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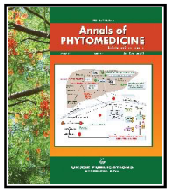

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# Morphoanatomical, pharmacotaxonomical, physiochemical and phytochemical profiles, including TLC and HPTLC analysis of *Ipomoea pes-tigridis* L.

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**Abstract**

Herbal drugs are becoming more popular around the world, particularly in developed countries, but one of the barriers to their acceptance is the lack of a standard quality control profile. The main goal of this study is to look at the morphoanatomical, pharmacotaxonomical, physiochemical, and phytochemical properties of the leaves of *Ipomoea pes-tigridis* L. The study represents macroscopical and microscopical evaluation along with estimation of its physicochemical parameters, such as ash values, extractive values, fluorescence analysis, and phytochemical screening, using WHO-prescribed standard procedures. The phytochemical screening includes preliminary phytochemical screening, thin-layer chromatography, and high-performance thin-layer chromatographic analysis. All the pharmacognostic standardisation parameters analysed gave satisfactory results. The preliminary phytochemical screening of various extracts of *I. pes-tigridis* showed the presence of various phytoconstituents such as carbohydrates, proteins, terpenoids, flavonoids, phenolic compounds, and resins. The thin layer chromatography (TLC) analysis of the hydroalcoholic extract showed the presence of flavonoids and polyphenolic compounds from the Rf values. Equally, the high performance thin layer chromatography (HPTLC) analysis showed that the 3D finger print profile of TLC reflects 8 peaks and 12 peaks at 254 nm and 366 nm, respectively. Among the spots produced in HPTLC, the Rf value of 0.7 may indicate flavonoid content. Hence, these findings reveal a standardisation profile for *I. pes-tigridis*, which would be useful in botanical identification and plant drug authentication, as well as in the preparation of herbal monographs and the prevention of adulteration. Eventually, the presence of phytocontituents may have a synergistic effect on the therapeutic potential and bioactivity of the plant.

## 1. Introduction

*Ipomoea pes-tigridis* L., commonly called "Tiger Foot" or "Morning Glory," belongs to the Convolvulaceae family. It is a spreading or twining herb. It is an herbaceous, hairy, annual vine, with all parts being more or less covered with brownish hairs. It almost runs the length of India, rising up to 4000 feet on the plains from the coast to 750-900 meters, often in arable land. The climber flowers throughout the year. The plant grows in fields, shrubs, waste areas, grasslands, hedges, and near the seashore. It flourishes during the monsoon in North India and stays fresh and succulent for 3-4 months (Sameemabegum *et al*., 2022; Babu *et al*., 2018).

The leaf's common name is derived from its five lobes, which resemble the claws of a tiger. The axillary head contains flowers that normally open at the same time. The green sepal tube measures around 1 cm in length. The limb measures 3 cm in diameter, and the flowers are white and 4 cm long. The fruit has a 6-7 mm diameter and is rounded (Singh and Gupta, 1995). The herb has been used to treat dog bites as well as boils and carbuncles. The leaves are used as poultices on boils, pimples, and wounds in the Philippines and Indonesia. The root has purgative properties (Sharma, 2002). Phytoconstituents are responsible for a plant's or a drug's medicinal effects (Takur, 2023). As a result, the pharmacognostic standardization, physio-chemical, phytochemical, and fluorescence analysis of *I. pes-tigridis* are evaluated. Figure 1 displays the habitat and leaf pictures of *I. pes-tigridis*.

## 2. Materials and Methods

### 2.1 Collection and authentification of plant material

The plant samples were acquired in Madurai. Dr. John Britto, Rapinet Herabarium, St. Joseph's College, Tiruchirapalli, and Dr. L. Stephen, Lecturer, American College, both verified the plant's identity and authenticity (PARC/2012/1222). The microscopic properties of this plant were examined and compared to the existing literature for additional validation (Nimmy *et al*., 2017).

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**Figure 1: Habitat and leaf of *I. pes-tigridis*.**

## 2.2 Pharmacognostic studies

### 2.2.1 Macroscopic study

Under a microscope, the leaves of *I. pes-tigridis* were studied. Shape, dimensions, surface features, appearance, colour, uniformity, odour, taste, and other aspects were all taken into account (Kumarai *et al*., 2017).

### 2.2.2 Microscopic study

*I. pes-tigridis* leaves were cut into transverse slices using a rotary microtome. Saffron and iodine were used as needed to create a permanent mount for the leaf. Paradermal cutting (sections taken parallel to the surface of the leaf) was used to examine the stomata shape, venation arrangement, and trichome dispersion. The preparation of leaves for stomatal morphology research included leaf washing with 5% sodium hydroxide or partial soaking using Jeffrey's maceration solution (OBrien *et al*., 1964).

### 2.2.3 Powder microscopy

After staining, the powdered components of the various parts were treated with sodium hydroxide and mounted in a glycerin medium. Research and measurements were undertaken according to standard procedures (Pratap *et al*., 2021).

### 2.2.4 Physiochemical research

The physicochemical parameters total ash value, loss on drying, water soluble ash, acid insoluble ash, alcohol, and water soluble extractive values were assessed in accordance with the quality control guidelines for herbal medicine (WHO) processes (Pratap *et al*., 2014).

### 2.2.5 Powder examination

It was observed how the powder behaved when exposed to various chemical reagents, as described by Kay and Johansen in 1938 and 1940, respectively (Kay, 1938; Jahansen, 1940).

### 2.2.6 Fluorescence evaluation

Using the Chase and Pratt (1949) approach, the fluorescence examination of the medication powder and the *I. pes-tigridis* plant extract was conducted (Chase and Pratt, 1940).

## 2.3 Phytochemical analysis

### 2.3.1 Preliminary phytochemical screening

The leaves of *I. pes-tigridis* were coarsely powdered and extracted with various solvents such as petroleum ether, chloroform, ethyl acetate, methanol, ethanol, and water using the cold maceration technique. Then it was filtered, dried, and stored in a refrigerator. The obtained extracts were used for preliminary phytochemical screening for the identification of various phytoconstituents by using standard procedures (Chatwal, 2000; Finar, 1996; Wadher *et al*., 2009).

## 2.4 Thin layer chromatography analysis of *I. pes-tigridis*

### 2.4.1 Preparation of hydroalcoholic extract

*I. pes-tigridis* dry powdered leaf weight of 500 g was defatted with 1.5 l petroleum ether (60-80ºC) by maceration. Filtration was used to get rid of the solvent, and the marc was dried. 1.5 l of 70% ethanol was added to the dried marc, and triple maceration was used to extract the mixture (a 72-h process). After filtering, rotavapour was used to evaporate the combined filtrate and create a cohesive aggregate.

### 2.4.2 Preparation of TLC plates

The adsorbent (silica gel G) was produced as slurry in water (1:2). As a pattern, a row of dry, spotless glass plates (20 cm x 5 cm) were placed out. The suspension was then poured into Stahl. They were covered with a single pass of the TLC spreader, which was set to 0.25 mm in thickness. These plates were dried by air on the template until the layer's opacity vanished. They were then dried at 110°C for 30 min and stored in a dessicator. When necessary, the plates were employed. *I. pes-tigridis* 70% ethanol extract was placed on the plate.

### 2.4.3 Development of chromatogram

The plates for TLC were produced in a chromatographic container using the various mobile phases, including solvent system I (12:6:0.05) toluene, ethylacetate, and formic acid; solvent system II (30:10:1) toluene, acetone, and formic acid; solvent system III (7.5:2.5) toluene and ethylacetate; and solvent system IV (60:40) chloroform and ethylacetate. The chromatogram was produced and assessed using different chemical reagents, UV light at 254 nm and 366 nm, or visually (Avalaskar *et al*., 2011; Das *et* al., 2019).

## 2.5 High performance thin layer chromatography

The most advanced development of thin-layer chromatography is the high-performance thin-layer chromatography method (HPTLC). Both the detection and application of the track were done using the CAMAG Linomat 5 sample applicator and the TLC Scanner 3 "Scanner3-070408 S/N 070408 (1.41.21). The chromatogram was

developed in a twin-trough plate development chamber. Win CATS 1.4.3 was the programme used. In the stationary phase, silica gel Merck G F254 with a 0.2 mm layer thickness was pre-coated onto aluminium sheets. The mobile phase for creating the chromatogram was composed of toluene, ethyl acetate, and 100% formic acid (12:6:0.1). A CAMAG twin-trough glass chamber was used to collect data during the mobile phase. At wavelengths of 254 and 366 nm, the developed plates were analysed (Bently and Drivers, 2011; Das *et al*., 2016).

### 2.5.1 Sample preparation

*I. pes-tigridis* hydroalcoholic extract was diluted in ethanol to a concentration of 10 mg/ml. From this solution, 2 il was applied as an 8 mm band and utilised to take an HPTLC fingerprint.

## 3. Results

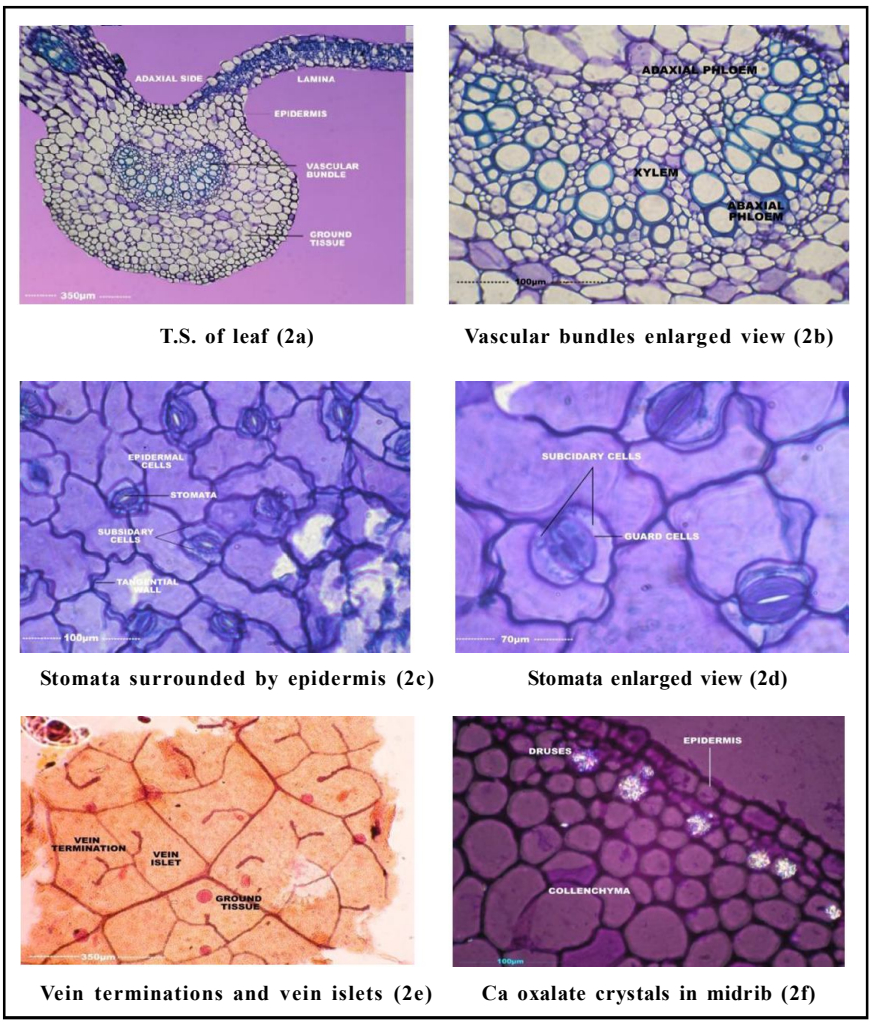
### 3.1 Pharmacognostic studies

### 3.1.1 Macroscopical characters

The flowers are white and cone-shaped, and the leaves are bushy and 5-9 septate. The leaf is made up of parenchymatous root tissue, thick, slightly curved epidermal cells, and a glabrous hanging midrib. The vascular strand is a single dome structure with bicollateral sheath cell bundles surrounding it. Crystals of calcium oxalate are seen in the parenchyma, which is spongy.

### 3.1.2 Microscopy

The epidermal cells that make up a leaf are unique, squarish, and somewhat thick (Figure 2). The ground tissue is parenchymatous and homogeneous. The vascular strand has one bowl-shaped bicollateral segment. It is made up of number of (about 11) brief lines of xylem components blended with sclerenchymatous ground tissue. The xylem components are lignified, circular tubes with thick walls. Along the bottom and top surfaces of the xylem strand, there are several little phloem nests (Figure 2b) and the thick lamina (Figure 2a). The mesophyll tissue often has calcium oxalate crystals in the shape of druses or sphaero crystals (Figure 2f). In parareal slices of the leaf, the cells of the epidermis and stomata were examined. The anticlinal structures of the cells of the epidermis are thick and wavy. The cells have the outline of an amoeboid, and the cutaneous marks are warts. The stomata range in size from 15-20 m and are primarily paracytic (Figure 2c). 48/mm$^2$ is the stomatal number (Figure 2d). The size and shape of the vein islets differ. The islets are polygonal, 5-sided, and rectangular shapes (Figure 2e). The well development is noticed in vein terminations (Figure 2e).




T.S. of leaf (2a) | Vascular bundles enlarged view (2b)

Stomata surrounded by epidermis (2c) | Stomata enlarged view (2d)

Vein terminations and vein islets (2e) | Ca oxalate crystals in midrib (2f)

**Figure 2: Microscopy of *I. pestigridis* leaves.**

### 3.1.3 Powder microscopy

The examination of the powder under a microscope revealed the existence of peltate-type glands that are observed detached from the epidermis (Figure 3), as well as unicellular and unbranched non-glandular epidermal trichomes (Figure 3a), subsessile glandular trichomes (Figure 3b), and calcium oxalate crystal dusts (Figure 3c).


**Epidermal trichomes (3a)** **Glandular trichomes (3b)** **Calcium oxalate crystals (3c)**

**Figure 3: Powder microscopy of *I. pestigridis*.**

### 3.1.4 Determination of leaf constants

Table 1 displays the values that were obtained from the quantitative microscopical characteristics. The results show that the total number of stomata in the top layer of epidermis was 34 ± 0.27, whereas the number in the lower epidermis was 48 ± 0.75. The vein terminal was identified as 23.1 ± 0.54, and the vein islet number was discovered to be 5.0 ± 0.54.

### 3.1.5 Determination of the physical constants

The results for the physical variables, including volatile oil, loss on drying, ash values, swelling index, etc., are shown in Table 2. The total amount of ash obtained was close to 10.75%, as shown in Table 2. It was discovered that the water-soluble ash content was 6.43%. Since the amount of acid-insoluble ash in the raw material was 0.25%, it may be concluded that there is no earthy debris adhering to the plant's leaves. The extractive values of *I. pes-tigridis* are shown in Table 3.

**Table 1: Leaf constant parameters of *I. pes-tigridis***

| S. No. | Parameters | Value |
|---|---|---|
| 1 | Stomatal number in upper epidermis | 34.00 ± 0.27 |
| 2. | Stomatal number in lower epidermis | 48.00 ± 0.75 |
| 3. | Stomatal index in upper epidermis | 32.23 ± 0.46 |
| 4. | Stomatal index in lower epidermis | 47.78 ± 0.22 |
| 5. | Vein islet number | 05.00 ± 0.54 |
| 6. | Vein termination number | 23.10 ± 0.54 |

Value expressed as mean ± SEM (n=3).

**Table 2: Physiochemical parameters of *I. pes-tigridis***

| S. No. | Parameters | Values expressed in % |
|---|---|---|
| 1 | Volatile oil | Nil |
| 2 | Foreign organic matter | 0.02 ± 0.01 |
| 3 | **Ash values** | |
| | i) Total ash | 10.75 ± 0.08 |
| | ii) Water soluble ash | 6.43 ± 0.08 |
| | iii) Acid insoluble ash | 0.25 ± 0.01 |
| 4 | Loss on drying | 2.72 ± 0.11 |
| 5 | Foaming index | Less than 100 |
| 6 | Swelling index | Initial volume- 2.08 ± 0.09 |
| | | Final volume- 9.78 ± 0.03 |

Value expressed as mean ± SEM (n=3).

**Table 3:** Extractive values of *I. pes-tigridis*

| S. No. | Extractive values | Values expressed as % |
|---|---|---|
| 1 | Petroleum ether | 0.40 ± 0.02 |
| 2 | Hexane | 0.96 ± 0.03 |
| 3 | Chloroform | 0.88 ± 0.01 |
| 4 | Acetone | 02.16 ± 0.02 |
| 5 | Ethylacetate | 03.60 ± 0.05 |
| 6 | Methanol | 18.96 ± 0.22 |
| 7 | Ethanol | 15.24 ± 0.18 |
| 8 | 70% ethanol | 28.08 ± 0.12 |
| 9 | Aqueous | 22.50 ± 0.05 |

Value expressed as mean ± SEM (n=3).

### 3.1.6 Powder analysis

Table 4 shows the powder's behaviour in interaction with several organic reagents and compounds. After being subjected to reagents, the powder examination showed that it comprised phytosterols, tannins, proteins, flavonoids, and phenolic substances.

### 3.1.7 Fluorescence analysis

The findings of the fluorescence investigation for the powder and several extracts of *I. pes-trigridis* are displayed in Tables 5 and 6. After being exposed to aqueous sodium hydroxide, the powder had an appearance of green and black when seen at 254 nm and 365 nm, respectively. The powder showed dark green when observed with iodine at 254 nm and violet when examined with iodine at 366 nm.

**Table 4: Reaction of *I. pes-tigridis* powder with various chemical reagents**

| S. No. | Powder + Reagent | Observation | Active principle |
|---|---|---|---|
| 1 | Picric acid | Yellow precipitate | Protein present |
| 2 | Con. sulfuric acid | Reddish brown | Phytosterols present |
| 3 | Lieberman Burchard | Reddish brown | Phytosterols present |
| 4 | Aqu. Ferric chloride | Greenish black | Tannins present |
| 5 | Iodine solution | Blue color | Starch present |
| 6 | Mayer's reagent | No cream color | Alkaloids absent |
| 7 | Spot test | No stain | Fixed oils absent |
| 8 | Sulfo salicylic acid | White precipitate | Protein present |
| 9 | Aqu. sodium hydroxide | Yellow | Flavonoids present |
| 10 | Mg-HCl | Magenta color | Flavonoids present |
| 11 | Aqueous lead acetate | White precipitate | Tannins present |

**Table 5: Fluorescence analysis of powder of *I. pes-tigridis***

| S.No. | Powder+reagent | Daylight | UV (254 nm) | UV (366 nm) |
|---|---|---|---|---|
| 1. | Powder of *I. pes-tigridis* | Green | Dark green | Greenish black |
| 2. | Powder+aqu. 1M sodium hydroxide | Green | Greenish black | Black |
| 3. | Powder+alc. 1M sodium hydroxide | Green | Green | Violet |
| 4. | Powder+iodine | Reddish brown | Dark green | Black |
| 5. | Powder+10%potassium hydroxide | Pale green | Green | Black |
| 6. | Powder+1M hydrochloric acid | Yellowish green | Green | Brownish black |
| 7. | Powder+gla. acetic acid | Yellowish green | Green | Black |
| 8. | Powder+ 50% sulphuric acid | Green | Greenish black | Black |
| 9. | Powder+50% nitric acid | Dark green | Greenish black | Black |
| 10. | Powder+50% hydrochloric acid | Dark green | Greenish black | Black |

**Table 6: Fluorescence analysis of *I. pes-tigridis***

| S.No. | Extracts | Consistency | Colour in day light | Colour under UV lamp | |
|---|---|---|---|---|---|
| | | | | 366 nm | 254 nm |
| 1. | Petroleum ether | Semi solid | Yellowish green | Violet | Green |
| 2. | Hexane | Semi solid | Greenish black | Black | Green |
| 3. | Chloroform | Semi solid | Greenish brown | Orange | Green |
| 4. | Ethylacetate | Semi solid | Yellowish green | Greenish black | Green |
| 5. | Ethanol | Semi solid | Yellowish green | Blackish green | Green |
| 6. | Methanol | Semi solid | Yellowish green | Black | Green |
| 7. | Aqueous | Semi solid | Brown | Black | Blackish green |

### 3.2 Preliminary phytochemical screening

Table 7 shows that the preliminary phytochemical screening results for *I. pes-tigridis* leaf powder extracted with different solvents (petroleum ether, chloroform, methanol, ethanol, ethyl acetate, and aqueous extracts) contained various phytoconstituents such as carbohydrates, proteins, terpenoids, steroids, phenolic compounds, flavonoids, resins, and tannins.

**Table 7: Preliminary screening of the various extracts of leaf powder of *I. pes-tigridis***

| Phytochemical tests | Pet. ether extract | Chloroform extract | Ethyl acetate extract | Methanol extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|---|---|
| Sterols | + | - | - | - | - | - |
| Carbohydrate | - | - | - | + | + | + |
| Proteins | - | - | - | - | - | + |
| Alkaloids | - | - | - | - | - | - |
| Polyphenol | - | + | + | + | + | + |
| Flavonoids | - | - | + | + | + | + |
| Terpenoids | - | + | + | + | + | + |
| Resins | + | - | - | - | - | - |
| Tannins | - | + | - | + | + | - |

(+) Indicates presence and (-) Indicates absence

#### 3.2.1 Phytochemical analysis by thin layer chromatography

Thin layer chromatography experiments were used to evaluate the 70% ethanolic extract of *I. pes-tigridis*. TLC plates were analysed using both UV and regular light sources. The spots visible in natural light are displayed, and Table 8 shows that the Rf values of the spots varied depending on the solvent system. According to Table 8, the presence of a flavonoid component may be the reason for the yellow colour spot visible on the TLC plate of the ethanolic (70%) extract of *I. pes-tigridis* at daylight.

**Table 8: Phytochemical evaluation of *I. pes-tigridis* by TLC studies**

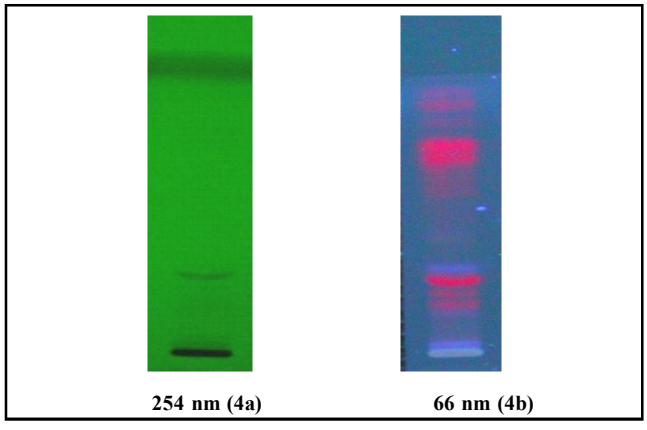
| Solvent system | UV | | | Day light | | |
|---|---|---|---|---|---|---|
| | No. of spots | Rf | Colour | No. of spots | Rf | Colour |
| Chloroform: Ethyl acetate (60:40) | 1 | 0.79 | Orange fluorescence | 1 | 0.79 | Yellow |
| | 2 | 0.61 | Orange | 2 | 0.61 | Yellow |
| | 3 | 0.38 | Yellowish orange | 3 | 0.38 | Green |
| | 4 | 0.12 | Pale orange | 4 | 0.12 | Pale green |
| Toluene: Ethyl acetate (7.5:2.5) | 1 | 0.72 | Orange fluorescence | 1 | 0.72 | Dark yellow |
| | 2 | 0.65 | Orange | 2 | 0.65 | Yellow |
| | 3 | 0.57 | Pale orange | 3 | 0.57 | Grey |
| | 4 | 0.12 | Pale orange | 4 | 0.51 | Green |
| | - | - | - | 5 | 0.46 | Light green |
| | - | - | - | 6 | 0.33 | Yellowish green |
| | - | - | - | 7 | 0.22 | Pale green |

| | | | | | | |
|---|---|---|---|---|---|---|
| Toluene:ethyl | 1 | 0.73 | Dark orange | 1 | 0.73 | Yellow |
| acetate: formic | 2 | 0.65 | Orange | 2 | 0.65 | Dark yellow |
| acid (12:6:0.1) | 3 | 0.53 | Pale orange | 3 | 0.53 | Pale yellow |
| | 4 | 0.47 | Orange | 4 | 0.47 | Grey |
| | - | - | - | 5 | 0.38 | Green |
| | - | - | - | 6 | 0.25 | Pale yellow |
| | - | - | - | 7 | 0.2 | Pale green |
| | - | - | - | 8 | 0.15 | Green |
| Toluene:acetone: | 1 | 0.82 | Dark orange | 1 | 0.82 | Yellow |
| Formic acid | 2 | 0.72 | Orange | 2 | 0.72 | Dark yellow |
| (30:10:0.1) | 3 | 0.64 | Orange | 3 | 0.64 | Pale yellow |
| | 4 | 0.54 | Pale orange | 4 | 0.54 | Pale green |
| | - | - | - | 5 | 0.45 | Green |
| | - | - | - | 6 | 0.41 | Green |
| | - | - | - | 7 | 0.33 | Pale green |

The TLC plates revealed the existence of various active ingredients in the 70% ethanolic extract of *I. pes-tigridis* when they were analysed following the use of various detecting chemicals (Table 9). There were four spots on Folin Ciocalteu's reagent-sprayed plate with Rf values of 0.07, 0.54, 0.76, and 0.90.

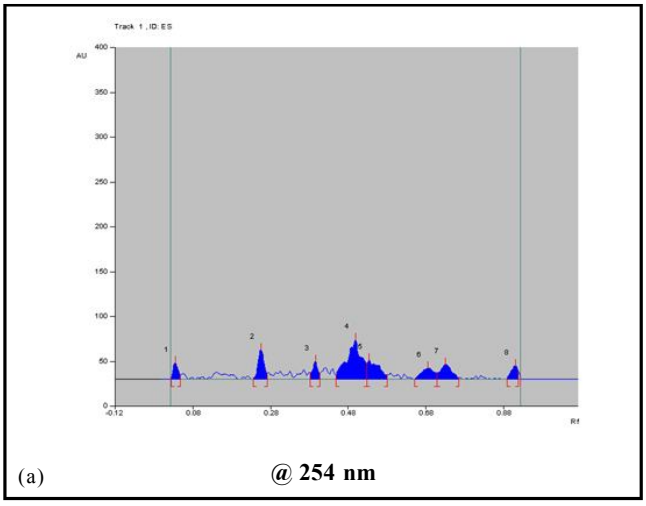
**Table 9: TLC finger profile of the 70% ethanolic extract of *I. pes-tigridis***

| Solvent system | Detectingagent | No. of spots | Color of spots | Rf values |
|---|---|---|---|---|
| Toluene:ethylacetate: | Folin Ciocalteu's | **4** | **Day light** | |
| formicacid (12:6:0.1) | reagent | 1 | Darkblue | 0.07 |
| | | 2 | Blue | 0.54 |
| | | 3 | Blue | 0.76 |
| | | 4 | Blue | 0.90 |
| | | **6** | **at 366 nm** | |
| | Under | 1 | Fluorescence | 0.07 |
| | UV light | 2 | Pinkish red | 0.52 |
| | | 3 | Pinkish red | 0.56 |
| | | 4 | Pinkish red | 0.66 |
| | | 5 | Pinkish red | 0.78 |
| | | 6 | Pinkish red | 0.83 |

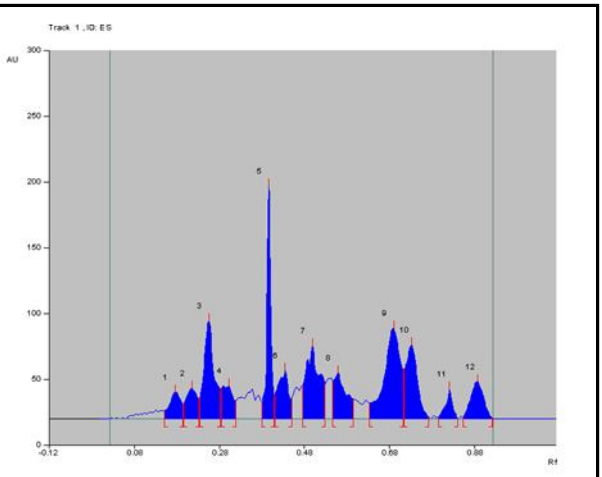


**254 nm (4a)** **66 nm (4b)**

**Figure 4: Visualization of 70% ethanolic extract of *I. pes-tigridis* in HPTLC.**



(a) **@ 254 nm**




(b) @ 366 mm

**Figure 5: HPTLC fingerprint of *I. pes-tigridis* at 254 nm and 366 nm.**

### 3.2.2 Phytochemical analysis by HPTLC studies

Figure 4 shows the TLC visualisation, a 3D presentation of the fingerprint profile, and peak displays at 254 nm and 366 nm. Eight spots are visible in the TLC plate at 254 nm, whereas 12 spots are visible at 366 nm. Figure 5 shows the peak displays of the 70% ethanolic extract of *I. pes-tigridis* at 254 nm and 366 nm, as well as the 3D presentation of the fingerprint profile. At 254 nm, the display indicates the existence of eight peaks; however, at 366 nm, it indicates the presence of 12 peaks.

## 4. Discussion

For the development of a standardised quality control profile for herbal medicine, the World Health Organisation encouraged the inclusion of the physicochemical and phytochemical evaluation of crude drug materials. The pharmacognostic standardisation of *I. pes-tigridis* leaves is considerably established by this investigation. The organoleptic character of the leaf powder of *I. pes-tigridis* showed that it was green in colour with a characteristic odour and taste. Macroscopic and microscopic results show that the leaves are bushy, 5-9 septate, made up of parenchymatous root tissue and thick, slightly curved epidermal cells. Crystals of calcium oxalate are seen in the parenchyma, which is spongy. The anticlinal walls of the epidermal cells are thick and wavy, and they give the appearance of being amoeboid in shape. The single bowl-shaped, bicollateral vascular strand has roughly 11 short lines of xylem components intermingled with sclerenchymatous ground tissue. The xylem components are lignified, circular tubes with thick walls. Along the bottom and top surfaces of the xylem strand, there are several little phloem nests. Additionally, powder microscopic characters of leaf powder show that calcium oxalate crystals, epidermal trichomes, and glandular trichomes should comply with the pharmacognostic standardisation. One of the standardisation parameters of physiochemical quantification is important for the detection of adulterated drugs or careless handling of raw materials (WHO, 1996). Another measurement, the ash value, provides information about the plant's inorganic composition and other contaminants. Less moisture content will be a sign of greater drug stability (Musa *et al*., 2006). According to the results of different solvent extractions, 70% ethanol and water extract had the highest percentage extractive value of all the solvents. As a result, menstrum was extracted using 70% ethanol to obtain active ingredients for phytochemical and pharmacological testing. Similarly, fluorescence analysis is a crucial method for identifying the chemical components of herbal medicines and giving insight into their nature as chemicals (Alarm and Saqib, 2015).

From the preliminary phytochemical screening, it was observed that the petroleum ether extract contained sterols, whereas the methanol and ethanol extracts contained carbohydrates, proteins, flavonoids, resins, and terpenoids. The aqueous extract exhibited the presence of flavonoids, proteins, and carbohydrates. The flavonoid compound may have been related to the yellow colour spot that was visible on the TLC plate of the ethanolic (70%) extract of *I. pes-tigridis* in daylight conditions. The existence of eight and twelve spots, accordingly, was shown by the 3D presentation of the HPTLC fingerprint profile and the peak show of the 70% ethanolic extract of *I. pes-tigridis* at 254 nm and 366 nm. The Rf value of 0.7 among the appropriate regions may indicate a flavonoid compound's probability. The development of drugs is facilitated by the existence of phytoconstituents that are connected to the plant's or products distinct medicinal properties (Upadhyay *et al*., 2019). For instance, flavonoids extracted from various plants exhibit a broad range of activities. According to several academic studies (Lin *et al*., 2009), they have been discovered to have antioxidant, antimicrobial, hypolipedimic, anticancer, aptosis-inducing effects (Galati Brien, 2004), and anti-inflammatory properties (Lazaro, 2009; Amara *et al*., 2009). Terpenoids have been found to have antibacterial and anti-inflammatory effects in plants (Singh and Singh, 2003; Neukirch *et al*., 2001). Plant tannins may also have effective antioxidant, anti-inflammatory, and wound-healing capabilities (Kaur and Arora, 2009; Zhang and Lin, 2008; Fawole *et al*., 2011; Leite *et al*., 2002). Numerous alkaloids were said to have an antidiabetic effect (Paolisso *et al*., 1985; Gulfraz *et al*., 2008). In addition, studies have shown that saponins have strong antimicrobial antioxidant properties, hypolipedemic activity, hemolytic activity, cholesterol binding properties, and bitterness (Gepdireman *et al*., 2005; Okwu *et al*., 2004; Gepdireman *et al*., 2005). Numerous polyphenolic and flavonoid chemicals have been proven in studies to have antioxidant and anti-inflammatory properties (Yano *et al*., 2008; Mandal *et al*., 2005). For establishing the variable within the same plant material, chromatography is one of the globally accepted methods. TLC and HPTLC methods find the majority of use in the pharmaceutical industry for method development, identification, and detection of adulterants in herbal products, and quality control of medicated herbs. Hence, *I. pes-tigridis* proved the standardisation parameters and HPTLC analysis from the results in a satisfactory way. The WHO mentioned that these studies are probably beneficial in the preparation of herbal broths and formularies.

## 5. Conclusion

The above findings can be used as appropriate quality control assessments to confirm the integrity, security, and effectiveness of this herbal medicine ingredient. The numerous researched elements can be used to recognise and authenticate *I. pes-tigridis*, a traditionally immense medicinal plant. According to the phytochemical investigations, it is a good plant to look into for phytochemical and pharmacological tests. Additionally, this research will be important in reducing the likelihood of adulteration of this beneficial medication when it is sold as a powder. Hence, on the basis of our study, *I. pes-tigridis* is the reference for future research.

## Conflict of interest

The authors declare no conflict of interest relevant to this article.

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