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Original Article

Macroscopical and microscopical evaluation of leaves of *Clerodendrum inerme Gaertn.*

Dinesh Kumar[a]*, Pravin V. Buge[b], Zulfikar Ali Bhat[a], Jeevan [S]. Dhumal[c], Yogesh [S]. katare[b], Santosh [S]. Bhujbal[d]

[a]*Department of pharmaceutical sciences, university of kashmir-190006*
[b]*JSPM's Hadapasar, Pune-28*
[c]*RJSPM's College of Pharmacy,Dudulagaol,Moshi Alandi Road Pune-412105*
[d]*Department of Pharmacognosy, Padamshree. Dr. D. Y. Patil Institute of Pharmaceutical Science and research, Pimpri, Pune-411018., (INDIA).*

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ABSTRACT

This study presents the detailed Macro and microscopical evaluation of leaves of crude drug *Clerodendrum inerme Gaertn*. belonging to family Verbenaceae, an important medicinal plant in Indian system of medicine. The leaves were evaluated using procedure of light, conofocal microscopy and with the help of sense organs. The study was help to identify and establish the authenticity of *Clerodendrum inerme* Gaertn. The parameters also help to standardize the crude drug and minimize the drug adulteration.

1. Introduction

During the past decade, the indigenous or traditional system has gained importance in the field of medicine. In most of the developing countries, a large number of populations depend on the traditional practitioners, who are dependent on medicinal plants to meet their primary health care needs. Although, modern medicines are available, herbal medicine retained their image for historical and cultural reasons. Since the usage of these herbal medicines has increased, issues and moto regarding their quality, safety, and efficacy in industrialized and developing countries are cropped up [1]. There has been rapid increase in the standardization of selected medicinal plants of potential therapeutic significance [2]. The use of plant drugs is subject to their correct identification. In general the potent drugs are always either adulterated are substituted depending upon morphological characters or biological activity [3]. Despite the modern techniques, of investigation, identification of plant drugs by. pharmacognostical studies is more reliable. *Clerodendrum inerme* (Verbenaceae), commonly known as "Vanajai" or "Garden quinine" is a perennial shrub. Different parts of this plant are used widely in traditional medicine for a variety of diseases Plant species such as *C. indicum* and *C. inerme* were used to treat coughs, serofulous infection, buboes problem, venereal infections, skin diseases and as a vermifuge, febrifuge and also to treat Beriberi disease [4-6]. It was also reported that tribals use *C. inerme* as an antidote of poisoning from fish, crabs and toads. It is also used for rheumatism and as substitute of quinine [7]. A glycoside ester namely Verbascoside has been isolated from the root of this plant, which has analgesic and antimicrobial properties [8]. However there is none or very minute pharmacognostical report on the Macro and Microscopical standards which required for the quality control of the leaves of crude drug.

2. Material and methods

2.1. Plant material

The material of C. inerme was collected from Hadapsar, Pune district, Maharashtra India. and herbarium of Plant material was prepared and identified by the Regional research institute of Ayurveda Kothrude, Pune (INDIA). A voucher specimen - 656 was authenticated and provided.

* Corresponding Author : Mr. Dinesh Kumar
Research Scholar, Department of pharmaceutical sciences,
University of kashmir - 190006.Contact No: + 91 9882120308(O)
+ 91 9419214116 (M)Fax no: +912027420261
E-mail ID: sharmadinesh82@gmail.com; Pravin_1buge@yahoo.co.in

2.2. Chemical and instruments

The different materials used for the study include basic microscopical instruments like compound microscope, trianacular microscope, glass slides cover slips, watch glass, and other common glasswares. Microphotographs were taken usingLecia DMLS microscope attached with Letiz MPS 32 camera. Common solvents like ethanol (95%), and reagent like glycerine, Toludine blue, Iodine solution, Phloroglycerinol, hydrochloric acid, chloral hyderate, and sodium hydroxide were procured from Ranbaxy fine chemical ltd, Mumbai (India).

2.3. Macroscopical analysis

The Macroscopy and morphplogy of the plant were studied according to the method of Brain and Turner [9].

2.4. Microscopical evaluation of leaves Clerodendrum inerme

The paraffin embedded specimens was sectioned with the help of Rotary Microtome [10-13]. The thickness of the sections was 10 – 12 µm. Dewaxing of the sections was done. The sections were stained with Toluidine blue. Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., wherever necessary sections also stained with safranin and fast-green and iodine (for starch) [14-15].

2.5. Leaf constants

For studying the stomatal methodology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated / cleared materials. Stomata, trichomes, and epidermal cells are important parameters of leaf constant for evaluation of leaf. Exposing the epidermis and studying the type of stomata present, nature of epidermal cell wall, type of trichomes, and their details can study these parameters. Apart from these parameters stomatal index, vein islet number, and vein termination number, that plays an important role in microscopical evaluation of leaf drug. Determination of leaf constants was done [16].

The leaf was studied for various leaf constants i.e.

i. Stomatal number
ii. Stomatal index
iii. Vein islet number
iv. Vein termination number

2.6. Photomicrographs

Photomicrographs of different magnifications were taken with Nikonlabphoto 2 microscopic unit. For normal observations bright light was used. For the study of crystals, starch grains and lignified cells, polarized light was employed as these structures have briefringent property under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books [17].

3. Results

The macroscopical and organoleptic features of C. inerme Gaertn. showed in the Table 1 & Plate 1.

Table1. Macroscopical and organoleptic features of *C.inerme Gaertn.*

Parameter	Leaf
Color	Greenish yellow
Odor	Charac teristic
Taste	Bitter
Size	4 to 8 cm long, 2 to 5 cm wide
Shape	The leaves are ovate, oblong, or elliptic-ovate

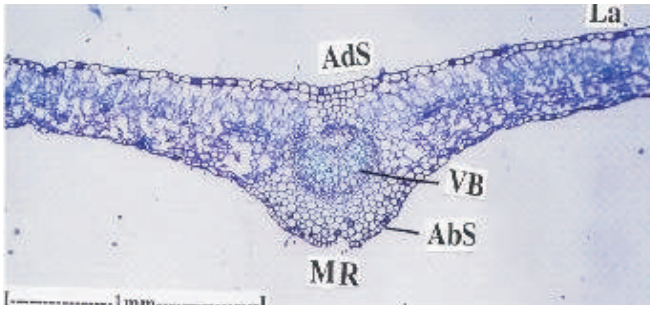
Plate 1. Leaves of *C.inerme. Gaertn*.

3.1. Microscopic evaluation of leaves of C. inerme Gaertn
3.2. Midrib

The leaf is bilateral with planoconvex midrib and smooth even lamina (Figure 1). The midrib has flat adaxial side and semicircular abaxial side (Figure 2). It is 500µm thick, the epidermal layer of the midrib has fairly thick walled circulat cells. The ground tissue consists of small thin walled compact parenchyma cells. The vascular strand is single, semicircular and consists of parallel radial files of wide angular thick walled xylem elements and this are of phloem (Figure 3). Xylem elements are up to 30µm wide.

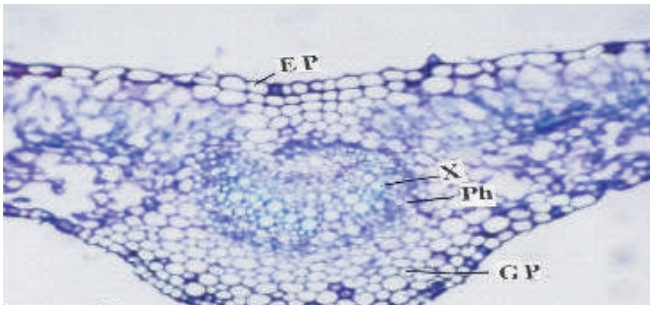
2.2. Chemical and instruments

Figure 1. T.S. of leaf through midrib with lamina



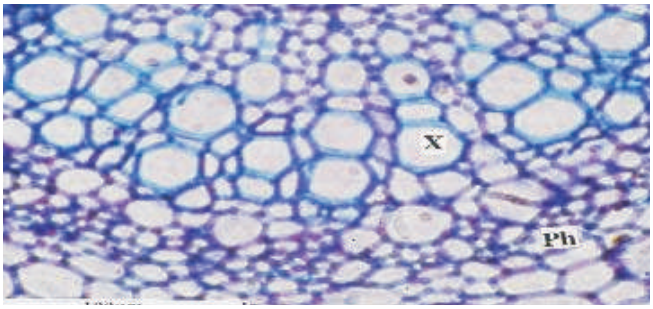
Where Abs-abaxial side, Ads-adxial side, La-lamina, MR-midrib, VB-vascular bundle

Figure 2. T.S. of leaf through midrib with lamina enlarged



Where Ep-epidermis, GP-ground parenchyma, Ph-phloem, X-xylem

Figure 3. T.S. of leaf through midrib with vascular bundle



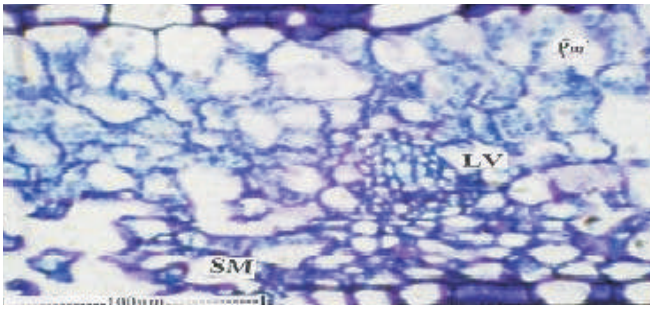
Where Ep-epidermis, GP-ground parenchyma, Ph-phloem, X-xylem

3.3. Lamina

The lamina is 170µm thick. The adaxial epidermis has wide rectangular cells which are 20µm thick. The abaxial epidermal cells are cylindrical measuring 10µm thick. The mesophyll consists of fairly wide abaxial some of two layered palisade cells and four or five layers of lobed arenchymatous cells. The lateral veins occur in the median position and has small collateral vascular bundle (Figure 4). The glandular trichomes occurs both on the upper and lower epidermis. The glands are sunsessile and situated in a shallow cavity (Figure 5). The secretory body of the gland pellate and four or eight triangular cells.

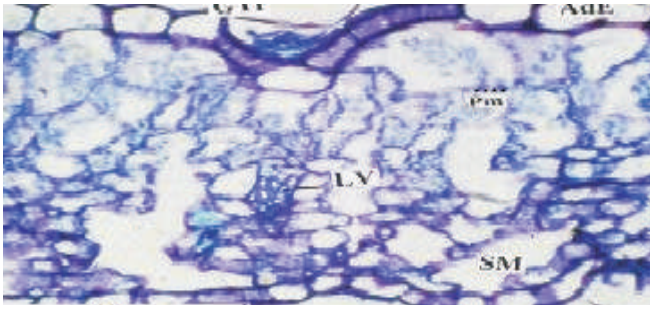
2.2. Chemical and instruments

Figure 4. T.S. of lamina through lateral vein



Where LV-Lateral Vein. Pm-Palisade mesophyll, SM-Spongymesophyll

Figure 5. T.S. of lamina showing adaxial galandular trichome

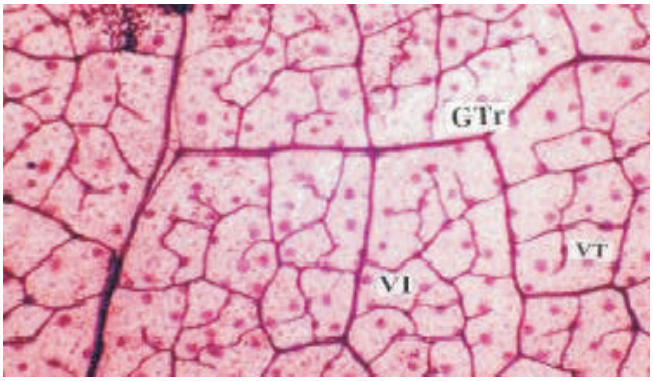


Where AdE-Adaxial epidermis, GTr-Glandular trichomes, LV-Lateral vein. Pm-Palisade mesophyil, SM-Spongy mesophyil.

3.4.Venation

The lateral vein branch profusely forming a dense reticulate venation pattern. The vein-islets are uniform, thin and straight. The vein islets are distinct and wide they vary in the shape and outline. The vein terminations are less frequent and occur only in some of the islets the terminations are short and straight (Figure 6) or less frequently long and wavy (Figure 7).

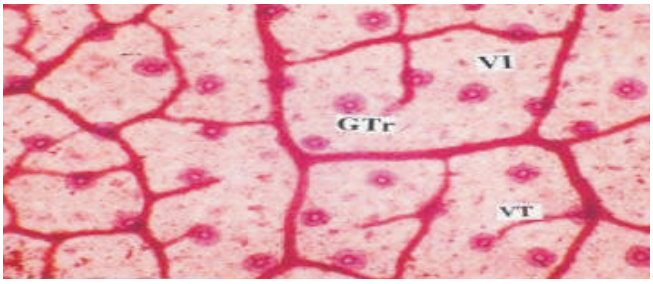
Figure 6. Clear leaf showing vein-islets and vein termination with galndular trichomes.



Where Etr- Glandular trichomes, VI-Vein islets, VT-Vein termination.

2.2. Chemical and instruments

Figure 7. T.S. of leaf showing vein-islets and vein termination with glandular trichomes enlarged

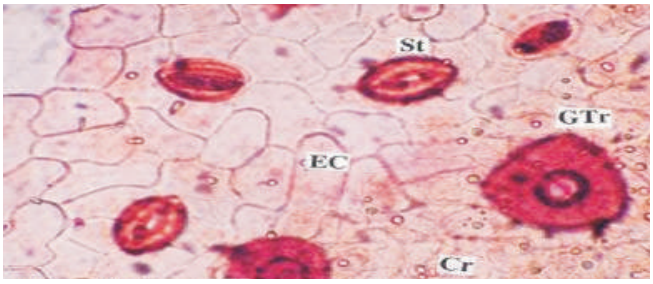


Where GTr-Glandular trichomes, VI-Vein islets, VT-Vein termination.

3.5. Stomata

Stomata are seen on the abaxial side of the lamina. They are anomocytic type. There are no distinct subsidiary cells for the stomata. The epidermal cells are wide and have thin undulate anticlival walls (Figure 8).

Figure 8. Abaxial epidermis with stomata and glandular trichome

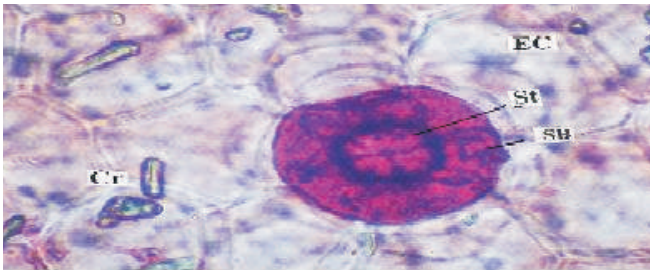


Where Ep-epidermis, GP-ground parenchyma, Ph-phloem, X-xylem

3.6. Epidermal glands

The leaf power shows fragments of lamina where we see the stomata venation pattern and epidermal glandular trichomes the glands are abundant and diffuse in distribution as seen in surface of view. The gland is circular and peltate type. The gland has a short stalke cell, which bears a spherical heads. The head part of gland appears plate like in surface view (Figure 9) and globular lateral view (Figure 10). The head is multicellualar and densely cytoplasmic. It is 90µm in diameter and 80µm in height.

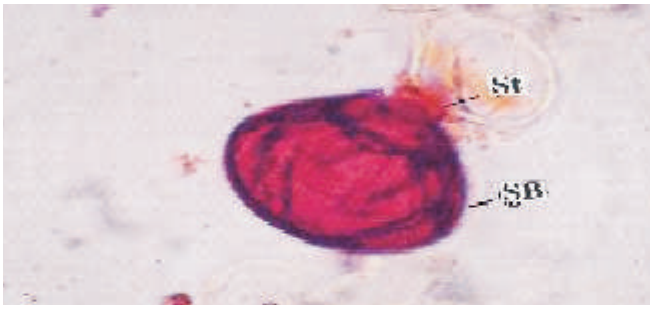
Figure 9. Glandular trichomes (Surfaceview)



Where Ep-epidermis cells, Cr-Crystals, SB-Spherical body, St-Stanlk

2.2. Chemical and instruments

Figure 10. Glandular trichomes (Lateral view)



Where Ec-Epidermal cells, Cr-Crystals, SB-Spherical body, St-Stalk

3.7. Crystals

Calcium oxalate crystals of rod, spherical and cubical types are fairly abundant in the leaf (Figure 11). Leaf constants are shown in the Table 2 Like Stomatal number, Stomatal index, Vein termination number, Vein islet number, Palisade ratio.

Figure 11. Cakinum oxalate crystals

Table 2. Leaf constants of *C.inerme. Gaertn.*

Leaf constant	Numbers
Stomatal number	46
Stomatal index	25.8
Vein termination number	9-11
Vein islet number	7-8
Palisade ration	9

4.0. Discussion

The quality control parameters for the crude drugs as raw materials were established with the help of several official determinations based on morphology, microscopy and physico-chemical studies. These studies were aimed at ensuring standardization of herbal drug under investigation. Morphological examination of drugs refers to evaluation of drugs by colour, odour, taste, size, shape and special features, like touch, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to

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