



## Studies on Histology and Phytochemical Evaluation of *Wattakaka volubilis* (L.f.) Stapf.

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### ABSTRACT

The present study was carried out to determine the requisite histological features of root, stem, leaf, petiole and phytochemical analysis for evaluating *Wattakaka volubilis*, an important medicinal plant, used in the traditional systems of medicine. This study provides referential pharmaco-botanical and phytochemical information for authentic identification of this plant.

**Keywords:** *Wattakaka volubilis*, Histology, Phytochemical, Authentication, Medicinal plant, Standardization.

### INTRODUCTION

Using plants as drugs has been a goal for mankind since prehistoric times. Most of the drugs in use have their origin from plants used by healers since time immemorial. The World Health Organization (WHO) has listed 21,000 plants, which are used for the medicinal purpose around the world. Among these 2500 species are from India and 150 species are used commercially on fairly large scale. The use of synthetic drug with impurities resulting in higher incidence of adverse drug reaction. This has motivated mankind to go back to nature for safer remedies. Therefore, quality control standards of various medicinal plants used in indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants. Standardization refers to the body of information and controls necessary to produce material of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agriculture and manufacturing processes. The development of parameters for quality control of herbal drugs is a big task involving biological evaluation for a particular disease area, chemical profiling to the raw materials and laying down specializations for the finished products.<sup>1-3</sup>

*Wattakaka volubilis* (L.f.) Stapf., (Syn. *Dregea volubilis* (L.f.) Benth. ex Hook.f., *Marsdenia volubilis* Cooke) belongs to the family Asclepiadaceae, is a tall woody climber, with densely lenticellate and pustular branches, leaves opposite, broadly ovate or suborbicular, cordate, acuminate, flowers bright yellowish-green, in lateral drooping, umbellate, cymes, follicle usually 2, lanceolate covered with brown, mealy, tomentum, turgid, c. 2cm long; seeds yellowish brown broadly ovate or broad elliptic, winged, comose.<sup>4</sup> The leaves are used in boils and abscesses. The roots and tender stalks are considered emetic and expectorant.<sup>5-6</sup> It is also used in eye diseases and snake bites.<sup>7</sup> Roots possess significant antibacterial

and antifungal activity.<sup>8</sup> Ram *et al.*,<sup>9</sup> reported neuropharmacological effects of drevogenins isolated from this plant. Antidiabetic and antioxidant activity was also reported in leaf ethanolic extract.<sup>10</sup> Sandhiya *et al.*,<sup>11</sup> reported the protective effect of leaf extract against stress induced amnesia & useful in combating the stress induced CNS disorders. The present study has been carried out to standardize the histological features of leaf, stem, petiole, root and phytochemical analysis to serve as a possible tool for proper identification of *Wattakaka volubilis* (L.f.) Stapf.

### MATERIALS AND METHODS

#### Histological studies

For the present study, fresh plant was collected and authenticated using regional flora.<sup>12</sup> The fresh samples of leaf, petiole, stem and root were cut in to small pieces and fixed immediately in Formalin-Acetic-Alcohol for 24h. After fixation they were washed thoroughly in distilled water, dehydrated, embedded in paraffin wax after infiltration and sectioned using rotary microtome to the thickness of 8 to 14  $\mu$ m.<sup>13</sup> Sections were stained with toluidine blue.<sup>14</sup> For the study of tracheary elements, the stem and roots were macerated employing Jeffrey's fluid and stained with safranin.<sup>15</sup> For the analysis of leaf epidermis in surface view and vein clearing, small rectangles were taken from the median third of the leaf-blade and dissociation proceeded by treated with 5% sodium hydroxide (NaOH) at 60°C for 3 to 4 hrs., thoroughly washed and stained with safranin. All the photomicrographs were taken using Nikon Eclipse 400 microscope.

#### Phytochemical studies

The whole plant was shade dried and powdered. Powdered samples were subjected to preliminary phytochemical screening using standard procedures.<sup>16-18</sup> Total alkaloid content was also determined according to



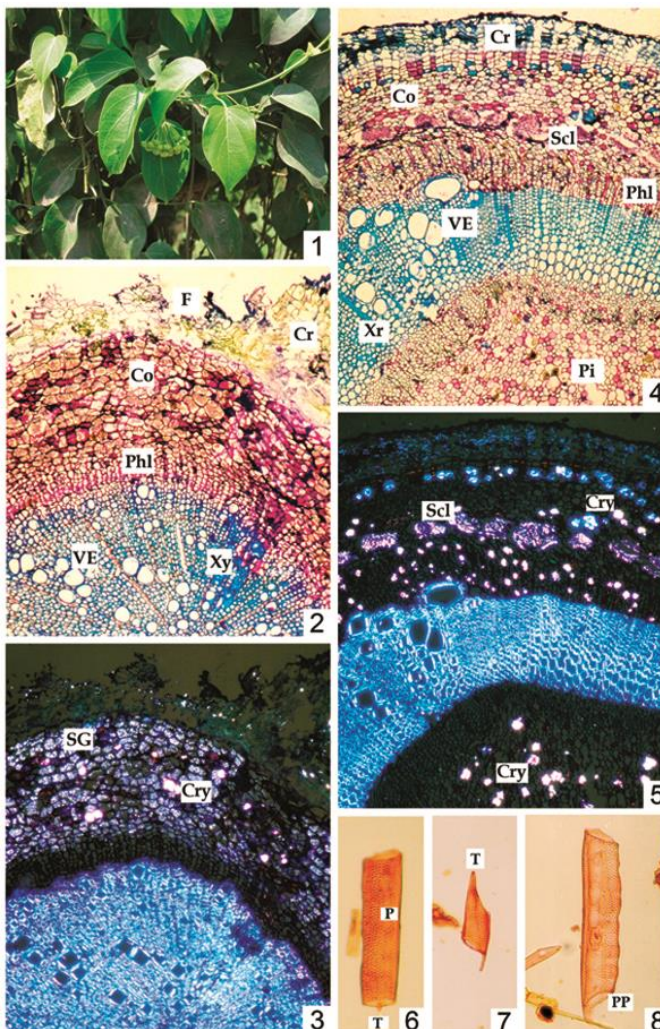
Abdelouaheb *et al.*<sup>19</sup> Cardiac glycosides (Cardenolide) were extracted as per the procedure followed by Wagner and Blatt,<sup>20</sup> and used for the TLC analysis. For TLC precoated Silica Gel F<sub>254</sub> (E.Merck) plate was used for stationary phase and Ethylacetate : methanol : water (100:13.5:10) used for mobile phase. After development the plate was sprayed with 10% ethanolic sulphuric acid and heated at 105°C in hot air oven for 5 to 10 min to develop the spots. For alkaloids, methanol : acetic acid (9.9:0.1) used as mobile phase and Dragendroff's reagent used for spray.

**OBSERVATIONS**

**Histology of Root (Plate – 1: 2 – 3; 6-7)**

A cross section of 5 mm thickness root shows well developed cork with fissures. Presence of druses type of calcium oxalate crystals and abundant starch grains in the cortex region. In secondary xylem, vessels are circular or polygonal in outline, wider or narrow, arranged in diffuse porous with pores solitary or short radial multiples. Presence of predominant uniseriate xylem rays and rarely biseriate. Vessel elements are narrow, long with simple pits, simple perforation plate and tailed.

**Plate 1**



**Plate 1:**

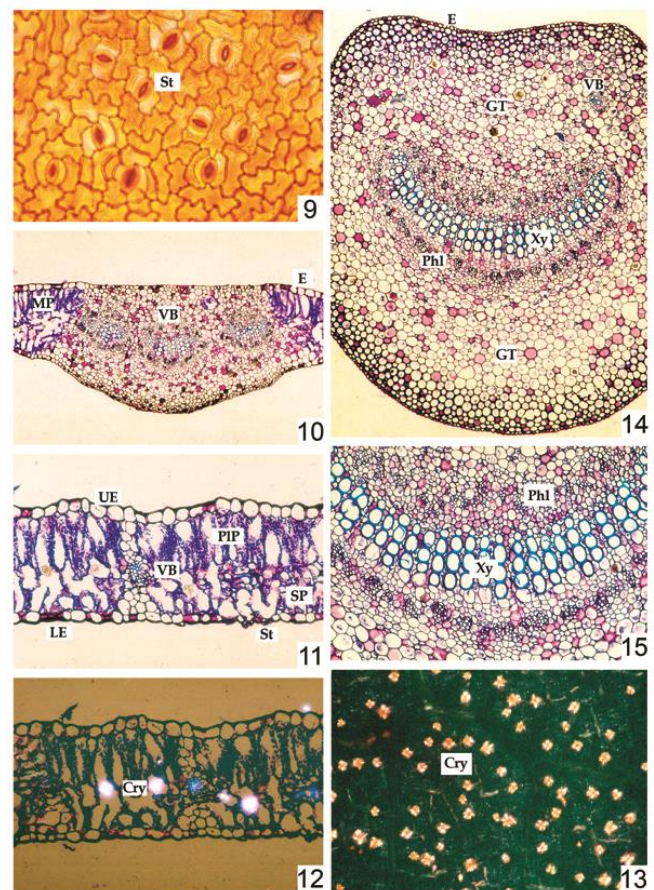
1. Habit
2. Cross section of root
3. Cross section of root under polarized light
4. Cross section of stem
5. Cross section of stem under polarized light
6. & 7. Vessel elements in root
8. Vessel elements in stem

(Co – Cortex; Cr – Cork; Cry – Crystals; F – Fissure; P – Pits; Phl – Phloem; Pi – Pith; PP – Perforation plate; Scl – Sclerenchyma; SG – Starch grains; T – Tail; VE – Vessel elements; Xr – Xylem rays; Xy – Xylem)

**Histology of Stem (Plate – 1: 4 – 5 & 8)**

A cross section of 6 mm thickness stem shows distinct cork layers without fissures. Presence of abundant druses type of calcium oxalate crystals and patches of phloem fibres, starch grains in the cortex region. Secondary xylem arranged in tetragonal shape, vessels are aggregated in the angled region, circular or polygonal in outline. Predominantly fibre tracheids are present. Vessel elements are wide, long with simple pits, simple perforation plate with tail.

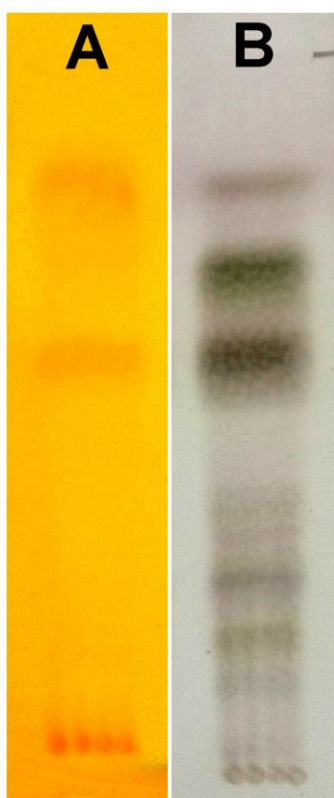
**Plate 2**



**Plate 2:**

9. Leaf epidermis
10. Cross section of leaf midrib
11. Cross section of lamina
12. Cross section of lamina under polarized light
13. Leaf vein clearing under polarized light
14. Cross section of petiole
15. Cross section of petiole (enlarged)

(Cry – Crystals; E – Epidermis; GT – Ground tissue; LE – Lower epidermis; MP – Mesophyll; Phl – Phloem; PIP – Palisade parenchyma; SP – Spongy parenchyma; St – Stomata; UE – Upper epidermis; VB – Vascular bundle; Xy – Xylem)

**Plate – 3.****Plate – 3.**

- A. TLC profile of crude alkaloid extract  
 TLC profile of cardenolides extract

**Histology of Leaf (Plate – 2: 9 - 13)**

The upper epidermis of the leaf consisted of polygonal, straight and thick walled cells. The lamina has narrowly cylindrical single layer of palisade parenchyma and spherical spongy parenchyma cells forming a network. Vascular bundles are various sizes. Three vascular bundles, one dorsal and two laterals and lateral bundles are comparatively small in size. Parenchymatous ground tissue is present. Crystals are scanty in the parenchyma. Vascular bundle consists of xylem and phloem.

Protoxylem toward the upper epidermis and metaxylem towards the lower epidermis. The stomata is paracytic type and hypostomatic (stomata occur only on lower sides) leaf. Trichomes present in lower epidermis.

**Histology of Petiole (Plate – 2: 14 - 15)**

The petiole in sectional view was circular with less distinct adaxial flat side. The surface was even and glabrescent. A large middle vascular bundle and two small lateral vascular bundle. Thus, the arrangement is expressed as 1 + 2. The middle vascular bundle consists of a central main bowl – shaped vascular strand. Next to the epidermis three layers of collenchyma and chlorenchyma cells are present. Ground tissue is parenchymatous. Vascular bundle consists of xylem and phloem. Xylem vessel elements are seen.

**Phytochemical analysis**

The presence and absence of different phyto-constituents, quantitative estimation of total alkaloids and TLC fingerprint profiles of cardenolide extract are presented in Tables – 1 & 2.

**Table 1:** Preliminary phytochemical analysis

S. No	Chemical constituents	<i>Wattakaka volubilis</i>
1	Tannins	+
2	Phlorotannins	-
3	Saponins	+
4	Flavonoids	+
5	Steroids	-
6	Terpenoids	+
7	Cardiac glycosides	+
8	Alkaloids	+
9	Quinones	-
10	Total Alkaloid content	0.030%

**Table 2:** TLC analysis

Crude Alkaloid Extract (Plate – 3:A)	
R <sub>f</sub> Values	Colour of the spot
0.57	Orange red
0.79	Orange red
Cardenolide extract (Plate – 3:B)	
0.12	Violet
0.18	Green
0.26	Violet
0.32	Pink
0.57	Dark green
0.70	Green
0.82	Light green

## DISCUSSION AND CONCLUSION

Traditional systems of medicine such as Ayurveda, Siddha and Unani uses majority of the crude drugs from plant origin. When a crude plant drug is subjected to pharmacological or pharmaceutical standardization its botanical identity becomes an imperative prerequisite. The role of plant anatomist is sought at this juncture to provide a set of microscopic features of the drug which will help to considerable extent to ascertain the botanical identity of the drug in question. Histology and phytochemical perspective of medicinal plants in an integral component of pharmacognosy, especially while proposing diagnostic protocols for establishing the botanical and chemical identity and ascertaining the quality control of raw materials.<sup>21</sup>

The following histology and phytochemical features of the above drug are the key features that can be used to diagnose this plant.

**Root:** Cork with fissures; druses type of calcium oxalate crystals and abundant starch grains in the cortex; vessels are circular or polygonal in outline, wider or narrow, arranged in diffuse porous with pores solitary or short radial multiples; predominant uniseriate xylem rays; vessel elements - narrow, long with simple pits, simple perforation plate and tailed.

**Stem:** Distinct cork layers without fissures; abundant druses type of calcium oxalate crystals and phloem fibres, starch grains in the cortex; secondary xylem - tetragonal shape, vessels - aggregated in the angled region, circular or polygonal in outline; vessel elements - wide, long with simple pits, simple perforation plate with tail.

**Leaf:** Druses type of calcium oxalate crystals in mesophyll; three vascular bundles, one dorsal and two laterals; stomata - paracytic and hypostomatic; trichomes in lower side of the midrib.

**Petiole:** Circular with adaxial groove; vascular bundle open type; three vascular bundles arrangement is 1 + 2. Dorsal bundle is large bowl shaped and laterals are very small circular; crystals are present.

**Phyto-constituents:** Tannins, Saponins, flavonoids, Terpenoids, cardiac glycosides and alkaloids are present.

**TLC profile:** Crude alkaloid – 0.57, 0.79 (Orange red); Cardenolides – 0.12 (Violet), 0.18 (Green), 0.26 (Violet), 0.32 (Pink), 0.57 (Dark green), 0.70 (Green), 0.82 (light green).

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