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# HPTLC Fingerprint Profile and Standardization of *Plantago Ovata* Forssk

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**ABSTRACT**

*Plantago ovata* belongs to the family Plantaginaceae and has notable medicinal properties as it is used for curing diarrhoea, dysentery and constipation due to its property of absorbing and retaining high amount of water. Methanolic extracts of *P. ovata* were obtained through reflux extraction to perform further experiments. Various experimental tests were carried out to understand the physicochemical, antimicrobial and fluorescence properties. HPTLC and phytochemical profiles were also obtained. Preliminary phytochemical analysis showed the presence of alkaloids, saponins, steroids and triterpenes. Microscopy of the powdered sample revealed the presence of starch granules, trichome and mucilage. The maximum antimicrobial activity of *P. ovata* was seen at a sample concentration of 10 µL where the activity was found to be more than standard against *Bacillus cereus* and *Aspergillus niger*. HPTLC profiling showed 9 bands each in 5 lanes of increasing concentration and a pattern unique to *Plantago ovata.*

**KEYWORDS**: *Plantago ovata,* Phytochemical, HPTLC profiling, Microbial activity, Physicochemical evaluation, Microscopical evaluation

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## 1. INTRODUCTION

*Plantago ovata* Forssk., commonly known as Psyllium, is a herbaceous annual plant belonging to the family-Plantaginaceae. Seeds are hard, translucent, smooth, mucilaginous; concave surface of the seeds contains the hilum covered with a thin membrane. The husk is thin, boat-shaped (2-3 mm x 0.5-1.0 mm.), white, translucent, odourless, with a bland mucilaginous taste [1].

Seed produced from *Plantago ovata* is known in trading circles as white or blonde psyllium, Indian Plantago, or Isabgol; the common name in India for *Plantago ovata*, comes from the Persian words “isap” and “ghol” which mean horse ear, which is descriptive of the shape of the seed. India dominates the world market in the production and export of psyllium. In India, *Plantago ovata* is cultivated mainly in North Gujarat as a “Rabi” or post-rainy season crop. An important environmental requirement of this crop is a dry open place. [2]

The mucilage of isubgol is colloidal in nature and its composition varies with the conditions of preparation. It is mainly composed of xylose, arabinose and galacturonic acid; rhamnose and galactose have also been reported. [1] Psyllium husk is used as Ayurvedic, Unani and Allopathic medicine. It is specially used in diarrhoea, dysentery and constipation due to its property of absorbing and retaining high amount of water. The efficacy of isubgol is due to the large quantity of mucilage present in the husk. In indigenous medicine, the seeds are considered cooling and diuretic as well and recommended in febrile conditions and the affections of kidneys, bladder and urethra. Psyllium fibre dietary supplements are used in veterinary medicine to treat sand impaction in horses to aid in elimination of sand from the horse's colon [3]. Psyllium can cause allergic reactions, including anaphylaxis [4]. Psyllium may act as a potent inhalant allergen capable of eliciting asthma symptoms [5]. Psyllium has been found by meta-analysis of clinical trials to significantly reduce blood pressure in people suffering from hypertension. Psyllium has not been shown to have the same effect on individuals with normal or elevated blood pressure [6]. The aim of this paper is to study and analyze the microbiological, HPTLC, pharmacognostic and phytochemical profile of *Plantago ovata* Forssk. to assess and understand it’s various applications and benefits

## 2. MATERIALS AND METHODOLOGY

Total ash is the amount of ash obtained after the sample has been incinerated and is devoid of carbon. It usually contains phosphates, carbonates, silicates and silica. An empty crucible was weighed after heating in the muffle furnace at 500 °C for 1 hour. 2 g of powdered sample was weighed and transferred into the dried and cooled empty crucible which was then placed in the muffle furnace with the temperature setting at 600 °C. After 4 hours, the crucible was removed from the furnace and cooled by placing in a desiccator for 30 min after which the following readings were noted.

Total Ash % $= \frac{(B-C)}{A} * 100$

where, A = Weight of sample (in grams)

B = Weight of crucible + contents after drying (in grams)

C = Weight of empty crucible (in grams)

### 2.3.2 Determination of Acid Insoluble Ash [10]

Total ash when treated with HCl reacts with materials to form soluble salts and insoluble residues consisting of mainly silica and acid insoluble ash. 25 mL of 2M HCl was added to the crucible containing the total ash obtained and covered with a watch glass. This setup was kept on a hot plate to boil gently for 5 min. The insoluble matter was filtered using a clean filter paper which was then rinsed repeatedly with hot water until it was neutral or free from acid. This filter paper containing the insoluble matter was then transferred into the original crucible. The crucible was dried on hot plate and placed in the muffle furnace at a temperature setting of 500 °C. After 4 hours, the crucible was removed and cooled by placing in a desiccator for 30 min after which the following readings were noted.

Acid Insoluble Ash % $= \frac{(B-C)}{A} * 100$

where, A = Weight of sample (in grams)

B = Weight of crucible + contents after drying (in grams)

C = Weight of empty crucible (in grams)

### 2.3.3 Determination of Alcohol Soluble Extract [11]

4 g of powdered plant material was weighed and macerated with 100 mL of 90% ethanol in a conical flask for 24 hours while shaking the contents frequently in the first 6 hours. Thereafter, the contents of the flask were rapidly filtered while taking precautions against loss of ethanol. An empty petri plate was weighed and the contents obtained after filtration was poured into it and placed on the hot water bath till all the liquid component had evaporated. The petri plate was then dried by placing it in a hot air oven at a temperature setting of 105 °C, followed by placing it in a desiccator for 30 min. The following readings were noted.

Alcohol Soluble Extractive value % $= \frac{(B-C)}{A} * 4 * 100$

where, A = Weight of plant material (in grams)

B = Weight of petri plate + residue

C = Weight of empty petri plate

### 2.3.4 Determination of Water Soluble Extract [11]

4 g of powdered plant material was weighed and macerated with 100 mL of chloroform water in a conical flask for 24 hours while shaking the contents frequently in the first 6 hours. Thereafter, the contents of the flask were rapidly filtered. An empty petri plate was weighed and the contents obtained after filtration was poured into it and placed on the hot water bath till all the liquid component had evaporated. The petri plate was then dried by placing it in a hot air oven at a temperature setting of 105 °C, followed by placing it in a desiccator for 30 min. The following readings were noted.

Water Soluble Extractive value % $= \frac{(B-C)}{A} * 4 * 100$

where, A = Weight of plant material (in grams)

B = Weight of petri plate + residue

C = Weight of empty petri plate

### 2.4 Preliminary Phytochemical Evaluation [12, 13, 14]

Phytochemicals, also known as secondary metabolites, are biologically active compounds found in plants like alkaloids, flavonoids, saponins, steroids, triterpenes, phenols, tannins etc., These phytochemicals are found in various parts of the plant like leaves, bark, seeds, flowers and roots. The methanolic extracts were subjected to various chemical tests to detect the chemical constituents present in them. Different tests were performed for different constituents i.e., alkaloids (Dragendorff' s test, Mayer' s test), flavonoids (Shinoda test), saponins (Froth test), steroids (Liebermann-Burchard' s test, Salkowski test), triterpenes (Liebermann-Burchard' s test, Salkowski test) and phenols (Ferric chloride test).

### 2.5 HPTLC Studies [15]

Sample solutions were applied to the Silica gel 60 F254 (E. Merck) precoated TLC plates as sharp bands by means of Aspire automatic sample applicator. The spots were dried in a current of air. Chromatography was carried out in a glass chamber (Aspire). The mobile phase (Toluene: Ethyl acetate (8:2 v/v)) was poured into a twin trough glass chamber. Whole assembly was left to equilibrate and pre-saturate for 30 min. The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of the silica plate at 20 ºC and 50% relative humidity. The plate was visualized for detection by observing it under UV light (254 nm) and at long UV (366 nm). Then the derivatization was carried with 10% $H_2SO_4$ solution. The densitometric scan was drawn using Just TLC software attached to Aspire HPTLC.

### 2.6 Microscopical Evaluation

#### 2.6.1 Powder Microscopy [16]

It is a quality control method used for medicinal plants to study the specific microscopic characters using different staining reagents. The powdered plant material was completely immersed in 10% HCl overnight. The sample was then filtered and rinsed with distilled water the next day. The soaked sample was completely drained of excess water, stained with safranin and observed in Magnus MLX Plus microscope under 4X magnification followed by 10X, 40X, and 100X.

**2.6.2 Powder Fluorescence [17]**

Powdered sample when treated with different chemical reagents gives characteristic color when visualized under UV light. On adding 1-2 drops of nitrocellulose on the sample and observed in UV Transilluminator, a change in color is noticed.

**2.6.3 Fluorescence analysis [17]**

The powdered sample of *P. ovata* was treated with various chemicals like water, concentrated $HNO_3$, $H_2SO_4$, and HCl, methanol, petroleum ether, hexane, chloroform, and ethanol. The powdered materials gave different colors with different chemicals when observed under UV light of short and long wavelengths.

**2.7 Microbial Limit Test [18, 19]**

Microbial testing is carried out for the detection of microorganisms in a product. We check for the amount of microbial activity in the sample chosen. The suitable media- Nutrient Agar, Potato Dextrose Agar and peptone water was prepared and sterilized. Serial dilution was performed by adding 10 g of powdered sample in 90 mL NaCl saline ($10^{-1}$). 9 mL of NaCl saline was poured in a series of 9 test tubes. 1 mL of sample from the first dilution was taken and mixed with the contents of second test tube ($10^{-2}$). This process was continued for up to 4 dilutions. 0.1 or 1 mL of sample was taken from each dilution and poured into the respective petri plates and inoculated with the media by pour plate method. Incubate the bacterial plates with Nutrient Agar at 37 °C for 24-48 hours and fungal plates with the Potato Dextrose Agar at room temperature for 3-5 days. Observe and count for bacterial and fungal colonies with the help of a digital colony counter after the incubation period.

**2.8 Microbial Activity**

**2.8.1 Antibacterial activity [20]**

The in-vitro antibacterial activity test was conducted against two different bacteria- *Escherichia coli* and *Bacillus cereus*, as these bacteria multiply rapidly and show results quickly. The sample was prepared by adding 40 mg of plant extract in 10 mL DMSO solution. The antibacterial activity was determined by disc method where gentamycin was used as standard and distilled water as control. 1.5 mL of sample was added at different concentrations of 10, 20, 30 mg in the respective vials and sterilized discs were allowed to soak for an hour. Nutrient Agar media was poured into petri plates and allowed to solidify followed by inoculation of bacteria by spread plate method. The discs were removed from vials, completely drained of excess liquid and placed on the agar gently. Further, the petri plates were incubated at 37 °C for 24 hours. The zone of inhibition around each disk were then measured.

**2.8.2 Antifungal activity [20]**

The in-vitro antifungal activity test was conducted against *Aspergillus niger*. The sample was prepared by adding 40 mg of plant extract in 10 mL DMSO solution. The antifungal activity was determined by disc method where Fluconazole was used as standard and distilled water as control. 1.5 mL of sample was added at different concentrations of 10, 20, 30 µL in the respective vials and sterilized discs were allowed to soak for an hour. Potato Dextrose Agar media was poured into petri plates and allowed to solidify followed by inoculation of bacteria by spread plate method. The discs were removed from vials, completely drained of excess liquid and placed on the agar gently. Further, the petri plates were incubated at room temperature for 48 hours. The zone of inhibition around each disk were then measured.

## 3. RESULTS AND DISCUSSION

### 3.1. Organoleptic evaluation

**Table 3.1. Organoleptic evaluation of *Plantago ovata***

| Sl. No | Parameter | Observation |
|---|---|---|
| 1 | Color | Light brown |
| 2 | Shape | Oval |
| 3 | Texture | Brittle |
| 4 | Taste | Tasteless |
| 5 | Odor | Odorless |

### 3.2. Physicochemical evaluation

**Table 3.2. Physicochemical evaluation of *Plantago ovata***

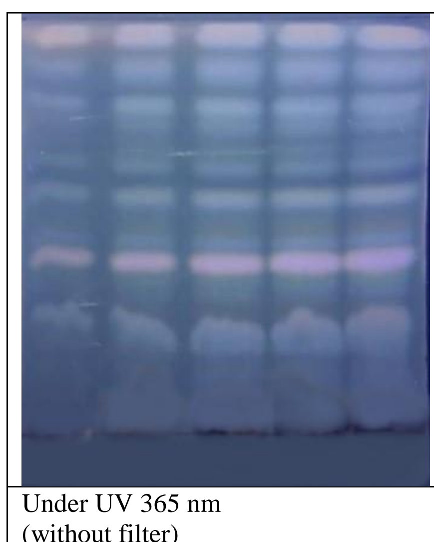
| Sample | Total ash (%) | | Acid insoluble ash (%) | | Water soluble extract value (%) | | Alcohol soluble extract value (%) | |
|---|---|---|---|---|---|---|---|---|
| | OV | LM | OV | LM | OV | LM | OV | LM |
| *Plantago ovata* | 3.2857% | <4.0% | 1.78% | <1.0% | 6.68% | NA | 8.44% | <2.0% |

### 3.3. Preliminary phytochemical evaluation

**Table 3.3. Preliminary phytochemical evaluation of *Plantago ovata***

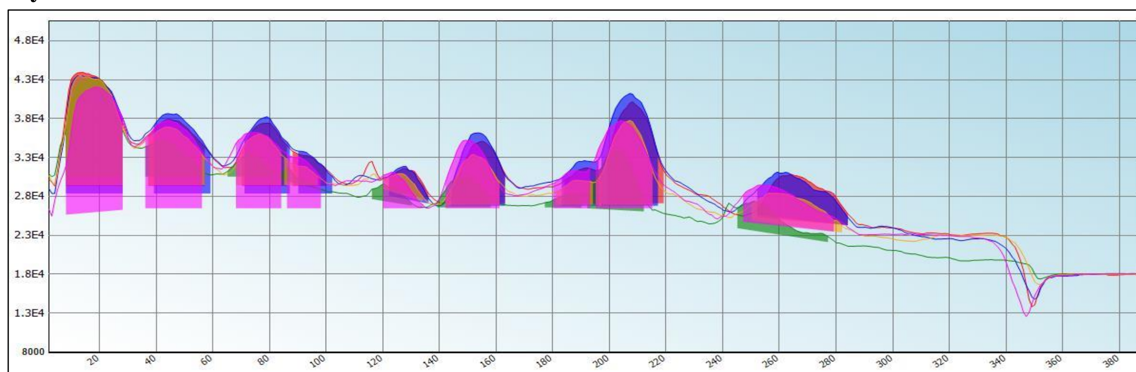
| Sl. No | Test | Result |
|---|---|---|
| 1 | **Alkaloids**<br>Dragendorff's test<br>Mayer's test | <br>Positive<br>Positive |
| 2 | **Flavonoids-** Shinoda test | Negative |
| 3 | **Saponins**- Froth test | Positive |
| 4 | **Steroids**<br>Liebermann-Burchard's Test<br>Salkowski test | <br>Positive<br>Positive |
| 5 | **Triterpenes**<br>Liebermann-Burchard's Test<br>Salkowski test | <br>Positive<br>Negative |
| 6 | **Phenols-** $FeCl_3$ test | Negative |
| 7 | **Tannins** | Negative |

### 3.4. HPTLC studies



Under UV 365 nm
(without filter)

**Analysis:**




Legend: Lane 1 Lane 2 Lane 3 Lane 4 Lane 5

**LANES**

| ID | Width | Bands | Volume |
|---|---|---|---|
| 1 | 59 | 9 | 346.02 |
| 2 | 63 | 9 | 562.8 |
| 3 | 68 | 9 | 744.76 |
| 4 | 60 | 9 | 754.95 |
| 5 | 68 | 9 | 841.95 |

### 3.5. Microscopical evaluation

#### 3.5.1. Powder microscopy of *Plantago ovata*

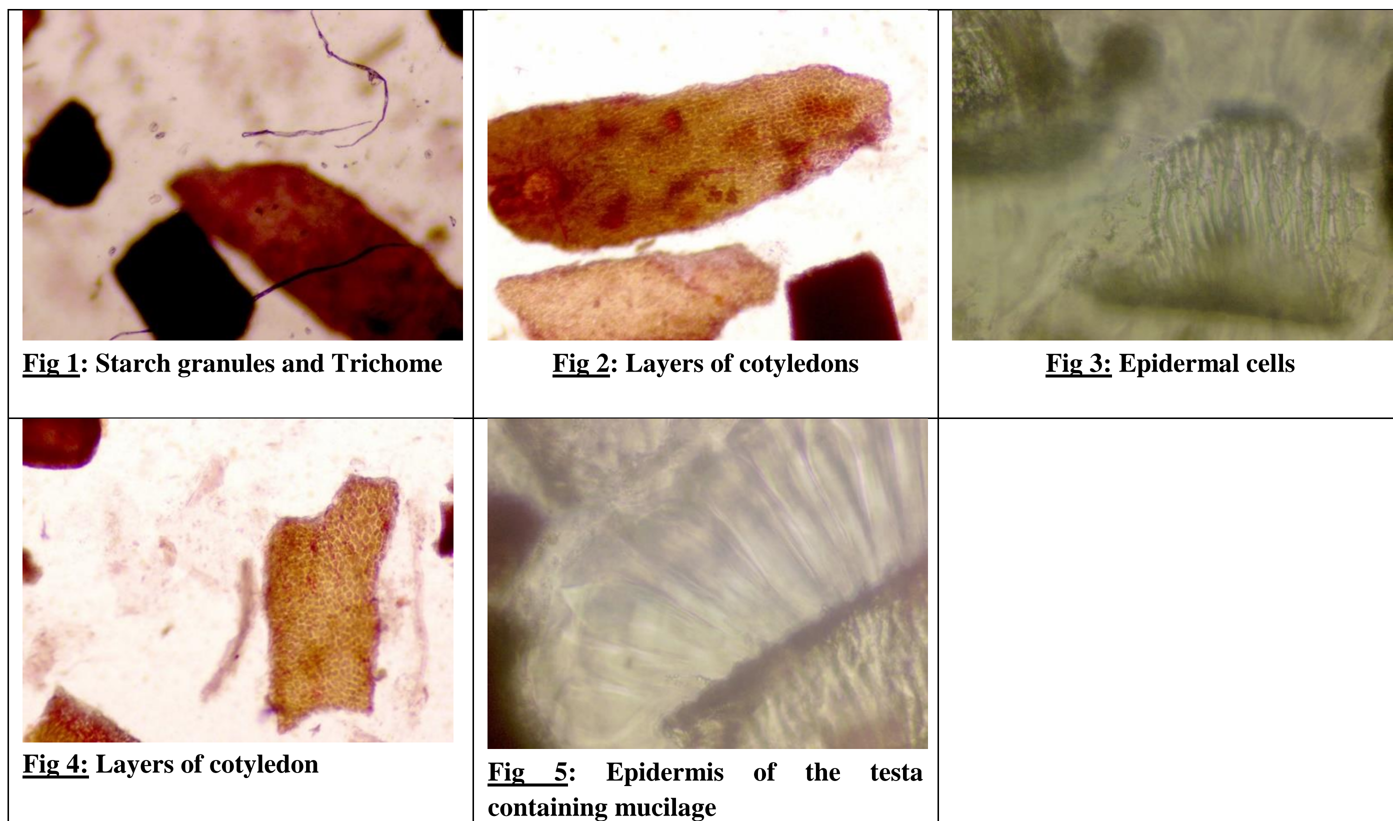


**Fig 1: Starch granules and Trichome**

**Fig 2: Layers of cotyledons**

**Fig 3: Epidermal cells**

**Fig 4: Layers of cotyledon**

**Fig 5: Epidermis of the testa containing mucilage**

#### 3.5.2. Powder fluorescence of *Plantago ovata*

**Table 3.5.2. Powder fluorescence of *Plantago ovata***

| Sl. No | Reagent added | Color |
|---|---|---|
| 1 | No reagent (raw sample) | Blue |
| 2 | water | Blue |
| 3 | Conc. $HNO_3$ | Yellowish-brown |
| 4 | Conc. $H_2SO_4$ | Blue |
| 5 | Dil. HCl | Light blue |
| 6 | Methanol | Bright blue |
| 7 | Petroleum ether | Bright blue |
| 8 | Hexane | Bright blue |
| 9 | Chloroform | Bright blue |
| 10 | Ethanol | Bright blue |
| 11 | Nitrocellulose | Bright blue |

### 3.6. Microbial limit test

**Table 3.6. Microbial limit test of *Plantago ovata***

| Sl. No | Dilution | No. of colonies | CFU |
|---|---|---|---|
| 1 | control | No growth | 0 |
| 2 | $10^{-1}$ | 25 | $25*10^{-1}$ |
| 3 | $10^{-2}$ | 18 | $18*10^{-2}$ |
| 4 | $10^{-3}$ | 10 | $10*10^{-3}$ |
| 5 | $10^{-4}$ | 4 | $4*10^{-4}$ |

TNTC: Too Numerous To Count

CFU: Colony Forming Units

### 3.7. Microbial activity

#### 3.7.1. Antibacterial activity

**Table 3.7.1. Antibacterial activity of *Plantago ovata* against *Bacillus cereus* and *Escherichia coli***

| Sl. No | Concentration of antibiotic | Zone of inhibition (cm) | |
|---|---|---|---|
| | | Against *Bacillus Cereus* | Against *E. coli* |
| 1 | Standard- Gentamycin | 1.2 cm | 1.3 cm |
| 2 | 10 µL | 1.8 cm | 1.4 cm |
| 3 | 20 µL | 1.8 cm | 1.3 cm |
| 4 | 30 µL | 0.8 cm | 1.3 cm |

#### 3.7.2 Antifungal activity

**Table 3.7.2. Antifungal activity of *Plantago ovata* against *Aspergillus niger***

| Sl. No | Concentration of antibiotic | Zone of inhibition (cm) |
|---|---|---|
| 1 | Standard- Fluconazole | 1.0 cm |
| 2 | 10 µL | 1.8 cm |
| 3 | 20 µL | 1.3 cm |
| 4 | 30 µL | cm |

## 4. DISCUSSION

The plant under study *Plantago ovata*, was identified and authenticated as per the study requirements. The organoleptic and physicochemical studies conform with the limits mentioned in the API. The preliminary phytochemical studies of methanolic extract of *Plantago ovata* showed varied results where except flavonoids, phenols and tannins, all other group of chemicals viz., alkaloids, saponins, steroids and triterpenes were present. As far as HPTLC fingerprinting profile is concerned, 9 bands were observed in all 5 lanes of 2 µL, 4 µL, 6 µL, 8 µL and 10 µL at Rf values 0.96, 0.89, 0.80, 0.77, 0.68, 0.61, 0.51, 0.47, and 0.32. The fluorescence studies of the powder of *P. ovata* on treating with various solvents and with nitrocellulose, indicated different colors when observed under visible and UV light (365 nm). In the microbial limit test, the colony-forming unit (CFU) both in bacteria and fungi, the ranges were well within the limits as mentioned in API. Having a large group of chemicals, *P. ovata* exhibited moderate to significant antimicrobial activity. The activity of *P. ovata* is 50% more than standard against *Bacillus cereus* in 10 µL concentration whereas against *E. coli*, the activity is almost the same as that of standard. In all three concentrations of 10 µL, 20 µL, and 30 µL, the activity is very high and significant against *Aspergillus niger*, notably higher and more than that of standard Fluconazole. At 10 µL, the activity of *P. ovata* is 80% more than that of standard. The antimicrobial activity of *P. ovata* may be attributed to the group of chemicals present in plant parts under study. The HPTLC fingerprint profile of methanolic extract of this plant showed 9 bands each in 5 lanes of increasing concentration and a pattern unique to *Plantago ovata* and it can be used as quality standard method for identification and authentication.

## 5. CONCLUSION

From the above study, it can be concluded that pharmacognostic and phytochemical evaluation will possibly help as a valuable resource for the identification, authentication, and preparation of the monograph of *Plantago ovata*. The present work was embraced with a perspective of setting down a benchmark which could be valuable in recognizing the authenticity of therapeutically important medicinal plants. Microscopical studies have demonstrated the presence of starch granules, trichome, epidermis of the testa containing mucilage, epidermal cells and layers of cotyledons.

The phytochemical investigation showed the presence of alkaloids, saponins, steroids and triterpenes. Thus, obtained results can be utilized for the quality control of the crude drug/ drugs. This type of study helps standardize the drugs and can be used to differentiate closely related or allied species The HPTLC results provide standard fingerprints that can be used as a reference result for the identification and QC of the drug. This can also be used as a good tool in preparing the monograph. The various physicochemical parameters were established which are also important in analyzing adulteration and mishandling of the crude drug. Further spectral studies and in vivo studies are required to know their exact chemical composition and therapeutic efficacy.

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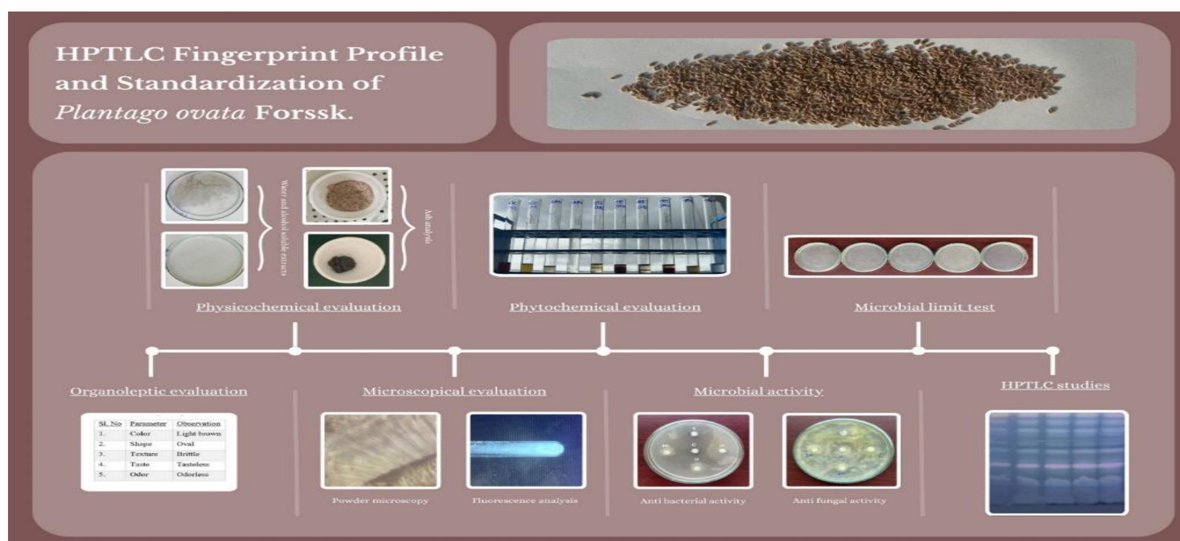


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