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Studies on *Wattakaka volubilis* (L. F.) Stap. A medicinally important plant

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ABSTRACT

The use of herbal medicine for the treatment of diseases and infections is a safe and traditional therapy. Hence, medicinal plants have been receiving great attention worldwide by the researchers because of their safe utility. The plant *Wattakaka volubilis* is a climbing shrub of the family Asclepiadaceae. Traditionally, the plant is useful in cold and eye disease. Leaves are used as an application to boils and abscesses. However, it has not yet been studied pharmacognostically. The study includes macroscopy and microscopy, phytochemistry including fluorescence and ash analysis of the leaf and stem of *W. volubilis*. Phytochemical screening of the plant revealed the presence of tannins, saponins and alkaloids. The detailed pharmacognostic account of *W. volubilis* which includes macroscopic and microscopic characters will be helpful for the correct botanical identification of the drug. In addition, the values of percentage extractive and ash analysis, results of fluorescence analysis and phytochemical data will be helpful for the standardization and quality control of precious indigenous drug. The study scientifically validates the use of plant in traditional medicine.

Keywords: *Wattakaka volubilis*, Pharmacognosy, Asclepiadaceae.

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INTRODUCTION

The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites [13,14]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals, [1,3]. *Wattakaka volubilis* (L. F.) Stap. (= Marsdenia volubilis Cooke) is a medicinal member of the family Asclepiadaceae, which is cosmopolitan in distribution. It is found to be growing in high rainfal as well as in low rainfall regions. Traditionally, leaves are used as an application to boils and abscesses. The plant is useful in cold and eye disease. It contains dregein alkaloids. The plant is being used very specifically in the indigenous systems of medicine such as Ayurveda, Siddha and Unani. However, it has not been studied pharmacognostically.

MATERIALS AND METHODS

Fresh plant material was collected from Western Ghat region of Maharashtra (India). Efforts were made to collect the plants in flowering and fruiting conditions for the correct botanical identification. The plant material was brought to the laboratory and identified with the help of flora of Maharashtra State [17], Flora of British India [10] and Fasicals of flora of India [11]. For microscopic studies, uniform and thin free hand section were taken from the fresh leaves and stems, dehydrated, double stained and finally mounted in Canada balsam by following the microtechniques method of Johansen (1940). Macro and microscopic characters were studied as per Wallis (1976) and Trease and Evanse (1982). For phytochemical investigation, mature and healthy leaves and stems were separated and dried in shade so as to prevent the decomposition of chemical constituents, powdered in blender and analysed qualitatively and quantitatively for different chemical parameters as per Trease and Evanse (1982). Detailed phytochemical studies were carried out by following Stahl (1969), Harborne (1973) and Manikam and Sadshivam (1991). Ash analysis constants were studied as per Anonymous [2] and fluorescence analysis on powdered drug was carried out as per Chase and Pratt [5].

Observations

Macroscopic characters

Wattakaka volubilis is a large climbing shrub. Stems are woody, much branched; older branches ash-coloured, very long, glabrous, young branches are green and slender. Latex is colourless and watery. The leaves are simple, opposite, broadly ovate or cordate, acuminate at apex and cordate at base. The venation patterns of leaves are observed as camptodromous – brochidodromus.

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Microscopic characters

Transverse section of leaf shows typical drosiventral structure. Uniseriate and multicellular hairs are present. Stomata are rubiaceous and present on the lower epidermis only. Mesophyll tissue is differentiated into two layers of palisade tissue on the upper and armpalisade tissue on the lower side. Midrib portion is bulged towards adaxial side. Collenchyma cells caps on upper epidermis (2 - 3 rows) and on lower epidermis (6 - 7 rows). Cortex is 14-16 rows of irregular parenchymatous cells. Mid rib projects considerably from the lower surface and contains crescent shaped vascular bundle.

Quantitative microscopy of the leaf

Stomatal index	16.88 per mm² area
Stomatal number	13 per mm² area
Vein-islets number	20 per mm² area
Veinlet termination number	11 per mm² area
Palisade ratio	13.25 per cell

The outline of the transverse section of stem is almost circular. The outermost layer shows single row of epidermal cells. A distinct endodermis with casparian strips is absent. Pericycles is represented in the inner region of the cortex by scattered groups of thick walled stone cells. Intraxylary phloem present at the periphery of the pith, in the form of separate strands. Medullary rays are uniseriate. Different types of crystals of calcium oxalate and stone cells are present. Laticiferous tubes are also found in this region.

Vessels in stems

The vessels are broad and drum shaped. Their average length varies from 150µ - 250µ; breadth varies from 40µ - 75µ. Perforation plates are restricted to the side walls as well as end wall. Late metaxylem elements in primary stem are drum shaped. Vessel length is increased in primary stem while it decreased in the lateral branches. This indicated that they follow the general trends of vessel specialization in dicot described by Bailey (1944).

RESULTS AND DISCUSSIONS

In the present investigation, the detailed pharmacognostic account of *W. volubilis* is given which includes macroscopic and microscopic characters, which will be helpful for the correct botanical identification of the drug. Phytochemical tests were carried out of water extractives for starch, tannins, saponins, proteins, anthraquinones and reducing sugars and on alcoholic extract for alkaloids, glycosides and flavanoids. Results are tabulated in Table No.1. In addition, carbohydrates, proteins and alkaloids were quantitatively estimated and their results

are given in Table No.2. Phytochemical screening portrays that most of the natural products tested for were present in the plant material except glycosides, anthroquinones and flavonoides which were not detected in any of the tested fractions. Analysis of tannins, alkaloids, saponins and proteins in the leaves and stem extracts was positive and the stem extract showed positive results for reducing sugar and starch while the leaves extract showed negative results for reducing sugar and starch (Table 1). Results of the quantitative estimation of proteins indicated that proteins are less in quantity in the leaves while more in the stems of studied plant. It is also observed that alkaloids are more in quantity in leaves than stem and also carbohydrates and starch are present in this plant but the percentage values are very less (Table 2). Mixture of such chemicals shows a spectrum of biological effects and pharmacological properties[7]. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (16). The growth of many fungi ,yeasts ,bacteria and viruses was inhibited by tannins [6]. Presence of tannins suggests the ability of this plant to play a major role for the treatment of some disease [3]. Alkaloids were present in the ethanolic extracts. On this premise it will be advisable to extract the leaf and stem of *W. volubilis* with ethanol in an attempt to exploit its detoxifying and antihypertensive properties since alkaloids is known to be effective for this purposes [19]. Saponins are a special class of glycosides which have soapy characteristics [8]. It has also been shown that saponins are active antifungal agents [16]. Percentage extractive and ash analysis were carried out and results are tabulated in Table No.3. Results of ash of *W. volubilis* indicated that percentage extractives are less in quantity in the leaves while more in quantity in the stem of *W. volubilis* . Results of acid insoluble ash of *W. volubilis* indicated that percentage extractives are less in quantity in the stem while more in quantity in the leaves .Fluorescence analysis of powdered drug was also observed and the results are shown in Table No.4. Results of Fluorescence analysis of the leaves and stem of W. volubilis showed green color for leaf and light yellow color for stem in powder as such, light green color for leaf and Greenish yellow color for stem in powder as such in UV-light, dark green color for leaf and greenish yellow for stem in powder mounted in nitrocellulose, greenish black color for leaf and yellowish green for stem in powder mounted in 1 N NaOH in methanol, greenish black color for leaf and yellowish green for stem in powder in 1 N NaOH in methanol and after drying for 30 min. mounted in nitrocellulose. This analysis suggests that, stem and leaves extract of *W. volubilis* probably contain active agent(s) and this provides the basis for their folkloric use as a cure for some human ailments [5]. The values of percentage extractive and ash analysis, results of fluorescence analysis and phytochemical data will be helpful for the standardization and quality control of precious indigenous drug.

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TABLE NO. 1: PHYTOCHEMICAL TESTS of *Wattakaka volubilis*

Sr. No.	Tests	Reagents used	Results of stem	Results of leaf
A) Water Extractives				
1.	Starch	I2-KI	-ve	+ve
2.	Tannins	Acidic FeCl3	+ve	+ve
3.	Saponins	H2SO4 + Acetic Anhydride	+ve	+ve
4.	Proteins	Million's test	+ve	+ve
5.	Anthraquinones	Benzene + 10%NH4OH	-ve	-ve
6.	Reducing sugars	Benedict's	-ve	+ve
B) Alcoholic Extractives				
1.	Alkaloids	Mayre's	+ve	+ve
		Wagner's	+ve	+ve
		Dragnendorff's	+ve	+ve
2.	Flavonoides	HCl + Mg turnings	-ve	-ve
3.	Glycosides	Benzene+hot ethanol	-ve	-ve

TABLE NO. 2: Quantitative Estimation of *Wattakaka volubilis*

Name of the Plant Species	Plant Part Used	
	Stem % / g / dry weight	Leaf % / g / dry weight
Alkaloids	1.03	1.46
Proteins	2.17	0.20
Reducing Sugar	00.00	0.020
Non-Reducing Sugar	0.020	00.030
Total Carbohydrates	0.020	0.050
Total Starch	00.00	0.114

The results are mean of three different readings.

TABLE NO. 3. Results of Ash and Acid insoluble ash of *Wattakaka volubilis*

Criteria	Leaf	Stem
% of ash analysis	7.97%	8.76%
% of acid insoluble ash	8.85%	7.88 %

TABLE NO. 4. Results of Fluorescence analysis of *Wattakaka volubilis*

Treatments	Leaf	Stem
Powder as such	Green	Light yellow
Powder as such in UV-light	Light green	Greenish yellow
Powder + Nitrocellulose	Dark green	Greenish yellow
Powder + 1 N NaOH in methanol	Greenish black	Yellowish green
Powder + 1 N NaOH in methanol dry for 30 min. + Nitrocellulose	Greenish black	Yellowish green

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