Pharmacognostical studies on leaves of *Cadaba trifoliata* (Roxb.) Wt. & Arn.

R.Mythreyi*[1], E.Sasikala[2], and A.Geetha[3]

[1] Vathsalya College of Pharmacy, Anantharam, Bhongir, Nalgonda Dist., Andra Pradesh.

[2]Central Research Institute for Siddha, Arumbakkam, Chennai 600 106.

[3]Bharathi womens College, North Chennai 600 108.

Abstract

Cadaba trifoliata known as Maraviluthi in Siddha system of medicine an important medicinal plant. In folklore, the leaves are used in the treatment of rheumatism, as an antiphlogistic, purgative and anthelmintic. Roots of this plant are used as purgative and plasters. The present study provides detailed pharmacognostical evaluation of this plant species that include microscopy, standard physicochemical determinations and authentic phytochemical parameters. These morphological characteristics could be used for rapid identification of the drug, particularly in case of powdered materials, and may possibly help to differentiate the drug from its other species. Preliminary phytochemical analysis revealed the presence of alkaloids in petroleum ether, chloroform and ethanol extracts, carbohydrates and glycosides in petroleum ether, chloroform, ethanol and aqueous extracts, flavonoids in chloroform, acetone and ethanol extracts and phenolic compounds and tannins in all extracts. Volatile oils and saponins are absent in all extracts.

Key words : *Stremia trifoliata*, Pharmacognosy, Diagnostic characters, *Cadaba trifoliata*

1.1 Introduction

Cadaba trifoliata (Roxb.) Wt. & Arn. (Maraviluthi) is an important siddha drug; the leaves are used in the treatment of rheumatism, as an antiphlogistic, anthelmintic, purgative and as febrifuge[1]. Leaves and roots of *C. fruticosa* (Viluthi) an allied species of *C. trifoliata* are included in various siddha formulations and in folklore *C. fruticosa* is used in the treatment of ailments such as rheumatism, as an antiphlogistic, anthelmintic, purgative and as febrifuge[2,3,4]. As per folklore *C. trifoliata* also possess similar uses and no scientific reports are available both on the chemical constituents and biological activity of this plant[5,6]. Hence the present investigation is undertaken. The objectives of the present study were to carry out pharmacognostical investigation including microscopical characters, powder analysis standard physiochemical characters and phytochemical parameters on the leaves of *C. trifoliata* which help in the identification of the drug.

2. Materials and methods

The fresh leaves of *Cadaba trifoliata* were collected in the month of September 2005 from Tinnelvely, Dist., Tamil Nadu, India. The botanical identity was confirmed by Department of Pharmacognosy, Central Research Institute for Siddha, Chennai, India. A voucher specimen of the leaf was deposited in the department of Pharmacognosy, M.S.Ramaiah College of Pharmacy, Bangalore for future reference (016).

2.1 Chemicals and Instruments

All chemicals and reagents used for testing were of analytical grade obtained from E.Merk. Collected fresh leaves were washed and used for study of macroscopical observations microscopical investigations, histochemical tests, macerate and powder analysis[7,8,9]. For microscopical studies free hand sections of leaves were taken using a sharp razor blade. Sections

were cleared by warming with a few drops of chloral hydrate, stained with phloroglucinol: conc. HCl (1:1), treated with iodine solution and safranin. Sections were then mounted in glycerin with cover slip for microscopical observations.

Photographs of the images were captured by observing different sections under a compound binocular microscope (Olympus – CH 20 I model) with CMOF analogue camera, AV-digitalizer. Special software (Grand VCD 2000+) was used for capturing the images. These were transferred to a CD after selection and photo prints were taken on Kodak paper. Measurements of tissues were recorded using Micro Image Lite image analysis software (Erma – Japan).

2.5 Preliminary analysis

Physico-chemical parameters like different extractive values, percentage of total ash, acid-insoluble ash, water soluble ash and sulphated ash were calculated as per the Indian Pharmacopoeia, 1996. Fluorescence analysis was carried out by following established protocols[10,11]. Preliminary phytochemical analysis was carried out by using standard procedures[12, 13, 14] .

3.0 Results and discussion

The genus *Cadaba* possess rigid wiry unarmed shrubs. Leaves simple or 3-foliolate. Flowers solitary, corymbose or racemed. Sepals 4, unequal, in 2 whorls, outer 2 valvate. Petals 4 or 2, clawed, hypogynous, disk large, colored, encircling the gynophore with its tubular stalk and expanded trumpet-wise at the top or spathulate. Stamens 4-6, inserted unilaterally on the slender gynophore. Ovary 1-celled, stigma sessile; ovules many, on 2-4 parietal placentas. Fruit a fleshy slender cylindric berry or sometimes dehiscing ultimately by two valves which fall away from the placentas. Seeds globose, testa horny; cotyledons convolute.

Leaves trifoliolate, petals 2, pure white with yellowish veins; disk limb bright yellow; stamens 6; fruit 2-4 inch long, dehiscent ~ 1. trifoliata.

C. trifoliata is a rigid, branched shrub or a small tree with hairy shoots. Leaves trifoliate; leaflets ovate or lanceolate, base cuneate with an entire margin and an acute apex; upper surface greenish and glabrous while the lower surface is pale green; flower – pure white with yellowish vein, petals 2, stamens 6; fruit capsule, cylindric, 2-4 inch long, blunt usually curved; seeds angular or reniform, finely muricate (Fig.1).

Leaves trifoliate; leaflets ovate or lanceolate, base cuneate, margin entire; apex acute; upper surface greenish, glabrous lower surface pale green and fibrous. Taste slightly bitter with a characteristic odour[12,13].

The petiole is abaxially circular with two thick, long adaxial horn-shaped wings. The epidermis is distinct comprising of circular cells with thin cuticle and dark tannin content. The ground tissue is homogeneous and circular, thick walled parenchymatous cells. Fairly large druses of calcium oxalate crystals are common in the ground parenchyma cells. This vascular bundle is single, large and circular with adaxial opening; the two free ends of the adaxial openings are invaginated into the central part (Fig.1.4 - 1.6). The xylem consists of several, thick walled, radial conical bands, the intervening gaps of the xylem bands has thin walled parenchyma cells. The xylem elements are circular, narrow and thick walled. Phloem occurs in discontinuous masses along the lower part of the xylem. Sclerenchyma sheath is absent or scanty, solitary sclerenchyma are seen.

The lamina is dorsiventral, smooth and even. The adaxial epidermis is slightly wider with squarish or rectangular cells and thick, smooth cuticle. The abaxial epidermis is thin and consists of small squarish cells. The mesophyll is not well differentiated into palisade and

spongy parenchyma. However, the adaxial half of the lamina has one or two layers of wide, rectangular palisade cells and the lower half has four or five layers of spherical, less compact spongy parenchyma cells. The vascular bundles of the lateral veins are placed in the median part of the mesophyll. The vascular bundles are small, collateral and have scleren caps on the lower and upper ends (Fig.1.7).

The midrib is planoconvex with flat adaxial side and wide hemispherical abaxial side (Fig.1.8). The epidermis of the adaxial side is thick with wide rectangular cells and heavy, smooth cuticle. The abaxial epidermis is also prominent with squarish and thick, warty cuticle. The vascular strand is large and bowl shaped. It consists of parallel bands of thick walled, circular, compact xylem elements separated from each other by thin parenchymatous cells. Phloem occurs as a thin arc along the basal part of xylem mass. Phloem is ensheathed by a bowl-shaped band of thick walled sclerenchyma cells on the adaxial part of the midrib, there is a small collateral vascular bundle which has a few xylem strands and a small nest of phloem. The phloem mass has a wide, druse arc of sclerenchyma cells. Small druses of calcium oxalate crystals are seen in the ground tissue. The palisade zone extends as narrow band above the vascular bundle. In lower part of the midrib, the ground tissue is parenchymatous, with circular, thick walled compact cells.

The adaxial epidermis is apostomatic (absence of stomata) and the epidermal cells are small with wavy cuticle. The anticlinal walls are thick and wavy (Fig.1.9). The abaxial epidermis is stomatiferous, and is deeply sunken stomata. Stomata are of paracytic (Fig.2). The secondary and tertiary veins become gradually thinner forming distinct vein-islets. The vein-islets are wide and polyhedral. The vein-islet has mostly single and multiple vein terminations. The vein-terminations are usually forked once or twice forming short dichotomies (Fig.2.1, 2.2).

The extreme end of each terminal has a cluster of rectangular tracheids. These tracheids are wide, thick walled, lobed or entire, and lignified. The long, thick sclereids are also seen in the mesophyll.

Sections of leaves of *C. trifoliata* were treated with different chemical reagents for histochemical and results are presented in Table-1. Macerate of leaf (including petiole) showed the elements (Fig.2.3) viz., fibres with narrow and broad lumen, vessels of different size and shape, having spiral thickenings, parenchyma cells, which are tangentially elongated or rectangular, foliar sclereids of different size and shape, druses of calcium oxalate crystal, collenchymas cells, which are thick walled and elongated, epidermal peel and tracheids, which are narrowed at both, end and pitted.

Dried leaf powder is green in color. Elements with specific colors were shown by the plant powder upon treatment with different chemical reagents (Fig.2.4). They are fibres, vessels with spiral thickenings, trichome, epidermal peel, parenchyma cells, sclereids, simple starch grains, globular in shape, appearing bluish (when treated with iodine) and druses of calcium oxalate crystal.

Leaf constants such as stomatal number, stomatal index, palisade ratio, vein islet number and veinlet termination number were found to be 187.5 – 230 – 255, 20 - 23.90 - 25.50, 9 - 11.23 – 12.21, 05 – 06.5 – 08 and 06.5 - 08 – 10.5. Physicochemical tests such as ash value, extractive value, moisture content and crude fibre content are provided in Table-2. Fluorescence analysis helps in identifying the drug in powder form and presented in Table-3. Successive solvent extractive values and nature of extracts were found and presented in Table-4. Preliminary phytochemical tests revealed the presence of different metabolites and are presented in Table-5.

Chemical constituents such as alkaloids, carbohydarates and glycosides, flavonoids, phenolic compounds, triterpenoids and tannins are present in the leaves that are similar to the reported allied species *C. fruticosa*[15].

4.0 Conclusion

The pharmacognostical parameters help in identification, standardisation and also aid in formulating pharmacopoeial standards of drugs. Exomorphological characters serve as useful tool to identify the species taxonomically. Pharmacognostical study comprises of taxonomical characters, macroscopical, microscopical characters including macerate studies, powder analysis and histochemical tests of *C. trifoliata*. Physicochemical constants, phytochemical investigations of the extracts rendered valuable information about the nature and intensity of phytoconstituents present in the plant. This also helps in preparing a comparative study amongst its species that are in progress.

5.0 Acknowledgement

Authors are thankful to M.S.Ramaiah College of Pharmacy for providing necessary facilities to carry out the work.

6.0 References

1. Kannusamy Pillai.C, *Padhartha Gunavilakkam* (Moola varkkam), *Materia Medica* (Vegetable Kingdom), Ratna, Nayagar & Sons, 1973, Chennai, 656.

2. Murugesa Mudhaliar.K.S. *Gunapadam, Siddha Materia Medica*, (Medicinal Plants Division), Chennai, 1936, 824.

3. Sharma PC, Yelne MB, Dennis TJ. *Database on medicinal plants used in Ayurveda*.vol.1, 2nd ed. Delhi, Central Council for Research in Ayurveda and Siddha, 2000, p. IX.

4. Yoganarasimhan SN. *Medicinal Plants of India*, Vol. 2, Tamil Nadu. Bangalore: Cybermedia, 1996, 82.

5. Mitra R, *Bibliography on Pharmacognosy of Medicinal Plants*. Lucknow, NBRI, 1985, 46.

6. Iyengar MA. (1950-75). *Bibliography of Investigated Indian Medicinal Plants*, Manipal, Manipal Power Press, 1976, 39.

7. Brain KR, Turner TD. *The practical evaluation of phytopharmaceuticals*, Wright Scientechnica, Bristol, 1975, 1-5.

8. Johansen DA. *Plant Microtechnique*, New York, McGraw Hill, 1940, 183-203.

9. Kokate CK. *Practical pharmacognosy.* 1st edition, Vallabh Prakashan, New Delhi: 1986, 112-120.

10. Chase CR, Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of system of identification, *J Am Pharm Assoc*; 1949, 38: 32.

11. Kokoski JC, Kokoski RJ and Slama FJ. Fluorescence of powdered vegetable

drugs under ultraviolet radiation, *J Am Asso*, 1958, 47(10): 715.

12. Harborne JB. *Phytochemical Methods*. 3rd ed. London, Chapman and Hall, 1998, 85-8.

13. Wagner H, Bladt S, *Plant Drug Analysis*. Berlin: Springer, 1996, 64, 83.

14. *Indian Pharmacopoeia* 4th ed., Vol. II, Government of India, Ministry of Health and Welfare, New Delhi, 1996, A48- A54.

15. Mythreyi R, Sasikala E, Geetha A, Madhavan V. Pharmacognostical studies of leaves of *C.fruticosa* (L.) Druce, Phcog J, Vol.1, 2009, 1; 8-14.

Fig.1. *CADABA TRIFOLIATA* (ROXB.) WT. & ARN

***Cadaba trifoliata* (Roxb.) Wt. & Arn.**

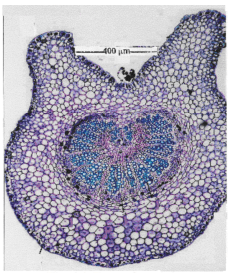
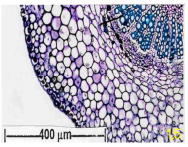


Fig.14.T.s . of petiole



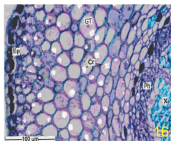


Fig. 15, 16. T.s. of petiole - a portion enlarged

Cadaba trifoliata (Roxb.) Wt. & Arn.

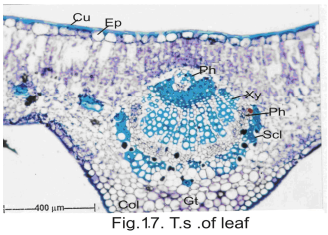
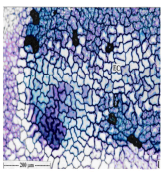


Fig.17. T.s .of leaf

***Cadaba trifoliata* (Roxb.) Wt. & Arn.**



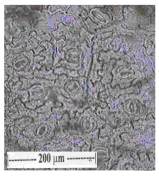


Fig.19, 20 Adaxial and abaxial epidermis

***Cadaba trifoliata* (Roxb.) Wt. & Arn.**

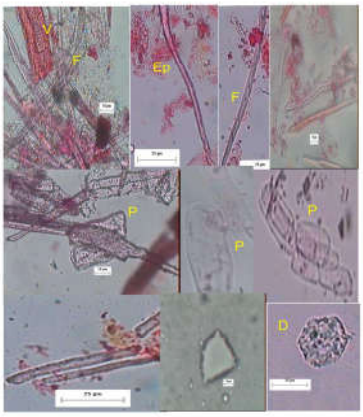


Fig.23 Leaf maceration
F- Fibre; Ep-Epidermis; V- Vessel; D-Druse of calcium oxalate crystal; P-Parenchyma

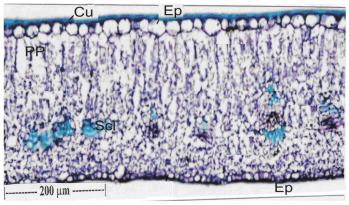
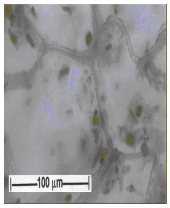


Fig. 18. T.s . of lamina



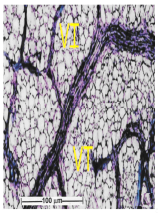


Fig. 21, 22 Venation pattern

***Cadaba trifoliata* (Roxb.) Wt. & Arn.**

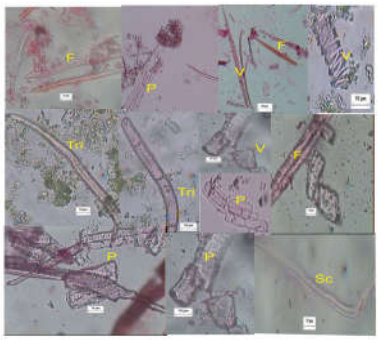


Fig.24 Powder analysis
F-Fibre; P-Parenchyma; V- Vessel; Sc-Sclereids; Tri-Trichome

Table-1 Treatment of sections of *C. trifoliata* with different chemical reagents

Drug	Reagent	Reaction	Test for	Result
Section	Iodine solution	Blue	Starch	+
Section	Ferric chloride	Bluish black	Tannin	+
Section	Phloroglucinol + dil. HCl	Pink	Lignin	+
Section	Millon's reagent	Red	Proteins	-
Section	Dragendroff's reagent	Brown	Alkaloids	+
Section	Rheuthenium red	Pink	Gums & Mucilage	-
Section	Toluidene blue	Blue colour	Polyphenol content	+
Section	HCl	Dissolved	Crystals	+

+ = present; - = absent

Table-2 Ash values

Test	*C. trifoliata*
Total ash	11.86%
Water soluble ash	7.09%
Acid insoluble ash	3.06%
Sulphated ash	1.02%
Water soluble extractive	8%
Alcohol soluble extractive	5%
Moisture content	5%
Crude fibre content	10.1%

Table-3 Fluorescence analysis

Treatment of powder	Ultra -violet light		Visible light
	254 nm	**366 nm**	
Powder as such	Nf	Tm	Tm
Powder + 50% H_2SO_4	Nf	Tm	Tm
Powder + 50% HNO_3	Nf	Mb	Mb
Powder + 5% KOH	Nf	Gy	Gy
Powder + Methanol	Nf	Tm	Tm
Powder + 1N HCl	Nf	Tm	Tm
Powder + 1N Methanolic NaOH	Nf	Wg	Wg
Powder + ethanol (70% v/v)	Nf	Tm	Tm
Powder + 1N Ethanolic NaOH	Nf	Tm	Tm
Powder + Acetone	Nf	Tm	Tm
Powder + ammonia	Nf	Tm	Tm

Tm-Tata mimosa; Ly-Lemon yellow; Mb-Mid buff; Cg-Casgade green; Gy-Golden yellow; Nf-No fluorescence; Jg-Jade green

NOTE: Colours mentioned in the Table are based on the "Asian paints" premium gloss enamel sheet, Asian paints limited, Mumbai.

Table-4 Successive solvent extractive values

	Nature of extracts		**Extractive value (%w/w)**
Solvent	**Colour**	**Consistency**	***C. trifoliata***
Petroleum ether	Greenish brown	Sticky mass	5.19
Benzene	Olive green	Sticky mass	3.8
Chloroform	Olive green	Solid	2.7
Acetone	Tata mimosa	Sticky mass	3.7
Ethanol 70% v/v	Yellowish brown	Solid	4
Water	Dark brown	Solid	4.4

Table-5 Preliminary phytochemical analysis of leaves of *C. trifoliata*

	Successive solvent extracts					
Type of compound	**Pet. ether**	**Benzene**	**Chloro form**	**Acetone**	**Ethanol**	**Water**
Alkaloids	-	-	+	- -	+++	-
Carbohydrates	-	-	- -	++	+++	++
Glycosides	-	-	- -	++	+++	++
Saponins	-	-	- -	- -	- -	-
Flavonoids	-	-	+++	+++	++	-
Steroids	-	-	+++	+++	+++	++
Phytosterols	++	+++	+++	-	-	-
Gums and mucilage	+++	-	-	-	+++	++
Phenolic compound and tannins	-	-	+++	+++	++	+++
Fixed oil and fats	+	+	-	-	-	-
Proteins and amino acids	++	-	-	-	-	-
Volatile oils	-	-	-	-	-	-
Triterpenoids	-	-	+++	-	+++	-

+ presence in low, ++ presence of moderate, +++ presence of high, - absence