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# Pharmacognostic evaluation of *Cayratia trifolia* (Linn.) leaf

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ABSTRACT

**Objective:** To present a detailed pharmacognostic study of the leaf of *Cayratia trifolia* (*C. trifolia*) Linn. (Vitaceae), an important plant in the Indian system of medicine. **Methods:** The macroscopy, microscopy, physiochemical analysis, preliminary testing, fluorescence analysis of powder of the plant and other WHO recommended methods for standardization were investigated. **Results:** Leaves are trifoliolated with petioles (2-3 cm) long. Leaflets are ovate to oblong-ovate, (2-8 cm) long, (1.5-5 cm) wide, pointed at the tip. The leaf surface shows the anisocytic type stomata covered with guard cells followed by epidermis layer. Leaf surface contents including veins, vein islet and vein termination were also determined. Transverse section of leaf shows the epidermis layer followed by cuticle layer and vascular bandles (xylem and phloem). The mesophyll is differentiated into palisade and spongy parenchyma. Abundant covering trichomes emerge from the upper epidermis. Trichomes are uniseriate and multicellular. Strips of collenchyma are present below and upper layer of epidermis. **Conclusions:** It can be concluded that the pharmacognostic profile of the *C. trifolia* is helpful in developing standards for quality, purity and sample identification.

## 1. Introduction

*Cayratia trifolia* (*C. trifolia*) Linn. Domin Syn. (Family: Vitaceae) is commonly known as fox grape in English, Amlabel, Ramchana in Hindi and Amlavetash in Sanskrit. It is native to India, Asia and Australia. It is a perennial climber having trifoliated leaves with (2-3 cm) long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white and brown in colour[1,2]. Fruits are fleshy, juicy, dark purple or black, nearly spherical, about 1 cm in diameter[3]. This perennial climber is also found in the hilly regions as well as the hotter part of India from Jammu and Rajasthan to Assam extending into the peninsular India up to 600 m height[4]. Whole plant of *C. trifolia* has been reported to contain yellow waxy oil, steroids, terpenoids, flavonoids and tannins by preliminary phytochemical screening. Leaves contain stilbenes, piceid, reveratrol, viniferin and ampelopsin. Stem, leaves, roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidins are reported in the leaves. This plant also contains kaempferol, myricetin, quercetin, triterpenes and epifriedelanol[4]. Root paste mixed with coconut oil can be used as decoction. Roots grounded with black pepper can be used as poultice on boils[5]. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. Paste of tubers is applied on the affected part in the treatment of snake bite. Whole plant is used as diuretic, in tumors, neuralgia and splenopathy[6]. Its climbers are wrapped around the neck of frantic bullock whereas poultice of leaves is used to yoke sores of bullock[7]. The bark extract has been reported to have antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activities in animal models[4]. For the standardization and quality assurance purpose, the following three attributes must be verified: authenticity, purity and assay[8]. Hence, in this work we make an attempt for the standardization of *C. trifolia* leaf by carrying out its pharmacognostic evaluation.

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## 2. Materials and methods

### 2.1. Chemicals and instruments

Phloroglucinol, glycerin, hydrochloric acid, potassium

hydroxide and all other chemicals used in the study were of analytical grade.

### 2.2. Plant material

The leaves of *C. trifolia* Linn. were collected from the Campus Kurukshetra University, Kurukshetra in the month of October 2010. The plant was authenticated by Dr. Singh HB, Scientist-F and Head, Raw Herbarium and Museum NISCAIR, New Delhi, India. A voucher specimen of the plant was preserved in the herbarium (NISCAIR/RHMD/Consult/-2010-11/1548/146) for further reference.

### 2.3. Macroscopic and microscopic analysis

The macroscopy and microscopy of the plant were studied according to the method of Brain and Turner[9,10]. For the microscopical studies, transverse sections were prepared and stained. The powder microscopy was performed according to the methods of Kokate and Khandelwal[11,12].

### 2.4. Physiochemical analysis

Physiochemical values such as the percentage of ash values and extractive values were determined according to the official method[13-15] and the WHO guidelines on quality control methods for medical plants materials[16].

### 2.5. Preliminary phytochemical screening

Preliminary screening was carried out using the standard procedure described by Kokate[11].

### 2.6. Florescence analysis

Powdered leaf material was analyzed under visible light, short ultra violet light, long ultra violet light after treatment with various organic/inorganic reagents like chloroform, methanol, petroleum ether, ethyl acetate, 50% sulphuric acid, 50% nitric acid, 50% hydrochloric acid, 10% sodium hydroxide, ethyl acetate and hydrochloric acid (1:1), acetone, *etc*[12,17,18].

## 3. Results

### 3.1. Macroscopic characteristics

*C. trifolia* is a weak herbaceous climber. Leaves are trifoliolated with petioles (2-3 cm) long. Leaflets are ovate to oblong-ovate, (2-8 cm) long, (1.5-5 cm) wide, pointed at the tip. Leaves are green in colour with agreeable odour and bitter taste (Figure 1).

### 3.2. Microscopic characteristics

The leaf surface shows the stomata covered with guard cells followed by epidermis layer (Figure 2A). Epidermal cells are rectangular, thin and straight walled cells. Stomata are anisocytic or unequal celled stomata, three subsidiary cells, one is smaller than other two. Leaf surface analysis also shows the presence of veins, vein islet and vein termination (Figure 2B). Transverse section of leaf shows the epidermis layer followed by cuticle layer and vascular bundles (xylem and phloem). Upper epidermis consists of rectangular cells and the outer wall which contains abundant covering trichomes and anisocytic stomata. Trichomes are uniseriate and multicellular. The mesophyll is differentiated into palisade and spongy parenchyma. Spongy parenchyma is two to three layered, compactly arranged. Strips of collenchyma are present below upper and above lower layer of epidermis. Collenchyma is thick walled with cellulose cells. Collenchyma tissue consists of thick walled rounded parenchymatous cells. Xylem are lignified whereas phloem are non-lignified. Lower epidermis is similar to upper epidermis (Figure 2C).


**Figure 1.** Macroscopic characteristics of *C. trifolia* Linn.
F: Fruit; TL: Trifoliate leaf.

### 3.3. Powder characteristic

The organoleptic evaluation of the leaf powder revealed the following characteristics. The leaf powder is pale green in color, with a characteristic odour and bitter taste. Fibers are elongated distributed (Figure 3A). Trichomes are unicellular, dagged shaped, warty (Figure 3B) and sometimes are in fragments or multicellular (Figure 3C).

### 3.4. Preliminary phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of steroids, terpenoids, yellow wax, fixed oils, flavonoids and carbohydrates (Table 1).

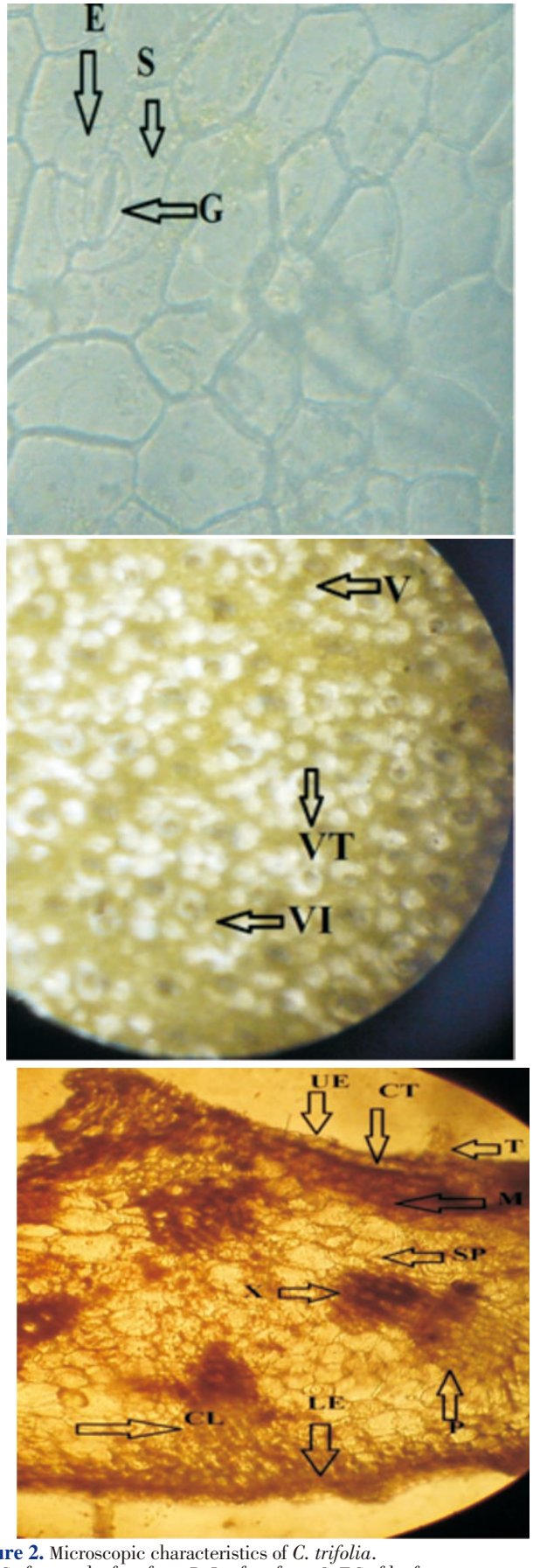


**Figure 2.** Microscopic characteristics of *C. trifolia*.
A: T.S of upper leaf surface; B: Leaf surface; C: T.S of leaf.
S: Anisolytic stomata; E: Epidermis cell; G: Guard cell; V: Veins; VT: Vein termination; VI: Vein islet; UE: Upper epidermis; T: Covering trichomes; CU: Cuticle layer; M: Mesophyll; SP: Spongy parenchyma; X: Xylem; P: Phloem; CL: Collenchyma; LE: Lower epidermis.

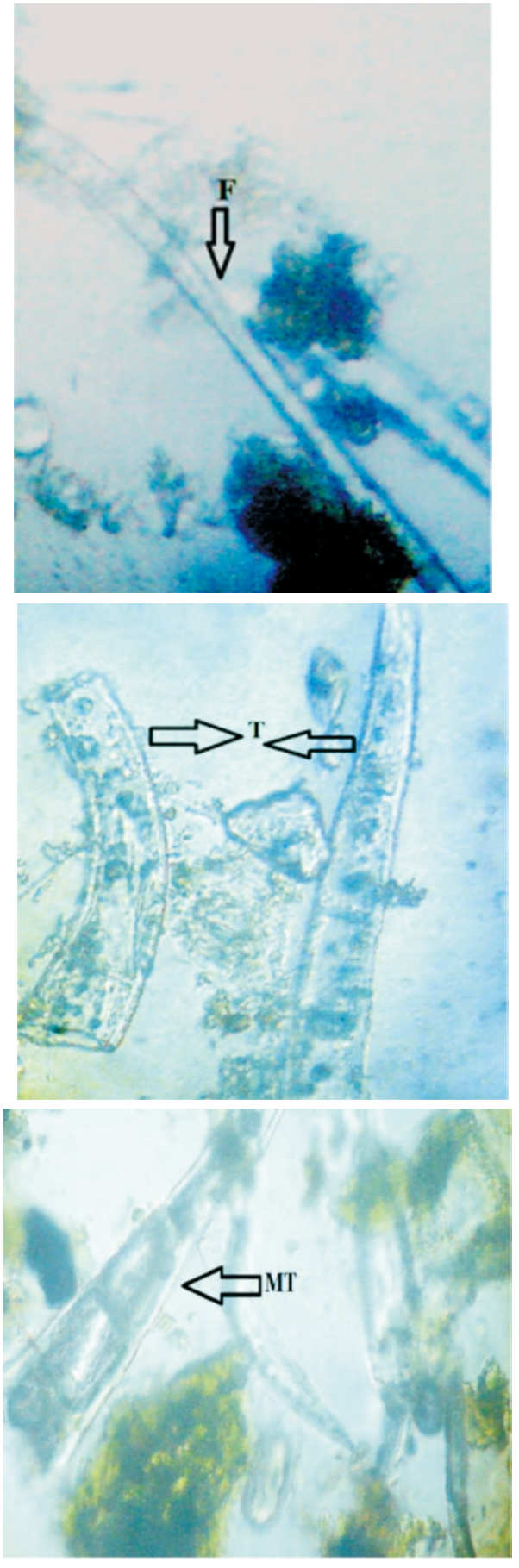


**Figure 3.** Powder characteristics of *C. trifolia*.
A: Powder microscopy; B: Powder study; C: Powder study.
F: Fibers; T: Trichomes; MT: Multicellular trichomes.

**Table 1**
Preliminary phytochemical screening of leaf extract.

| Chemical constituents | Chemical tests | Petroleum ether extract | Hydro-alcoholic extract |
|---|---|---|---|
| Alkaloids | Dragendorff's test | – | – |
| | Mayer's test | – | – |
| Glycosides | Keller-killiani test | – | _ |
| | Borntrager's test | – | _ |
| Saponin glycosides | Foam test | _ | – |
| Flavonoids | Shinoda test | _ | + |
| | Sodium hydroxide test | _ | + |
| | Lead-acetate test | _ | + |
| Tannins | Ferric chloride test | – | + |
| | Bromine solution test | – | + |
| Steroids | Salkowaski test | + | + |
| | Liebermann-burchard test | + | + |
| Carbohydrates | Benedict's test | _ | + |
| | Fehling's test | _ | + |
| | Molisch test | – | + |
| | Selivnoff's test | – | + |
| | Test for pentoses | _ | – |
| | Killer-killiani test | _ | + |

+: Positive; –: Negative.

**Table 2**
Physiochemical parameters of *C. trifolia* Linn.

| Paramters | Value |
|---|---|
| Foreign matter | 0.20% w/w |
| Loss on drying | 2.05% w/w |
| Total ash value | 12.20% w/w |
| Acid insoluble ash | 0.24% w/w |
| Water soluble ash | 9.07% w/w |
| Sulphated ash | 1.03% w/w |
| Swelling index | 4 mL |
| Foaming index | – |
| Stomatal index, lower surface | 25 |
| Stomatal index, upper surface | 33.3 |

**Table 3**
Extractive values of *C. trifolia* leaf.

| Extract | Plant powder taken (g) | Yield (% w/w) |
|---|---|---|
| Pet ether | 2 | 16.65 |
| Chloroform | 2 | 25.10 |
| Methaholic | 2 | 35.40 |
| Water | 2 | 19.45 |

**Table 4**
Florescence analysis of powdered leaf of *C. trifolia* Linn.

| Reagents | Visible | Short ultra violet | Long ultra violet |
|---|---|---|---|
| Chloroform | Green | Green | Red |
| Methanol | Dark green | Pale green | Red |
| Pet ether | Green | Green | Red |
| Ethyl acetate | Green | Fluorescent green | Orange |
| 50% Sulphuric acid | Green | Green | Black |
| 50% Nitric acid | Pale green | Black | Green |
| 50% Hydrochloric acid | Green | Black | Green |
| 10% Sodium hydrooxide | Green | Dark green | Green |
| Ethyl acetate: Hydrochloric acid (1:1) | Green | Green | Orange |
| Acetone | Pale green | Green | Orange |

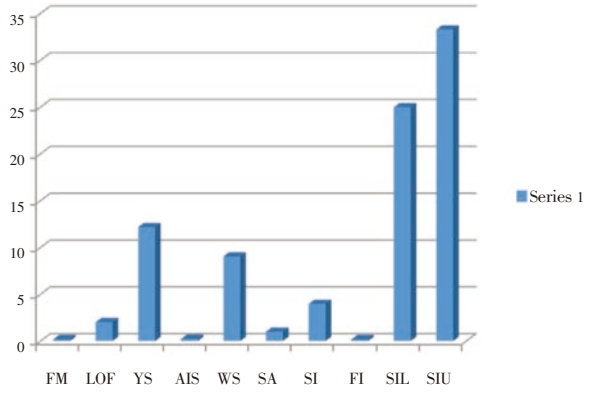


**Figure 4.** Physiochemical parameters of *C. trifolia* Linn.
FM: Foreign matter; LOD: Loss on drying; TA: Total ash value; AIS: Acid insoluble ash; WS: Water soluble ash; SA: Sulphated ash; SI: Swelling index; FI: Foaming index; SIL: Stomatal index of lower surface; SIU: Stomatal index of upper surface.

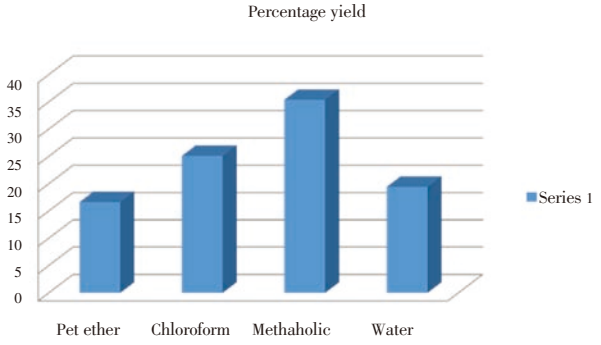


**Figure 5.** Extractive values of *C. trifolia* leaf.

### *3.5. Physiochemical constants*

Ash value of a drug gives an idea about earthy matter or

inorganic composition and other impurities present along with the drug (Table 2, Figure 4). The physiochemical parameters were shown in Table 2, such as total ash value was 12.20%, water soluble ash 9.07%, acid insoluble ash 0.24%, swelling index 4 mL, loss on drying 2.05% and foreign matter was 0.20% w/w, respectively whereas stomatal indexes of upper and lower surfaces were 33.3 and 25, respectively. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Petroleum ether soluble drug was 16.65%, chloroform soluble 25.10%, water soluble 19.45% and methanolic soluble 35.40% w/w (Table 3 and Figure 5). The fluorescence analysis observed in visible, short and long ultra violet was depicted in Table 4.

## 4. Discussion

Standardization is an essential measure of quality, purity and authenticity. Microscopic method is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials[19]. As there is no pharmacognostic work recorded on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Macro and micro standards here can be identifying parameters to substantiate and authenticate the drug. The information obtained from the preliminary phytochemical screening will reveal the useful findings about the chemical nature of the drug. Total ash value, fluorescence analysis and extractive values will be helpful in identification and authentification of the plant material[20,21]. The extractive values are useful to evaluate the chemical constituents of crude drug[22]. Preliminary phytochemical chemical screening ascertained the presence of steroids, tannins, flavonoids, fatty acid and terpenoids in the plant leaf. Transverse section of leaf confirmed the presence of uniceriate trichomes and anisolytic stomata which are characteristics of the family Vitaceae.

In conclusion, the present work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Pharmacognostic evaluation can be useful to substantiate and authenticate the drug.

## Conflict of interest statement

We declare that we have no conflict of interest.

## Acknowledgement

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