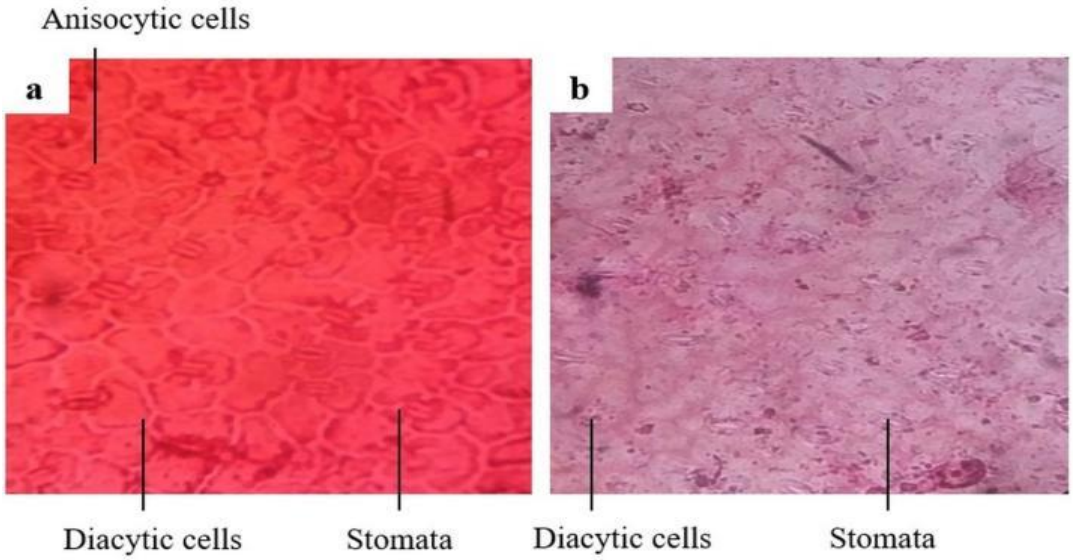

Fig. 3: Transverse section of the upper (a) and (b) lower epidermis of *P. major*

Stem anatomy

Transverse section of stem is circular in outline, has outer epidermis 3 mm wide. After epidermis, 10 mm cortical region is present. Vascular bundles consist of xylem and phloem cells irregularly arranged. Pith was present in center consist of massive cells (Fig. 4a).

Root anatomy

The epidermis was 5 mm thick; the cortical region was 20 mm in diameter. Vascular bundles were 15 mm in diameter; diameter of pith was 10 mm as shown in Fig. 4b.

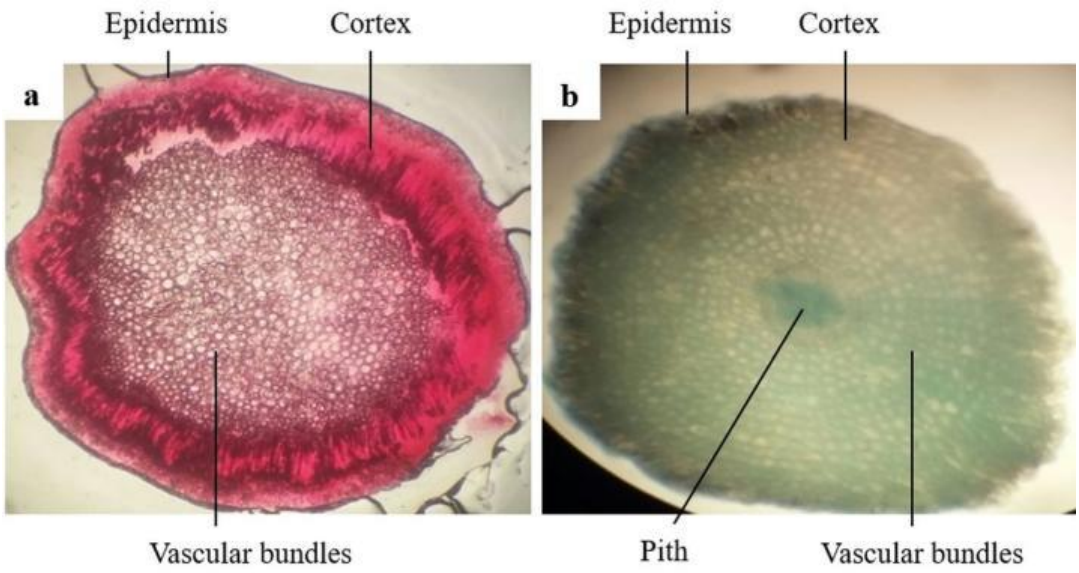

Fig. 4 Transverse section of stem (a) and (b) root of *P. major*

Table 1. Morphological description of *P. major* and *P. lanceolate*.

Morphological traits	***Plantago major***	***Plantago lanceolate***
Leaf type	Ovate with 3-9 parallel Venation	Lanceolate with 5-7 parallel venation
Plant height (cm)	34 ± 7.31	44.8 ± 1.56
Leaf length (cm)	19.6 ± 2.5	15.6 ±1.32
Leaf width (cm)	7.98 ± 0.88	4.6 ± 0.67
Flower length (cm)	11 ± 0.66	4.54 ± 0.53
Flower width (cm)	3.16 ± 0.67	2.24 ± 0.43
Fruit width (mm)	3.32 ± 1.49	3.96 ± 0.38
Seed width (mm)	0.6 ± 0.14	2.76 ± 0.22
Color	Green	Light green to dark green
Odor	Unspecific	Similar to silage
Taste	Bitter	Salty

Plantago lanceolata

The average length of the plant was 44.8±1.56 cm. Leaves were lanceolate with 5-7 parallel veins. The size was 15.6 ± 1.32 × 4.6 ± 0.67 cm. The stem was smoothing herbaceous and leafless. Flowers were born on the tip of the stem. The fruit was 4.54 ± 0.53 mm long and 3.96 ± 0.38 mm wide. Seeds 2-3.2 mm wide average width of seeds was 2.76 ± 0.22 mm. Plant color is light green to dark green; the odor is similar to hay and taste is slightly salty as shown in Table 1.

Leaf anatomy

The upper epidermis has stomata per unit area. Stomata length was 20 mm and the width was 10 mm. The lower epidermis has 45 numbers of stomata. Length of stomata was 5 mm, width was 2 mm and the type of stomata was anisocytic (Fig. 5).

Stem anatomy

Transverse section of stem showed epidermis (9mm) followed by cortex (30 mm). Vascular bundles were arranged in a circle having 20 mm in diameter. Pith was a hollow circle, 40 mm in diameter (Fig. 6a).

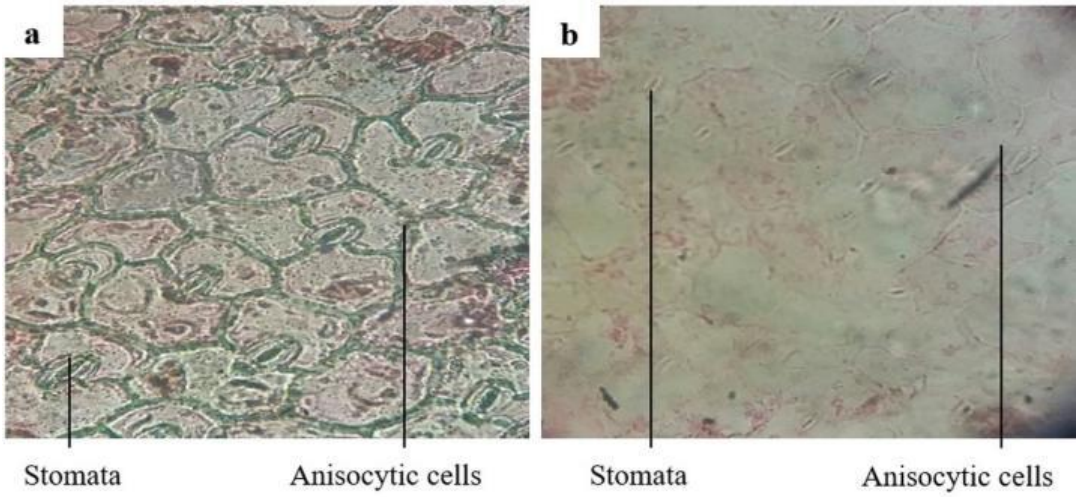

Fig. 5 Transverse section of the upper (a) and lower (b) epidermis of *P. lanceolata*

Root anatomy

The epidermis was 5 mm in thickness. The cortical region was 10 mm and pith area was 40 mm as shown in Fig. 6b.

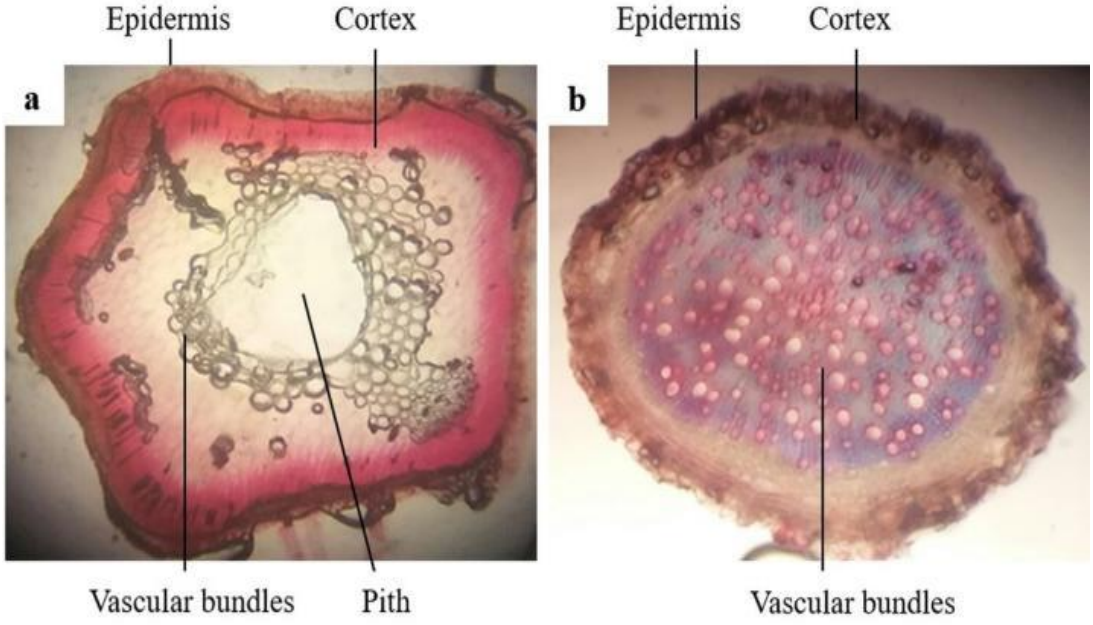

Fig. 6. Transverse section of stem(c) and root (d) of *P. lanceolata*

Physiochemical analysis

Ash values

It gives information about moisture contents, total ash, acid insoluble ash, and water-soluble ash. Moisture contents were observed in percentage while total ash, water soluble ash, acid insoluble ash was calculated in mg/g (Table 2). The moisture contents were 78.43 ± 0.06 and 47.00 ± 0.07% in *P. major* and *P. lanceolata* respectively. The amount of total ash, acid insoluble ash and water-soluble ash was 0.38± 0.01, 0.03 ± 0.002 and 0.11 ± 0.003 mg/g in *P. major* and 0.33 ± 0.03, 0.03 ± 0.001 and 0.15 ± 0.02 mg/g in *P. lanceolata*.

Table 2. Determination of ash soluble contents

Physicochemical contents	***P. major*** **Average ± SEM**	***P. lanceolata*** **Average ± SEM**
Moisture contents (%)	78.43 ± 0.05831	47 ± 0.066
Total ash (mg/g)	0.38 ± 0.01	0.33 ± 0.03
Acid insoluble ash (mg/g)	0.03 ± 0.002	0.03 ± 0.001
Water soluble ash (mg/g)	0.11 ± 0.003	0.15 ± 0.02

Extractive values

Extractive values such as chloroform extractive, water extractive, ethanol extractive, and petroleum ether extractive were calculated and percentage with a standard error was presented in Table 3. The highest extractives were found in water as 42.00 ± 0.03 % in *P. major* and 37.00 ± 0.02 % in *P. lanceolata*. Other extractives were in low amount.

Table 3. Determination of extractive values of *P. major* and *P. lanceolata*.

Extractive contents	***P. major*** **Percent ± SEM**	***P. lanceolate*** **Percent ± SEM**
Chloroform soluble extractive (%)	15.82 ± 0.02	10.70 ± 0.004
Water soluble extractive (%)	42.00 ± 0.03	37.00 ± 0.02
Ethanol soluble extractive (%)	26 .00± 0.01	27.00 ± 0.01
Petroleum soluble extractive (%)	7.00 ± 0.003	8.80 ± 0.0008
pH	5.3-7.5	4.2-7.8

Powder characteristics

Powder characteristics include the taste, color, and odor of the powder. The smell and taste were carried out with 1 mg of powder taken between the thumb and finger. The odor was first tested by the following parameters: No, Low, Sharp, and Strong" Then was determined odor type: Aromatic, fruity. For taste. 1 gram of drugs placed and kept in the mouth without swallowing for 10 to 30

seconds. After the spittle sample, the mouth was rinsed (bitter, sweet, and salty). Results are shown in Table 4.

Table 4 Powder characteristics of *P. major* and *P. lanceolatata*.

Powder characteristics	***P. major***	***P. lanceolate***
Taste	Slight bitter	Little Salty
Color	Light green	Light green
Odor	Odorless	Unspecific

Phytochemical analysis

Qualitative analysis of the whole plant of both species was carried out to show the presence or absence of phytochemicals. This study showed that phenols and flavonoids were present in the whole plant. Alkaloids were absent. Saponins and carbohydrates were also present in the whole plant of both species (Table 5). Total phenolic contents in *P. major* were 0.95 ± 0.20 mg/g and in *P. lanceolata* were 0.44 ± 0.12 mg/g. Flavonoids contents of *P. major* and *P. lanceolata* were 0.69 ± 0.05 mg/g and 0.80 ± 0.15 mg/g respectively (Table 6).

Table 5 Qualitative analysis of phytochemicals.

Phytochemicals	**Solvent (methanol)**	
	Plantago major	***Plantago lanceolate***
Flavonoids	+	+
Phenols	+	+
Alkaloids	–	–
Saponins	+	+
Carbohydrates	+	+

Table 6 Quantitative analysis of phytochemicals of *P. major* and *P. lanceolata*.

Chemical constituents	***P. major***	***P. lanceolata***
Phenols (mg/g)	0.95 ± 0.20	0.44 ± 0.12
Flavonoids (mg/g)	0.69 ± 0.05	0.80 ± 0.15

Discussion

The easiest way to identify and authenticate the plant material is through pharmacognostic studies. These studies are helpful in establishing the standard and quality of medicinal plant material (Panda, 2004). Morphological and anatomical investigations offer valuable and inclusive information that is supportive of the authentication of even closely related species (Thirumalai *et al.,* 2013). The genus Plantago is one of the most important medicinal plants. It grows in almost every type of habitat (Primack, 1978). *P. major* is widely used to cure many ailments like skin diseases, digestive tract diseases, and blood circulation diseases (Sagar and Harper, 1964). As described in the results, *P. lanceolata* is also an herb. As described by Banach *et al*. (2012), roots were thicker, shorter, thread-

like, and shallower adventitious. Venation in the leaves of *P. lanceolata* was 5-7 parallel. The taste was slightly salty to bitter. The color was yellow to brown, and the odor was unspecific, similar to hay. Paulina *et al*. (2017) predicted similar results.

The microscopic method can serve as the simplest and cheapest method to serve as a diagnostic feature (Bragadeeswaran *et al*., 2011). The anatomical study indicated the presence of stomata on the upper and lower epidermis of leaves of both species. Studies of stomata can have great taxonomic and pharmacognostic value in the proper identification of different plant taxa, including medicinal plants (Inamdar, 1970). In *P. major* leaves were amphistomatic. Stomata are small kidney-shaped pores that help in gaseous exchange and transpiration and are surrounded by subsidiary cells. The number of stomata in the lower epidermis was higher than in the upper epidermis. Both surfaces have anisocytic and diacytic types of stomata. The size and types of stomata on both surfaces were the same (Harish *et al*., 2010).

Physicochemical studies provide valuable information about moisture content, total ash, acid insoluble ash, and water-soluble ash. The moisture content is a very important factor for the stability of crude drugs (Ismail *et al*., 2001). The longer shelf life can be achieved only by reducing the moisture content. Higher moisture content is because of less transpiration of water. This makes *Plantago* more resistant to drought, and the plant is able to grow in dry places. Higher moisture contents are also the cause of microbial attack, so a plant cannot be stored for a long period of time. Fewer values of these plants show that they are safe and contain less toxicity, so they can easily be used against wound healing and many other diseases (Nazarizadish *et al*., 2013). Ash values give an idea of the inorganic composition and other impurities in a plant drug (Alam and Najam, 2015).

In particular, extractive values are useful for estimating the constituents present in the crude drug and also help in the estimation of specific constituents soluble in particular solvents (Kumar *et al*., 2011). The extractive values were determined to find petroleum, ethanol, water, and chloroform-soluble compounds. Water-soluble extractives show more water-soluble compounds than ethanol soluble compounds, chloroform soluble compounds, and petroleum soluble extractives (Vivekanand *et al.*, 2012).

The important medicinal properties of plants are due to the presence of various secondary metabolites such as flavonoids, phenolics, saponins, etc. An analysis of plant extract revealed that phenols, flavonoids, and saponins were present in the whole plant of both species. These results were similar to the results of Aruna *et al.* (2013). This reported that phenols, flavonoids, and saponins are present and alkaloids are absent in *Trichodesmum indicum*. Khan (2013) also described similar results. Alkaloids were present, but this study showed the absence of alkaloids. According to Alam and Najam (2015), phytochemical constituents of the plants are an important parameter, which gives an indication of the pharmacologically active metabolites present in the plant (Liu *et al*., 1992).

Phenolic compounds are one of the largest and most important groups of plant metabolites (Sing *et al.*, 2007). According to Yadav and Agarwala (2011), phenols possess properties such as antiapoptosis, antiaging, and anti-inflammation. Several studies have described the antioxidant properties of medicinal plants, which are rich in phenolic compounds (Brown *et al*., 1998). This shows that both species of *Plantago* may have antiapoptosis, antiaging, and anti-inflammatory activities. Flavonoids are important components of medicinal plants. They have antimicrobial substances against a wide array of microorganisms. They also have anticancer activities (Salah, 1995). Several biological activities are attributed to *Plantago* leaves, including anti-inflammatory, antiviral, anti-cancer, anti-fever, and anti-tumor (Beara *et al*., 2010). The presence of flavonoids in both species of *Plantago* shows that it can be used as an anti-viral, anti-tumor, anti-cancer, and for many other disorders.

Conclusion

In order to maintain the value of its crude drug, genus *Plantago* deserves to be properly recognized and conserved due to its numerous medicinal uses. The macroscopical, microscopic, physiochemical, and phytochemical pharmacogenetic parameters examined in this study will help in identifying the plants and preventing their contamination. This study also offers useful guidelines for standardizing the development of herbal formulations as well as useful data for the identification of these herbs. Alkaloids and flavonoids, that are necessary for treating a variety of ailments, are present in this herb, which will aid scientists in developing treatments for these conditions.

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