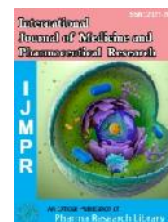




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Research Article

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Pharmacognostical and Phytochemical Evaluations of *Catharanthus Pusillus* (Murr.) G. don (*Apocynaceae*) Aerial Parts

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ABSTRACT

Catharanthus pusillus (Murr.) G. Don commonly known as *Vinca pusillus* belongs to the family Apocynaceae, is a annual erect herb distributed in India and also cultivated in gardens. The crushed powder material of plant or decoction used as folk medicines by tribes for the treatment of cancer, ulcers, paralysis and hysteria. In spite of its reputation in these ailments it has not yet been investigated scientifically hence it was thought worth to study it in detail. The present paper highlights the pharmacognostical and phytochemical details and their role in laying down standardization and Pharmacopoeial parameters. The physicochemical parameters like Ash values, Extractive values and moisture content are determined. Preliminary phytochemical screening revealed that *Catharanthus pusillus* contains Alkaloids, Flavonoids, Carbohydrates, Saponins. Pharmacognostic investigation of fresh, powdered and anatomical sections of leaves of *C. pusillus* were carried out to determine its macromorphological, micromorphological profiles. Qualitative studies indicated the presence of Aboxial phloem, collenchymatous tissue, Epidermis, Lamina, Xylem, Vascular Strand, Spongy mesophyll, stomata etc., and macroscopical examinations green colour leaves with characteristic odour and bitter taste. These findings will be useful towards establishing pharmacognostic standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

Keywords: *Catharanthus pusillus*, Pharmacognostic Investigation, Physicochemical and phytochemical evaluation.

ARTICLE INFO

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1. Introduction

Medicinal plants, which have been found on earth, have renowned medicinal significance and their usage is increasing day by day to day in our daily life. Due to this tremendous potential they offered new drugs against diseases that the effect the health of mankind. Different researches are going on to explore the beneficial pharmacological medicinal properties of herbal drugs (Shaukai Mahamud *et al.*, 2010). Standardization of natural products is complex task to their heterogeneous composition, which is in the form whole plant, plant parts or extracts obtained by thereof. To ensure that reproducible quality of herbal products proper control of starting material is utmost essential. The first step towards ensuring quality control of starting material is authentication. Thus in recent years there has been a rapid increase in standardization of selected medicinal plants of potential therapeutic significance. Despite of modern techniques, identification of plant drugs by pharmacognostic studies more reliable. According to WHO the macroscopic and microscopic description of medicinal plant is first step towards establishing the identify and degree of material and should be carried out before tests are undertaken (Venkatesh *et al.*, 2008).

Catharanthus pusillus (Murr) G. Don is commonly known as *Vinca pusillus* belongs to the family Apocynaceae. It is widely distributed in West Bengal and throughout part of India as weed. Sanghakupuli is indigenous to India. This Indian medicinal plant is found in western Himalayas through the Gangentic plain Deccan Peninsula to Sri Lanka. In India it is found in Andhra Pradesh, Delhi, Haryana, Himachal Pradesh, Karnataka, Kerala, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh (Khare, 2009).

These are erect herbs, branchlets terete below, quadrangular above, glabrous, the whole plant contain milky sap and it grows up to 1 foot, Leaves are elliptic, lanceolate, entire acute at both ends with rough margin, lateral nerves 5-7 pairs, flowers are in white colour, auxiliary, solitary or in pairs, sepals, petals, and stamens 2 each, anthers sagittae. Corolla is white and tubes are usually 0.5 to 1 cm long. Flowers are tiny, small and flat with 5 petals. Flowering starts from July-August, Ovary is bicapellary sub-apocarpous, bilocular, ovules are numerous per locule, marginal contains dumbel shaped stigma. Folicules are ribbed, puberulous and fruits are small, divaricated, membranous folicles in pairs which seeds are cylindrical, black colour ribbed (Madhava Chetty *et al.*, 2001).

Catharanthus pusillus (Murr.) G. Don used as oncolytic. It acts resoling agent and also decoction of dried plant boiled in oil used in the treatment of lumbago. It is acts as cytotoxic. The chemical plants pusillune cause marked depression of the heart (Khare, 2006), whole plant is used in treatment of lumbago, used in the treatment of hysteria. About 20gm of fruit powder administered orally for three months it relives hysteria (Neelima *et al.*, 2011), used in the treatment of paralysis, epilepsy and ulcers (Pullaiah, 2002), and the alkaloids of these plant active against Eagles 9 KB

carcinoma of the nasopharynx in cell culture. Mainly Lochnerine from *Catharanthus pusillus* (Murr.) G. Don alkaloids having this activity against 9KB carcinoma (Tinwa *et al.*, 2006).

2. Materials and Methods

Fresh plant material of *Catharanthus pusillus* were collected from natural habitat from the forest of Tirupati, Chittoor District, Andra Pradesh, in the month of December, 2011. The collected samples were identified, authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi. The roots are separated and leaves, stems are cut in to small pieces and shade dried, some portion of Collected leaves kept aside for physicochemical analysis coarsely powdered drug was used for powder analysis and isolation of various vascular elements. The rest of the sample was kept in preservation solution (FAA) (Khandelwal, 2008). Standard protocol/methods prescribed in pharmacopoeia were followed for pharmacognostical, phytochemical and physico-chemical studies. Specifications for pharmacognostic and physico-chemical values prescribed in Ayurvedic Pharmacopoeia of India were taken as standard values.

Pharmacognostic Evaluation

Organoleptic evaluation

Various sensory parameters of the plant material, such as size, shape, colour, odour, and taste of the whole plant were recorded.

Microscopic evaluation

Microscopical examinations is helpful in identification of drugs and detecting the adulteration various cellular tissues, trichomes, stomatas, starch granules, calcium oxalate crystals and grains are some of important parameters which play vital role in identification of drugs.

Physical Evaluation

In physical evaluation, moisture content, ash values viz., total ash, acid insoluble ash, and extractive values viz., alcohol soluble extractive value, water soluble extractive values were determined. The ash value represents the inorganic salts present in the drug. Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. The percentage w/w values were calculated with reference to the air-dried drug.

Preliminary Phytochemical Screening

The plant powder was subjected to successive extraction in a soxhlet apparatus using methanol (60- 80°C), chloroform, ethanol and water for 8 hrs and the extracts were evaporated to dryness. The dried extracts were weighed, and percentage yields were calculated (Harborne, 2005). The extracts were used for preliminary phytochemical screening with a battery of chemical tests viz., Dragendorff's Mayer's, Hager's and Wagner's tests for alkaloids; ferric chloride, lead acetate, potassium dichromate and dilute iodine tests for tannins and phenolics; foam test for saponin glycosides (Trease and Evens, 1983).

3. Results and Discussion

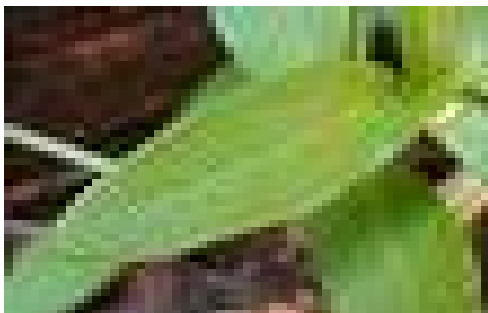
Macroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almost entirely on macroscopical characters. The macroscopical description of a crude drug includes size, shape, nature of outer and inner surfaces, type of fracture and organoleptic characteristics like colour, odour, taste, consistency, etc. The macroscopical feature of the fresh areal part as well as the powder of dried areal parts of *Catharanthus pusillus* (Murr.) G.Don (Apocynaceae) was studied and the results were shown in table 1. The photographs of whole plant and its aerial parts are shown in fig. 1.1



Catharanthus Pusillus Plant



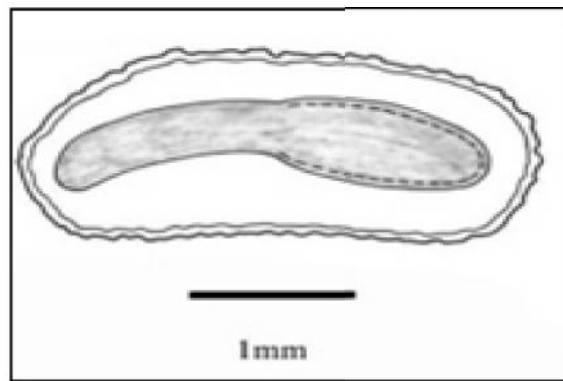
Aerial Parts



Leaves



Flowers



Seed Embryo

Fig 1.1: whole plant of *Catharanthus pusillus* and its aerial parts

Microscopical evaluations of leaf reveals that, which contains following characteristics, the leaf is basifacial with adaxial and abaxial distinction (Fig- 1.1). The midrib is flat on the abaxial side and much wider bowl shaped on the abaxial part (Fig- 1.1.2). It is 700 µm thick; the adaxial part is 600µm wide. The epidermal layer consists of small squarish thin walled cells. Inner to the epidermis is wide zone of 4 or 5 layers of collenchymas cells inner to the collenchyma. The ground tissue is parenchymatous (Fig- 1.1.2). The vascular strand is flat and wide. It is thin and bicollateral. It comprises of horizontal, thin, short, parallel rows of xylem elements. Along the adaxial and abaxial portions of the xylem band occurs small several of phloem elements (Fig-1.1.3). Occurrence of phloem elements on the adaxial and abaxial sides of xylem is called bicollateral vascular bundle. The lamina is bifacial with distinction of adaxial and abaxial side. It is amphistomatic i.e., stomata occur both on the adaxial (Fig-1.2.3) and abaxial sides. The upper epidermis is wide and thick, the cells are rectangular and fairly thick walled. The adaxial epidermis is thin. The cells are narrow, circular and cylindrical (Fig- 1.2.1). The mesophyll tissue is differentiated into adaxial zone of thick, cylindrical less compact palisade cells. The marginal portion is slight bent down. It is conical and thick. The epidermal cells are thick-walled and squarish. The marginal part is 80µm thick. The mesophyll tissue consists of angular thick walled compact hyaline cells (Fig-1.2.2).

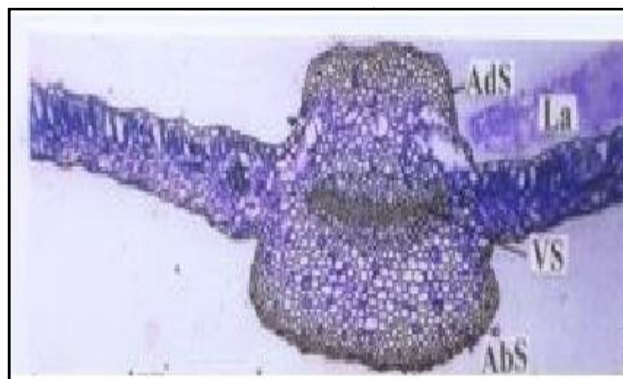


Figure 1.1.1 Transverse Section of Leaf through midrib (AdS–Adaxial Side, La–Lamina, VS–Vascular Strand, AbS–Abaxial Side)

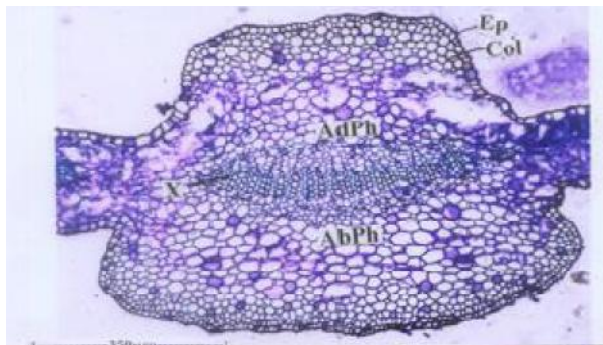


Figure 1.1.2. Transverse Section of Leaf through midrib (AdPh – Adaxial Phloem, X - Xylem, Ep- Epidermis, Col - Collenchyma, AbPh - Abaxial Phloem)

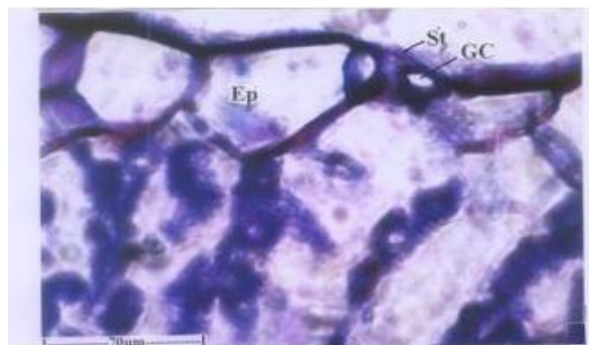


Figure 1.2.3 Transverse Section of Lamina showing Adaxial Stomata (St - Stomata, GC - Guardcells, EP - Epidermis)

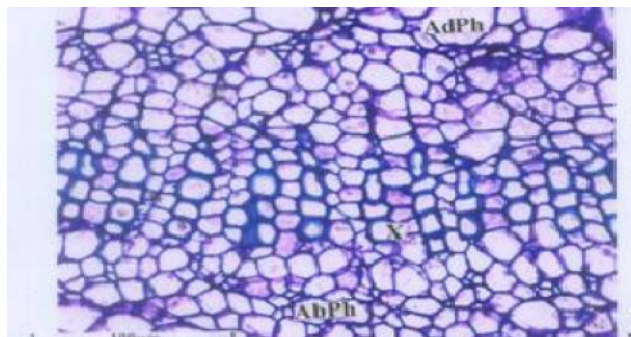


Figure 1.1.3. Transverse Section of Midrib Vascular Strand enlarged (AbPh-Abaxial Phloem, AdPh-Adaxial Phloem, X- Xylem)

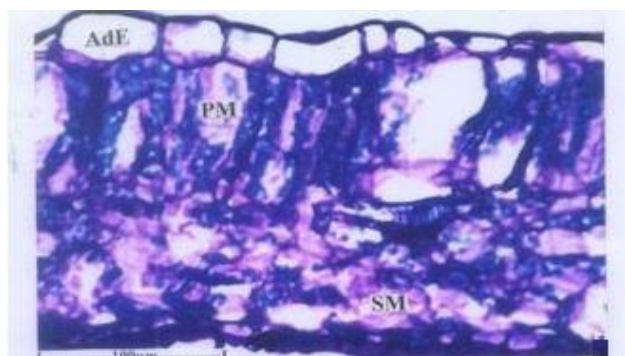


Figure 1.2.1. Transverse Section of Lamina – Enlarged (AdE-Adaxial Phloem, PM–Palisade Mesophyll, SM–Spongy Mesophyll)

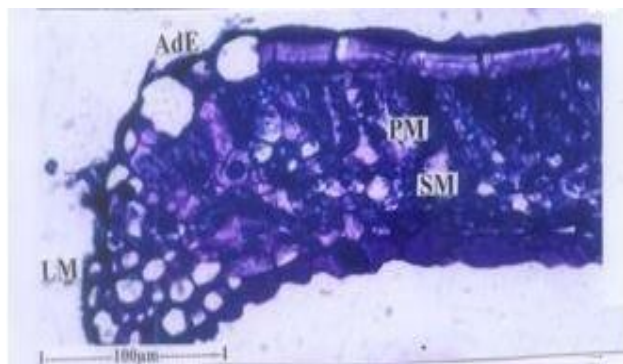


Figure 1.2.2. Transverse Section of Leaf Margin (AdE - Adaxial Phloem, PM - Palisade Mesophyll, SM - Spongy Mesophyll, LM – Leaf Margin)

Discussion

Physicochemical constant is an important parameter in detecting adulteration on improper handling of the drug. In the evaluation of crude drug ash values, extractive values and moisture contents are important parameters. The ash content of crude drug is generally taken to the residues remaining after incineration. Ash value determination furnishes a basis of judging the identity and cleanliness of a drug and gives information related to its adulteration with inorganic matter. The estimation of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy matter. Hence ash values are helpful in determining the quality and purity of crude drugs in the powder form.

The determination of extractive values with a range of solvents gives information about extractable non-polar and polar as well as total extractable plant constituents. Determination of moisture content indicates the percentage of active chemical constituents in crude drugs mentioned on air-dried basis. The moisture content of a plant drug should be minimized in order to avoid decomposition of crude drugs either due to microbial contamination or chemical change. The various physicochemical parameters of aerial parts powder of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) were evaluated and the values tabulated in table 2

The percentage yield and consistency of various extracts of aerial parts of *Catharanthus pusillus* (Murr.) G. Don were presented in the table 2.1. The methanolic extract gives the high percentage of yield and it was found it was found to be 34% w/w. The results of phytochemical studies of various extracts of aerial parts of *Catharanthus pusillus* (Murr.) G. Don were presented in the table 2.2., it is concluded that the medicinal value of this of plant may be attributed due to the presence of various phytoconstituents viz., Alkaloids, Carbohydrates, Flavonoids, Saponins.

4. Conclusion

In case of herbal drugs it is very important to collect the genuine plant material. Morphological and anatomical standardisation is very important. The pharmacognostic profile of crude drug has key role in standardisation for

quality, purity, drug identification. Various pharmacognostic studies of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) were carried out in order to establish parameters for its identification and to check adulteration or contamination of other species. Macromorphology and

microscopy along with ash values, extractive values moisture content are simplest methods for identification of plant materials. So the results obtained from the present study are helpful for future workers in identifying and selecting the correct herbal medicine.

Table-1: Macroscopical Features of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae)

Characteristics	Stem	Leaves	Flowers	Powdered plant material
Colour	Green	Green	White	Pale green
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Taste	Bitter	Bitter	Slightly bitter	Bitter
Texture	Slightly rough	Smooth	Smooth	-----
Shape	Cylindrical	Lanceolate Acute	Slaves or Funnel	-----
Size	0.2-0.5cm	Length 7-8cm	0.5-1cm	-----

Table- 2: Physicochemical parameters of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) crude drug

Parameters	Determined values(%w/w)
Ash values	
Total ash	7.21
Acid insoluble ash	1.96
Water soluble ash	1.58
Sulphated ash	3.06
Extractive values	
Alcohol soluble	16.23
Water soluble	10
Moisture content	
Loss on drying	4

Table-3: Percentage yield and colour of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) extracts

Parameter	Extracts			
	Methanol	Ethanol	Chloroform	Dist. Water
Percentage of yield (w/w)	34	32	15	20
Colour	Black	Light Green	Light Green	Dark Green

Table-4: Results of Phytochemical studies of *Catharanthus pusillus* (Murr.) G. Don (Apocynaceae) extracts

Tests	Methanol	Ethanol	Chloroform	Dist. Water
Alkaloids	+	+	+	-
Carbohydrates	+	+	-	+
Steroids	-	-	-	-
Triterpenoids	-	-	-	-
Flavonoids	+	+	+	+
Glycosides	-	-	-	-
Saponins	+	+	+	+
Tannins	-	-	-	-

-/ + = Absence / Presence

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