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PHARMACOGNOSTIC STANDARDISATION OF *DIDYMOCARPUS TOMENTOSUS* WIGHT (GESNERIACEAE)

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ABSTRACT

The present study deals with the microscopic analyses of all parts of *Didymocarpus tomentosus* Wight of Gesneriaceae, collected from the hills of W.Ghats of Thirunelveli, Tamilnadu. It is a common scapegerous herb growing in the crevices of the rocks. *D.pedicellata*, an Himalayan element, has been claimed by many investigators as a potential medicine for Kidney stone and urinary problems. *D.tomentosus* being a co-species was believed to possess properties similar to *D.pedicellata.* The investigation provides structural profile of leaf, midrib, petiole rhizome and root. The preliminary phytochemical evaluation was also carried out. The results are discussed critically with reference to the botanical identity of the taxon.

Keywords: Gesneriaceae- *Didymocarpus tomentosus*- Pharmacognostic studies.

INTRODUCTION

Gesneriaceae is one of the taxa which seem to have received little recognition with reference to its biochemical and biological evaluation. According to Evans (1996), members of Gesneriaceae possess tannins, naphthoquinones, chalcones, and anthroquinones, but no alkaloids. *Didymocarpus pedicellatus*, an element restricted to the Himalayan ranges, is considered as the source of the Ayurvedic drug, Pashanbhed, a potential herbal for kidney stone and other urinary problems. (Visweswara and Seshadri, 1948; Shah *et al*; 1972; Bhaskar and Seshadri, 1973; Bhattacharya *et al*; 1979; Amrit Pal Singh, 2007). However, Madhavan, *et al*. (2010) claimed that the authentic source of *Pashanbhed* is *Nothosaerva brachiata.*Voluminous literature is available for the chemical constituents of *D.pedicellata*. (Paul and Sankar, 2010)

Didymocarpus tomentosus Wight is a common species wide spread in the hills of Western Ghats of Thirunelveli, TamilNadu. As a co-species of *D.pedicellatus*, it was hoped that *D. tomentosus* may possess compounds that were identified from the former species.A study was initiated to investigate different aspects of *D. tomentosus*.As a preliminary step, pharmacognostic aspect of the plant was undertaken. Biological and phytochemical studies are being under process for the species.

MATERIALS AND METHODS

Didymocarpus pedicellatus samples for the present study were sourced from Kedavetti Parai, a Kani settlement in the W. Ghats of Thirunelveli, TamilNadu. The specimens were brought to the lab and identified with the help of the regional flora (Gamble, 1967; Mathew, 1983). The identification was confirmed and the authentication certificate was given by Prof. P. Jayaraman, Director, Institute of Herbal Botany.Chennai. Specimen plant is deposited at Madras Herbarium (MH), Botanical Survey of India, Coimbatore, Voucher No.1118 and at St. John's College Herbarium (JCH) Palayamkottai. Different parts of the plant were fixed in FAA in the field itself. The samples were dehydrated, infiltrated and subjected to serial microtome sections of 10µm thick following customary technique (Sass, 1940). The sections were stained with Toludine blue'O (O' Brien *et al*; 1964). Photographs were prepared with NIKON Digital Photo Unit System.Physicochemical constants, Fluorescent analysis and Qualitative tests were carried out by following the prescribed methods (1- 4, 8, 10, 13, 15, 16, 21, 23, 27 & 28)

Observations

D. tomentosus is an *acualescent* herb growing in the crevices of moist rocks. The leaves arise from a rosette. The leaves are ovate to broad elliptic, measuring 3-10 X 2- 6 cm in size (Fig.1,2). The veins are prominent on both surfaces, densely tomentose below, leaf margins are crenate to irregularly lobed; petiole winged; flowers are in panicle; corolla pale bluish purple. Capsules are slender and curved.

Anatomical features

(i) Leaf: The leaf is, mesomorphic, dorsiventral and hypostomatic. The *midrib*, sectional view shows planoconvex shape with flat adaxial side and thick semicircular abaxial part which hangs down from the lamina (Fig.3). It is 1.7 mm thick and 1.6 mm wide. The epidermal layer of the adaxial side consists of vertically oblong cells. The abaxial epidermal cells are narrow and cylindrical. The ground tissue is parenchymatous, the cells being circular and less compact. The vascular system of the midrib is multistranded comprising many discrete, collateral strands of varying size and diffuse in distribution (Fig. 3). There is a gradation in shape and size of the vascular strands from base to the upper part. Those at the lower end are wider, flat or semicircular; those towards the upper end are smaller and circular. Within the strands the xylem elements are in several compact parallel lines, the elements are angular, thick walled and lignified. Phloem elements are located along outer periphery of the vascular bundles.

Fig. 1: Entire plant with root and shoot systems

Fig. 2: Inflorescence highlighted

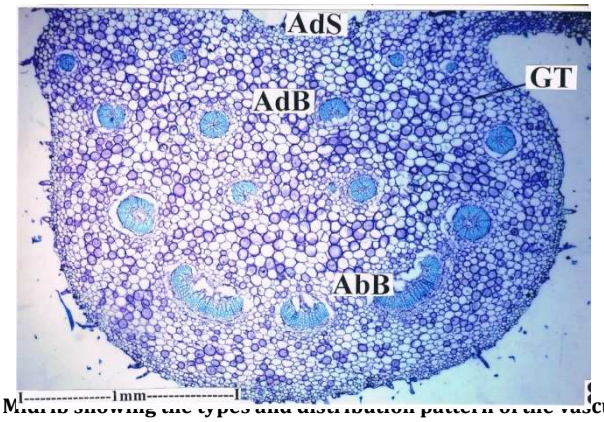

Fig. 3: T.S. of Midrib showing the types and distribution pattern of the vascular bundles

(AbS: Abaxial Side; AdE: Adaxial Epidermis; AdS: Adaxial Side; GT: Ground Tissue)

The lamina is undulate in sectional view with abaxially projecting lateral veins. The lamina is 200µm thick. It is dorsiventral with thick, rectangular thin walled upper epidernis and thin narrow abaxial epidermis (Fig 4). Both epidermal layers bear multicellular, uniseriate unbranched nonglandular trichomes. The mesophyll shows differential pattern with adaxial single row of short palisade cells and abaxial four or five layers of spongy parenchyma cells.

Epidermal tissue, as seen in surface view exhibit cells with wavy anticlinal walls. The stomata are circular with narrowly elliptical aperture; they are 30µm in diameter (Fig. 5). The stomata are cyclocytic type: a stoma is encircled by one or two rings of subsidiary cells. Venation is reticulate type; the *vein islets* are distinct and are rectangular to squarish in outline. The vein terminations are slender, long, branched or unbranched (Fig. 6).

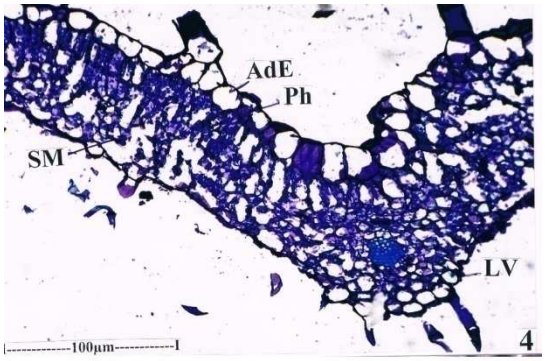

Fig. 4: TS of Lamina with lateral vein

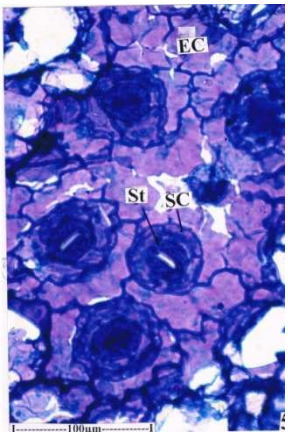

Fig. 5: Epidermal cells and stomata as seen in paradermal section

(AdE: Adaxial Epidermis; LV: Lateral Vein; (EC: Epidermal cell; SC: Subsidiary Cells; St: Stomata) Ph: Phloem; SM: Spongy Mesophyl)

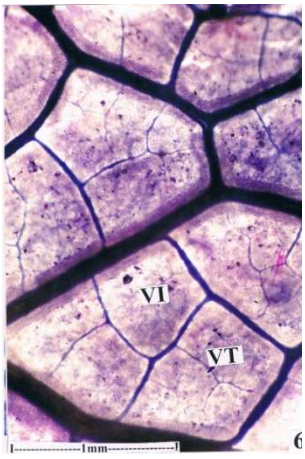

Fig. 6: Venation pattern of the lamina

(VI: Vein Islets; VT: Vein- Terminations)

Petiole (Fig. 7 & 8):- The petiole is boat shaped in transectional view with convex abaxial side and adaxial side with wide shallow median groove. Towards the proximal part, the petiole assumes plano-convex cylindrical shape with lateral wings. The wide distal part of the petiole consists of thin epidermal layer and less compact parenchymatous ground tissue. The vascular strands are discrete; there is a wide shallow arc of abaxial bundles ranging up to 10 strands and adaxial smaller many scattered vascular strands. The abaxial strands are semicircular and adaxial strands are circular. All the strands are collateral and possess several compact rows of xylem and thin layer of phloem elements.

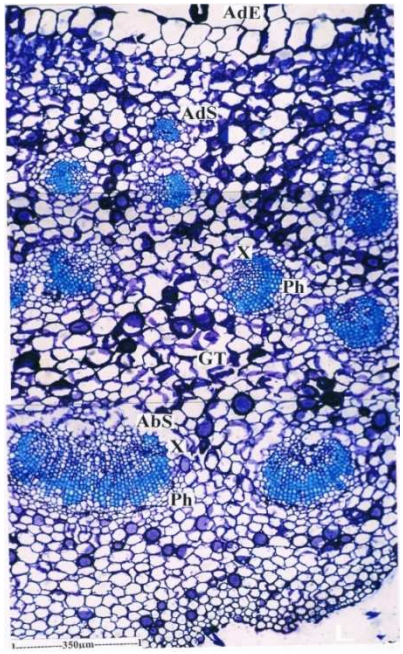

Fig. 7: T.S. of distal (terminal) part of the petiole

(AbB: Abaxial Bundle; AdB: Adaxial Bundle; AdG: Adaxial Groove; GT: Ground Tissue)

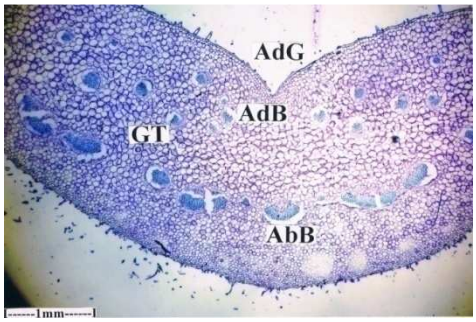

Fig. 8: T.S. of petiole

(AbB: Abaxial Bundle; AdB: Adaxial Bundle; AdS: Adaxial Side; GT: Ground Tissue)

Rhizome (Fig.9):- The rhizome is thick and fleshy and is covered by sheathing leaf-base. In sectional view it is uneven in outline. The epidermis and a few layers of outer cortical cells are disintegrated forming a dark region. The rhizome consists of a wide parenchymatous cortex and central pith. The vascular cylinder is thin and is broken by leaf traces and leaf- gaps. Root traces are also seen emerging from the vascular cylinder. The leaf traces are arc shaped while the root traces are solid and circular. The xylem cylinder consists of compact, radial rows of thick walled xylem elements as well as narrow lignified xylem fibres. Phloem is in thin continuous layer encircling the xylem cylinder.

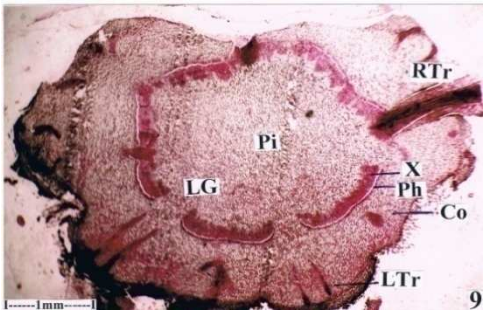

Fig. 9: T.S. of rhizome - entire view

(Co: Cortex: LG: Leaf Gap; LTr: Leaf Trace; Ph: Phloem; Pi: Pith; RTr: Root Trace; X: Xylem)

Root (Fig.10):- The root is 1.9mm thick. The epidermis is broken at several places exposing a thick periderm which consists of 10 layers of radial series of tabular suberised cells. Secondary phloem is preserved only in some of the places of the vascular cylinder where it includes thin radial rows of small sieve elements.The xylem cylinder is characteristic in having a narrow sclerenchymatous cells in the centre and several uniseriate radiating rows of vessels and thick walled fibres. The vessels are *angular* and thick walled; they are mostly in radial multiples or solitary. The vessels are up to 40µm wide.

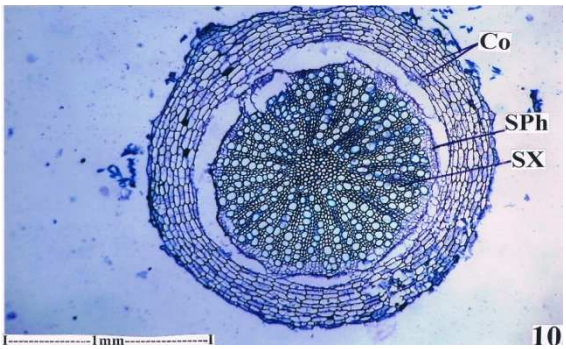

Fig. 10: T.S. of root – entire view

(Co: Cortex; SPh: Secondary Phloem; SX: Secondary Xylem)

Powder Microscopic observations

The powder preparation of the plant includes the following components when viewed under the microscope.

(1) **Epidermal Trichomes**: Two types of trichomes are evident in the powder. Some are thick walled, sturdy pointed trichomes (Fig. 11). They are two celled with wide lumen and arise from cluster of epidermal cells raised from the leaf surface. The thick walled trichomes are 280µm long and 25µm thick.A second type of trichome is cotony type. They are quite long of unlimited growth and have thin walls and wide lumen.

(2) **Fibres** (Fig.12). Short, thick walled fibres, most of them septate type, are frequently seen in the powder. They have tapering ends and lignified walls. The fibres are 270µm long.

(3) **Vessel elements** (Fig. 13) Long, cylindrical vessel elements with horizontal or oblique end walls are frequently seen in the powder. They have **scalariform lateral** wall pits. The vessel elements are 60-100µm long and 20µm wide.

(4) **Ground parenchyma cells** (Fig.14) Fairly large masses of speherical parenchyma cells are common in the powder. The cells are thin walled and possess dense, darkly stained amorphous inclusions. The cells are about 50µm thick.

(5) **Pollen grains** (Fig.15) Pollen grains are wide spread in the powder. They are spherical with thin smooth exine. The grains are 15µm in diameter.

Physico- Chemical Characters such as loss on drying, total ash, water soluble ash, acid insoluble ash and extractive values of petroleum ether, hexane, Chloroform and Ethyl acetate are given in Table-I. Flourescence analysis is tabulated in Table- II and preliminary phytochemical analysis in Table- III.

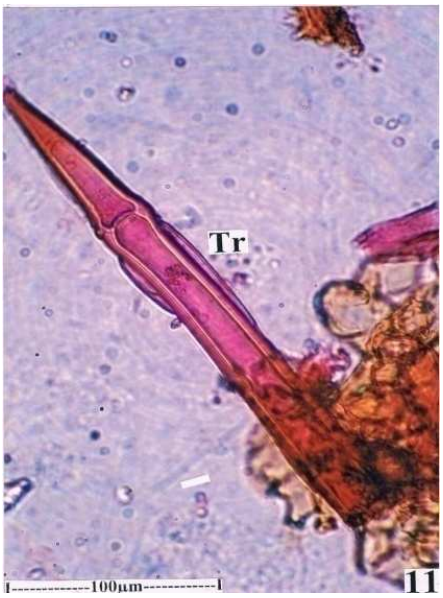

Fig. 11: Epidermal Trichome in the powder

(Tr: Trichome)

Fig. 12: A Septate Fibre

(Se: Septum)

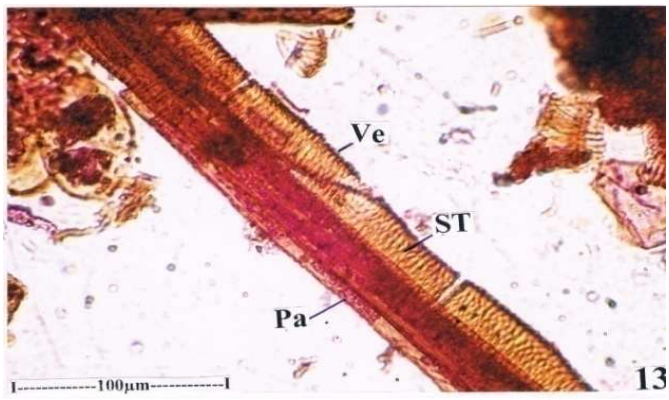

Fig. 13: A vessel associated with fibres

(Pa: Parenchyma; ST: Scalariform Thickening; Ve: Vessel)

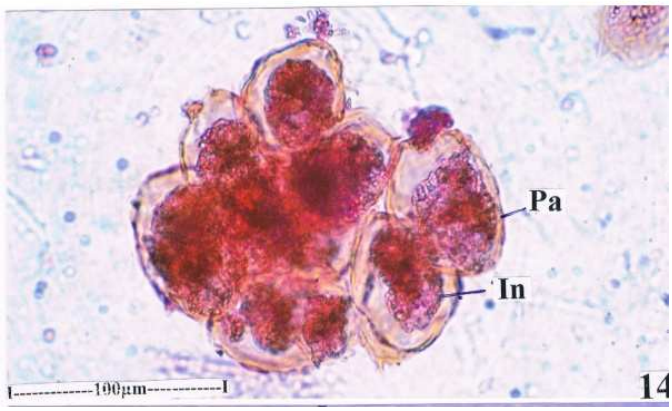

Fig. 14: Parenchyma cell masses

(In: Inclusion; Pa: Parenchyma)

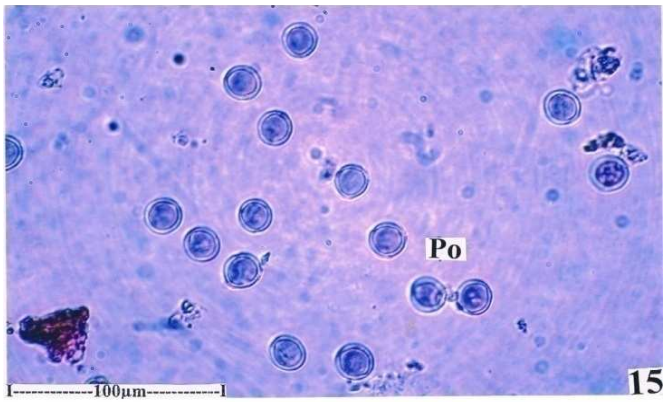

Fig. 15: Pollen grains seen scattered in the powder

(Po: Pollen)

Table I: Physico – Chemical Character of *Didymocarpus tomentosus.* Wight

S. No.	Parameter	Whole Plant Value
1.	Loss on drying	82.27
2.	Total Ash	13.12
3.	Water soluble ash	1.89
4.	Acid insoluble ash	6.14
5.	Extractive value	
	a. Petroleum Ether	2.0
	b. Hexane	0.72
	c. Chloroform	1.72
	d. Ethyl acetate	4.88

Table II: Fluorescence Analysis of *Didymocarpus tomentosus*. Wight

S. No.	Chemical Treatment	Visible	UV 254nm	UV 366nm (Long wave length)
1.	Powder	Grey	Grey	Grey
2.	Powder + H_2So_4	Dark Brown	Reddish Brown	Reddish Brown
3.	Powder + HCl	Green	Yellowish Green	Yellowish Green
4.	Powder + NH_4OH	Yellowish Red	Yellowish Green	Brown
5.	Powder + HNO_3	Yellowish Orange	Yellowish Green	Yellowish Brown
6.	Powder + CH_3 COOH	Pale Yellow	Pale Brown	Brown
7.	Powder + Iodine	Yellow Brown	Brown	Maroon
8.	Powder + $FeCl_3$	Yellowish Green	Yellowish Green	Yellowish Green
9.	Powder + Picric acid	Lemon Yellow	Fluorescent Yellow	Fluorescent Yellow
10.	Powder + NaOH	Maroon	Fluorescent Yellow	Yellowish Green

Table III: Preliminary Phytochemical Analysis of Various extract of *Didymocarpus tomentosus*. Wight

S. No.	Plant Constituent	Reagent used	Extracts				
			Petroleum Ether	Hexane	Chloro form	Ethyl Acetate	Ethanol
1.	Alkaloid	Mayer's	-	-	-	-	-
		Wagner's	-	-	-	-	-
		Dragendroff's	-	-	-	-	-
		Hager's	-	-	-	-	-
2.	Carbohydrates	Molisch's	-	+	-	-	-
3.	Reducing Sugar	Fehling's	+	+	+	+	+
		Benedict's	+	+	+	+	+
4.	Flavonoids		+	+	+	+	+
5.	Glycosides		+	+	+	+	+
6.	Phenolic Compounds	$FeCl_3$	-	+	+	+	+
7.	Saponins	Foam Test	-	+	-	-	+
8.	Steroids		+	+	-	-	-
9.	Proteins	Biuret	-	-	-	-	-
10.	Aminoacids	Ninhydrin	-	-	-	-	-
11.	Tannins	Lead Acetate	-	-	-	+	+
12.	Terpenoids		+	-	-	+	+
13.	Quinones		-	-	+	-	-
14.	Phlobatamin		-	-	-	-	-
15.	Triterpenoid		-	-	-	-	+
16.	Fixed oils & fats		+	+	-	-	+

DISCUSSION

Preliminary qualitative phytochemical studies of plants are an integral part of pharmacognosy. The objectives of qualitative evaluation of phytodrugs are twofold. It gives a preliminary insight into various compounds present in a plant, based on which a researcher can proceed further towards the biological activities of the compounds. Secondly, the study yields information on the purity of the drug as well as the genuineness of the drug.

During the present studies on *D.tomentosus*, the several preliminary phytochemical tests were carried out which are given in Tables I, II, & III. These studies offer corrobora evidences to the microscopic observations. The water content and total ash-values of *D.tomentosus* were found to be 82.27% and 13.12% respectively. The estimation of the residue left up on combustion is of great practical value, especially for the vegetable powders. For, every portion of the plant furnishes an amount of ash which fluctuates with in definite and often narrow limits. The weight of the same may therefore afford information whether an adulteration with other vegetable or inorganic materials has taken place (Fluckiger and Tschirch, 1913). A total ash figure is useful in to exclude drugs which have been coated with chalk, lime or calcium sulpalte to improve their appearance. The acid insoluble ash value will provide evidence of the presence of excessive earthy matter which likely to occur with root and rhizome drugs.

Extractive values of the plant with different solvents give a preliminary picture of the percentage of the compounds extracted. In *D.tomentosus* maximum extractive value was found with ethyl acetate (4.88%) and minimum (0.72%) with hexane. This result shows the solvent ethyl acetate is preferable to other solvent for the yield of more of the compounds. Fluorescence is phenomenons exhibited by many substances like quinine, when suitably illuminated emit fluorescent light (Evans 1996). When a compound is subjected to the fluorescent effects, it will indicate the presence/ absence of fluorescent molecules. The powder of *D.tomentosus* exhibits fluorescent effects with all chemical treatments under short wavelength. This provide as evidence for the presence of fluoresent compounds in the plant handled at present.

Extracts of different solvents when treated with certain specific reagents one can detect the presence/absence of various constituent in a plant. Reducing sugar, flavonoids and glycosides were found to be present in all extracts (Table III). Alkaloids are totally absent in the extracts. Microscopic studies of *D.tomentosus* provide certain features of diagnostic values of the plant. Multistranded vascular system and scattered distribution pattern of the strands in the midrib & petiole and their unique shape and size variation, cyclocytic stomata, amoeboid outline of the epidermal cells, thin wide cylinder of vascular tissues with wide cortex and pith in the rhizome and dense solid cylinder of xylem with regular radial seriation of thick walled angular vessels constitute the reliable features for botanical identity of the plant.

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REFERENCES

1. Anonymous, British Pharmacopoeia Press, London. 1968.pp. 1365.
2. Anonymous, Quality control methods for medicinal plant materials, WHO, Geneva, 1998. pp. 28.

3. Anonymous, Quality control methods for medicinal plant materials, WHO, Geneva, 1998. pp.30.
4. Anonymous, The Ayurvedic Pharmacopoeia of India, Part – I, Vol. – II (First Edition), Government of India, Ministry of Health and Family Welfare, Department of Indian System of Medicine and Homoeopathy, New Delhi. 1999.
5. Amrit Pal Singh, Didymocarpus pedicellata; The Lithontriptic Ethnomedicine, Ethnobotanical leaflet. 2007. 11: pp. 72-75
6. Bhaskar, A, Seshadri, TR, New chemical components of Didymocarpus pedicellata leaves. 1973. volume 11, Issue 4
7. Bhattacharyya, A. Choudhuri, A, Adityachandhury, N, Didymocarpus-A, a new flavanone from Didymocarpus pedicellata, Chemistry and industry, 1979. (10)
8. Chase, CR, Pratt, R. Flourescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Am.Pharm.Associ. Sci. 1949. 38: 324 – 331.
9. Evans,WC. Trease and Evan's Pharmacognosy, WB. Saunders Company Ltd., London. Philadelphia, Toronto, Sydney, Tokyo. 1996.
10. Evans, WC, Treas, Evans Pharmacognosy. 14thedn. Harcourt Brace and company. Asia Pvt. Ltd. Singapore. 1997.
11. Friedrich A, Fluckiger, Alexander Tschirch, The principles of Pharmacognosy. 1973. pp. 1- 279.
12. Gamble, JS. Flora of the Precidency of Madras (Rep. ed) 1967. pp.691-697.
13. Govindachari, TR. Laboratory Manual of Organic Chemistry, Central Art Press. 1959.
14. Harborne, JB., Phytochemical Methods, 3rd Edition, Chapman & Hall Publication. 1973.
15. Khandelwal, KR., Pawar, AP., Kokate, CK., Gokhale, SB. Practical Pharmacognosy Techniques and Experiments. Nirali Prakasam, 3rd Edition. pp. 1996. 140, 141.
16. Kokate, CK. Practical Pharmacognosy, PBH Press, Delhi. 1989.
17. Kokate, CK. Practical Pharmacognosy, 3rd Edition, 1991.10 – 13: 107 – 121.
18. Madhavan V, priyanka Goswami K, Gurudeva MR, Yoganarasiman SN, Pharmacognostical studies on the root of Nothosaerva brachiata wt.- A botanical source of the Ayurvedic drug, Pashanabheda, Indian Journal of traditional knowledge. 2010. Vo. 9(4), pp.629-634.
19. Mathew, KM. The Flora of TamilNadu Karnatic Vol.3. Gamopetalae & Monochlamydae pp.1124- 1125. The Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, India. 1983.
20. O' Brien, TP, Feder, N, Mc Cull, M E. Polychromatic Staining of Plant Cell walls by toluidine blue – O. Protoplasma; 1964. 59: 364 – 373.
21. Ramakrishnan, S, KG. Prasannan, R. Rajan. Text book of medicinal biochemistry. Orient Longman, New Delhi.India. 1994.
22. Raman, N, Phytoctemical Techniques, New India Publishing Agency, Pitam Pura, New Delhi. 2006.
23. Rasch, E, H. Swift. Micro Photometric analysis of the cytochemical million reaction.J. Histochem. Cytochem. 1960. 8: 4 – 17
24. Sass, JE. Elements of Botanical Microtechnique. Mc Graw Hill Book Co; New York. 1940. pp.222.
25. Shah, CS., N.Shah, K D. Mody, Pharmacognostic study of pashambad: {I}—{III}: Bergenia ciliata and Didymocarpus pedicellata,Quarterly Journal of crude Drug Research. 1972. 12,1 pp.182-193.
26. Visweswara Rao, K, Seshadri, TR., Nuclear oxidation in flavones and related compounds Part XII. Constituion and Synthesis of pedicin and Its Allies, Mathematics and Statistics, Proceedings Mathematical sciences. 1948. Volume 27, Number 5 , pp.375-390
27. Wagner, H. Pharmazeutische Biologic 5. (ed), AUFI.15 BN 3 – 437 – 20 498 –X.Gustav fisher Vwelag, Stuttgart, Germany. 1993.
28. Wagner, H, Baldt, S., Plant Drug Analysis, 2nd Edition, Springer Publications, Germany. 1996.