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Preliminary Phytochemical Screening, Fingerprinting and Pharmacognostic Evaluation of *Nothapodytes nimmoniana* Leaves, Stem and Root Collected from Different Geographical Region

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ABSTRACT

Nothapodytes nimmoniana (Graham) belonging to family icacinaceae is commonly known as Amruta and locally as Maharashtra, Goa, Kerala, Karnataka, Assam, Tamilnadu in India. It is an important medicinal plant used in various types of cancer, for HIV, in malaria and antibacterial activity. As the herb is used widely in the Indian traditional system, it was thought worthwhile to undertake the standardization of its leaves, stem and root parts. Aerial parts consist mainly of leaves that are simple, alternate, spiral, clustered at twig ends, petiole 1.2-6 cm long, flat above, puberulous, lamina 9-30 x 5-14 cm, flowers are yellowish, in terminal cymes, emitting an unpleasant odour. In the powdered form it had unpleasant odour and exceedingly sweet taste, fruit Drupe, purplish red, smooth, oblong, 1.5-1.8 cm. Microscopical examination of powder of aerial parts showed fragments of epidermis, glandular trichomes, stone cells, lignified xylem elements, anomocytic stomata and abundant calcium oxalate crystals. Successive extractive value was highest in aqueous extract (35% on dry weight basis). Mean ash values (%) were 2.50 (total), 0.05 (acid insoluble ash) and 1.29 (water soluble ash) are lowest in stem and greater in leaf. Loss on drying was found to be 8.34% and pH values of aqueous extract was 6.50. Foaming index was less than 100. Screening of all extracts indicated the presence of all phytoconstituents except resin. TLC fingerprints of extracts of all parts were also developed. The proposed parameters presented in this paper may help to establish the authenticity of drug, differentiate the drug from other camptothecin containing species and drawing the pharmacopoeial standards for this species.

Key words: *Nothapodytes nimmoniana*, TLC Fingerprints, Mappia Foetida, Camptothecin, 9-Methoxy-Camptothecin.

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INTRODUCTION

Nothapodytes nimmoniana (J. Graham) Mabberly (Icacinaceae) [formerly, *Mappia foetida* Miers] is a rich source of the potent alkaloid Camptothecin (CPT), 9-methoxy camptothecin and mappicine [1]. It is the endanger medicinal tree from Western Ghat (Karnataka) and vulnerable in Kerala & Tamilnadu of India which produce CPT [2]. CPT is a potent antineoplastic agent, which is a water insoluble pentacyclic monoterpene derivative indole alkaloid. CPT was first isolated from the Chinese tree Camptothecia acuminate [3], and Later it was isolated from a variety of plant species including Merriliodendron megacarpum [4,5] and Nothapodytes nimmoniana [6] (family Icacinaceae), Ophirrohiza mungos [7] and O. pumila [8] (family Rubiaceae), Eravatamia heyneana (family Apocynaceae) and Mostuea brunonis (family Loganiaceae) [4] in these species the highest concentration of CPT 0.3% (w/w) has been reported from *Nothapodytes nimmoniana*, a small tree distributed in the Western Ghats, South India [9]. In India CPT was first isolated and structure of CPT was settled by professor Govindachari from Indian species Nothapodytes foetida from its spectral data as well as by direct comparison with the authentic compound [10, 11]. CPT is an inhibitor of the DNA-replicating enzyme topoisomerase I and is believed to act by stabilizing a topoisomerase I-induced single strand break in the phosphodiester backbone of DNA. CPT binds reversibly to a Topo-1-DNA cleavable complex to form a stable ternary complex [11].

It numerous analogs have been synthesized as potential therapeutic agents [12]. Irinotecan [13,14] and topotecan [15] are two water-soluble derivatives of CPT, have been approved by the Food and Drug Administration (FDA) of the United States of America for treating colorectal and ovarian cancer [16-18]. In fact, CPT is regarded as one of the most promising anticancer drugs of the twenty-first century [6]. In the recent year CPT as a promising drug used in AIDS chemotherapy [19]. The anti HIV activity of CPT is due to the inhibition of Tat-mediated transcription from the viral promoter [20]. It is also active against parasitic trypanosomes and Leishmania [21]. CPT inhibits retroviruses such as the HIV and the equine infectious anaemia virus. CPT also hinders the synthesis of RNA [22]. CPT is also active against the malaria [21], antibacterial activity [23], and anti-inflammatory activity from the leaves of Nothapodytes foetida Miers [24]. Roja and Heble reported about 0.075% CPT in shoots of mature trees. Padmanabha et al determined CPT content in different parts of N. foetida. Japanese species of *Nothapodytes obscura, N. obtusifolia, N. piltosporsides, N. tomentosa and N. collina* [25].

The faster increasing pharmaceutical market demand of the CPT is very heigh due to unavailability of synthetic CPT. An estimated 20% of the population of this species is believed to have declined over the last decade. Recently, it has been assigned the threat status of 'Vulnerable' [26]. It have identified that the species is polygamous in nature [27].

For reduce the cost of this alkaloid few studies addressing this possibility have been carried out by plant tissue culture by some researcher such as Van Hengel et al., Ciddi & Shuler., Fulzele et al., Thengane et al., [28-31] . The projected global demand for CPT in 2002 was

valued at US$ 4045 million [32]. Thus, it was thought worthwhile to undertake the preliminary pharmacognostical standardization of *N.nimmoniana* aerial parts. Moisture content, extractive, ash value, and R_f values are relatively simple parameters for development of preliminary standards [33]. Standardisation of amrut aerial parts was carried out for widely accepted parameters. The aim of present study was to evaluate pharmacognostic studies on N. nimmoniana using macroscopical, microscopical, physicochemical and phytochemical parameters [33] with the aim of drawing the pharmacopoeial standards for this species.

MATERIALS AND METHODS

Materials

Leaves, stem, root parts were collected from *N.nimmonian* trees growing in natural habitat in Maharashtra, Karnataka, Tamilnadu, India, in july-August, 2010 and identified by R.Vasudeva, Taxonomist, Sirsi College of forestry, Karnataka.

Methods

All parts of *N.nimmoniana* were examined systematically to observe morphological characters followed by microscopy of all parts of plants which collected from different geographical sources. Extractive values were determined for hot and successive extraction methods. Standard methods were followed to determine the total, acid insoluble and water-soluble ash. The foreign matter percentage in the crude drug was determined. Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts and loss on drying was noted. Standard procedures were followed for recording the swelling and foaming indices were carried out as described by WHO.

The powder of all parts were subjected to the fluorescence analysis after being separately treated with water, NaOH, H_2SO_4, HCl, picric acid, ammonia solution, methanol, 5% iodine solution chloroform and examined under UV light as well as in daylight. Different colors were observed after treating the powder with NaOH, H_2SO_4, HCl, HNO_3, glacial acetic acid, chloroform, picric acid, ammonia solution, methanol and 5% iodine solution. The petroleum ether, chloroform, acetone, methanol and aqueous extract residues of all parts were subjected to phytochemical screening for detection of plant constituent's viz., sterols, alkaloids, tannins, flavonoids, proteins, amino acids, carbohydrates (including sugars) and lipids. TLC profiling was done as per the method described by Stahl[34]. Methanolic extracts were subjected to TLC to find out the nature and approximate number of the compounds present.

RESULTS AND DISCUSSION

The morphological examination revealed that leaves were simple, alternate, spiral, clustered at twig ends, petiole 1.2-6 cm long, flat above, puberulous, lamina 9-30 x 5-14 cm, broadly ovate-oblong to elliptic-oblong and sometimes obovate, apex acuminate, base acute to

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rounded attenuate or asymmetric, margin entire, chartaceous (at low elevations) to coriaceous with strong nerves (at high elevations), dark green above, pale beneath, glabrous, midrib flat, secondary nerves 6-11 pairs, tertiary nerves, distantly percurrent. Fruits were Drupe, purplish red, smooth, oblong, 1.5-1.8 cm. Morphological studies are in agreement with the previous findings [35, 36]. On the basis of these morphological details *N.nimmoniana* can be differentiated from other plants when intact. However, the identification becomes difficult in bailed or powdered samples. Therefore, it is tried to establish the pharmacognostical and phytoanalytical parameters for its standardization and quality control, especially in the powder form. Powder microscopy of *N.nimmoniana* showed abundant fragments of thin walled, polygonal epidermis with thick striated waxy cuticle, covering trichomes and sunken anomocytic stomata. Lignified fibres with simple pits, aseptate, 40-50 µ diameter were also present. Vessels were lignified with simple pits, 50-120 µ in diameter. Calcium oxalate crystals were irregular shaped and abundant.

1.Morphology

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Figure:1 (a) N. nimmoniana fresh leaf (b) N. nimmoniana dry leaf (c) N. nimmoniana stem wood (d) N. nimmoniana root wood (e) N. nimmoniana stem bark (f) N. nimmoniana root bark (g) N. nimmoniana flowers (h) N. nimmoniana fruits

2. Microscopy

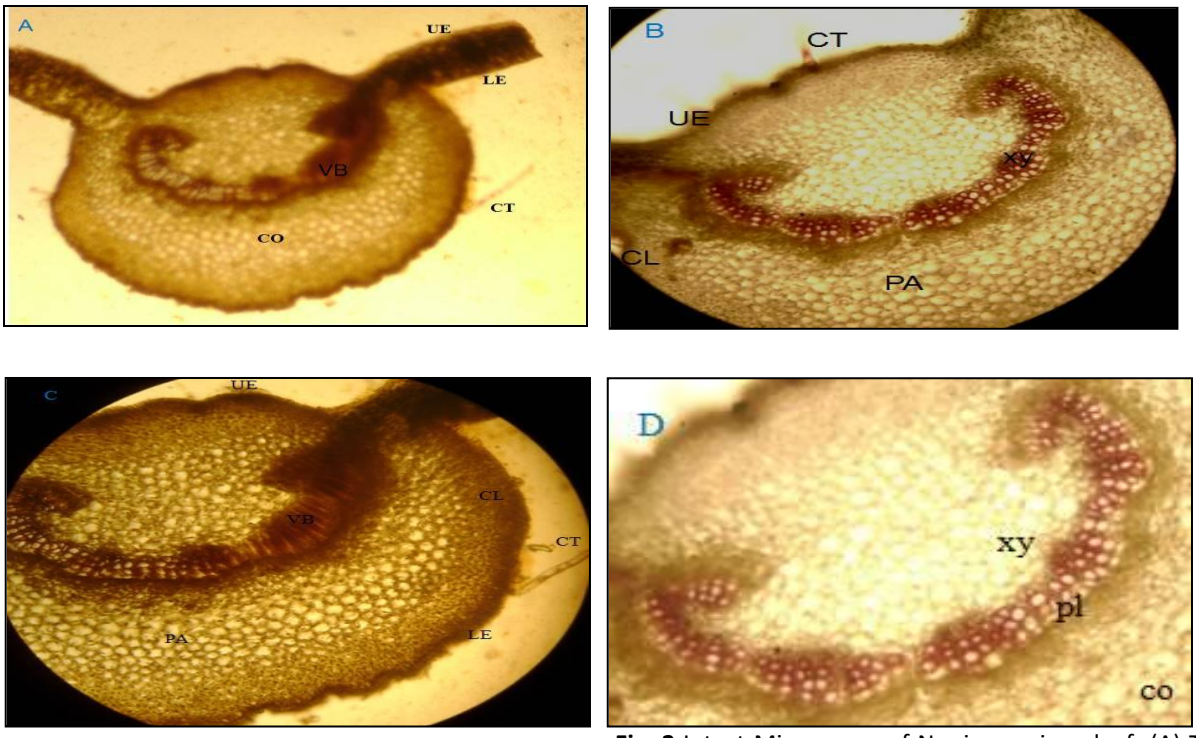

Fig: 2 Intact Microscopy of N. nimmoniana leaf (A) T.S of N. nimmoniana leaf (B) T.S showing CT=covering trichome, VB=vascular bundle, UE= upper epidermis, PA=parenchyma and XY=xylem (C) T.S showing CT=covering trichome, VB=vascular bundle, UE= upper epidermis, LE= lower epidermis, PA=parenchyma, and CL=collenchymas (D) T.S showing XY=xylem, Pl=phloem and CO=cortex

Habit : Trees up to 8 m tall.

Trunk & Bark : Bark brownish, lenticellate; blaze light orange.

Branches and Branchlets : Young branchlets subterete, lenticellate, puberulous.

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Leaves : Leaves simple, alternate, spiral, clustered at twig ends, petiole 1.2-6 cm long, flat above, puberulous, lamina 9-30 x 5-14 cm, broadly ovate-oblong to elliptic-oblong and sometimes obovate, apex acuminate, base acute to rounded attenuate or asymmetric, margin entire, chartaceous (at low elevations) to coriaceous with strong nerves (at high elevations), dark green above, pale beneath, glabrous, midrib flat, secondary nerves 6-11 pairs, tertiary nerves, distantly percurrent.

Flower: Flowers yellowish, in terminal cymes, emitting an unpleasant odour.

Fruit: Drupe, purplish red, smooth, oblong, 1.5-1.8 cm.

Table 1: Organolyptic evaluation of N. nimmoniana

Parameter	Leaf	Stem	Root
Colour	Green on fresh, blade black on drying	Yellowish brown to dark brown	Light yellow to brownish
Odour	Unpleasant	Unpleasant	Unpleasant
Taste	Sweet	Sweet	Sweet
Size	10-30 cm in length, 5-14 cm in width	Up to 8 m or more in length and 25 cm or more in width	80-110 cm in length and 15- 30 cm width
Shape	Ovate, elliptic or lanceolate-oblong	Cylindrical, rough and fibrous	Cylindrical, rough and fibrous

Leaf: The transverse section of leaf showed the dorsiventral characters. The important tissues in the lamina and midrib region are as follows-

A. Lamina:

Transverse section of lamina shows single layered epidermis. It is composed of rectangular, barrel shaped cells. It is followed by single layered palisade cells. It is followed by spongy tissue. Spongy mesophyll is 2-3 layered. The secretory cell is present in the mesophyll region. It is followed by single layered, rectangular lower epidermal cells. Lower epidermis shows the presence of anamocytic stomata.

B. Midrib:

Transverse section of midrib shows single layered rectangular, barrel shaped upper and lower epidermal cells. It is followed by 4-5 layered collenchyma cells. Cortex is composed of loosely arranged rounded to oblong parenchyma cells with intercellular cells. Cortex containing cluster crystals of calcium oxalate and starch grains. It is followed by patches of sclerenchyma cells. It is followed by vascular bundle. Vascular bundle composed of phloem and xylem elements. Phloem consists of cluster of calcium oxalate crystals (crystal sheath fibres). Xylem is composed of vessels and xylem parenchyma cells. It is followed by cortex. It is composed of rounded, oblong parenchyma cells with intercellular spaces. It is followed by collenchymas cells. Simple and unicellular trichomes were present on lower epidermal cells

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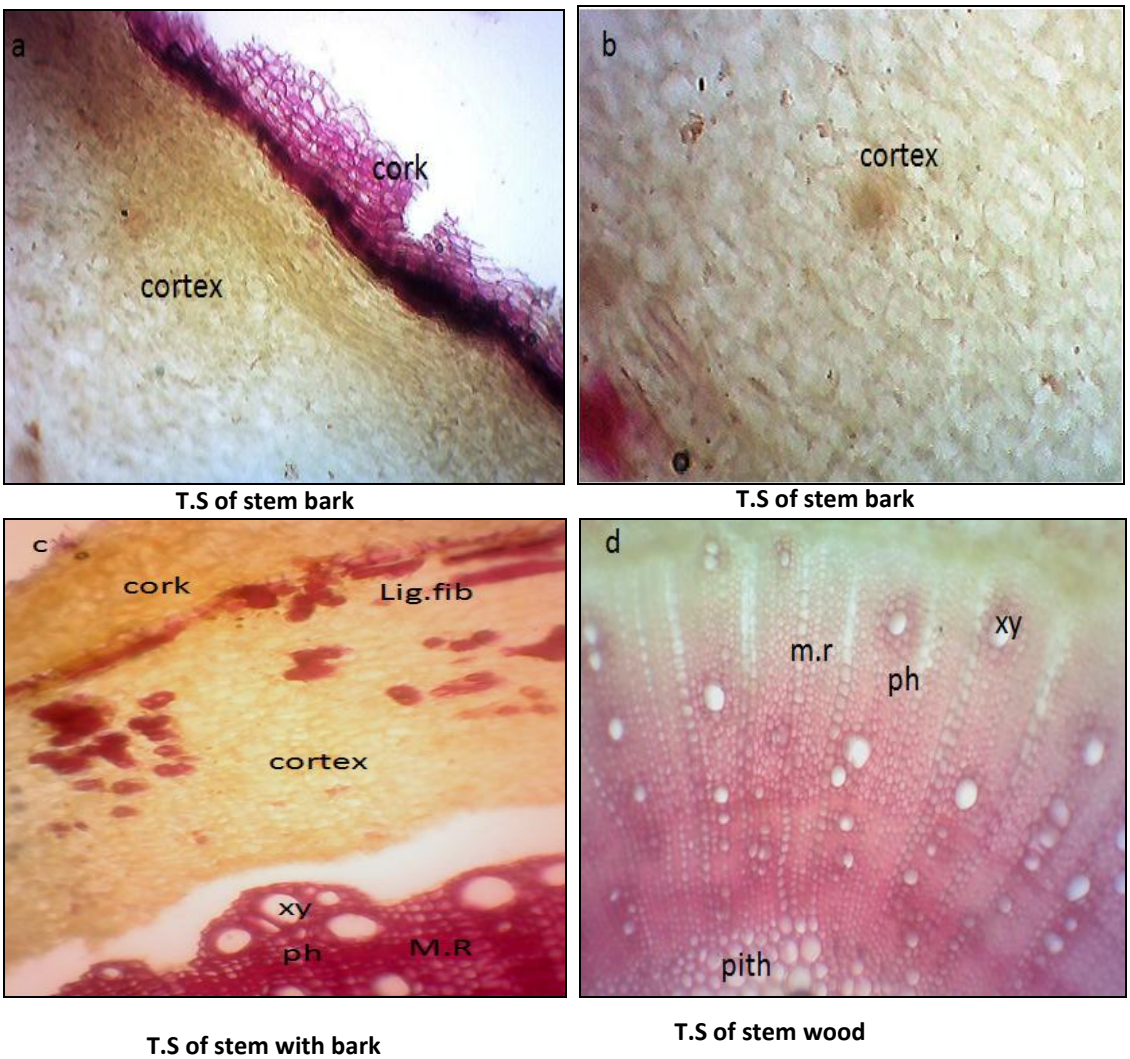

Fig: 3. Intact Microscopy of N. nimmoniana stem bark and stem wood (a) T.S showing cork and cortex (b) T.S showing cortex cells (c) T.S showing cork , cortex ,lignified fiber , xylem (xy), phloem (ph) and medullary rays (M.R) (d) medullary rays (m.r), xylem (xy), phloem (ph) and pith

C. Petiole:

Transverse section of petiole shows single layer of epidermis composed of rectangular, barrel shaped cells. Epidermis is followed by 3-4 layered collenchymatous hypodermis. Cells of collenchyma are rounded. Cortex is composed of loosely arranged rounded parenchyma cells with intercellular spaces. Patches of sclerenchyma cells are present. It is followed by phloem containing calcium oxalate crystals. Xylem is composed of xylem vessels and xylem parenchyma cells. It is followed by parenchyma cells and collenchyma cells. It is followed by lower epidermal cells. Trichomes are present on lower epidermis.

Stem: The transverse section of stem is almost circular in shape. The important features are as follows:

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a. Cork: it contains multiple rows of flattened closely arranged parenchymatous cells.
b. Cortex: The cells are more or less spherical in shape. The inner most layer of cortex i.e.endodermis is made up of closely arranged radially elongated parenchymatous cells.
c. Vascular bundles: collateral, conjoint vascular bundle arranged in form of ring. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Xylem consists of tracheids, fibres and few vessels.
d. Pith: pith region is large and made up of thin walled polygonal parenchymatous cells with intracellular spaces.

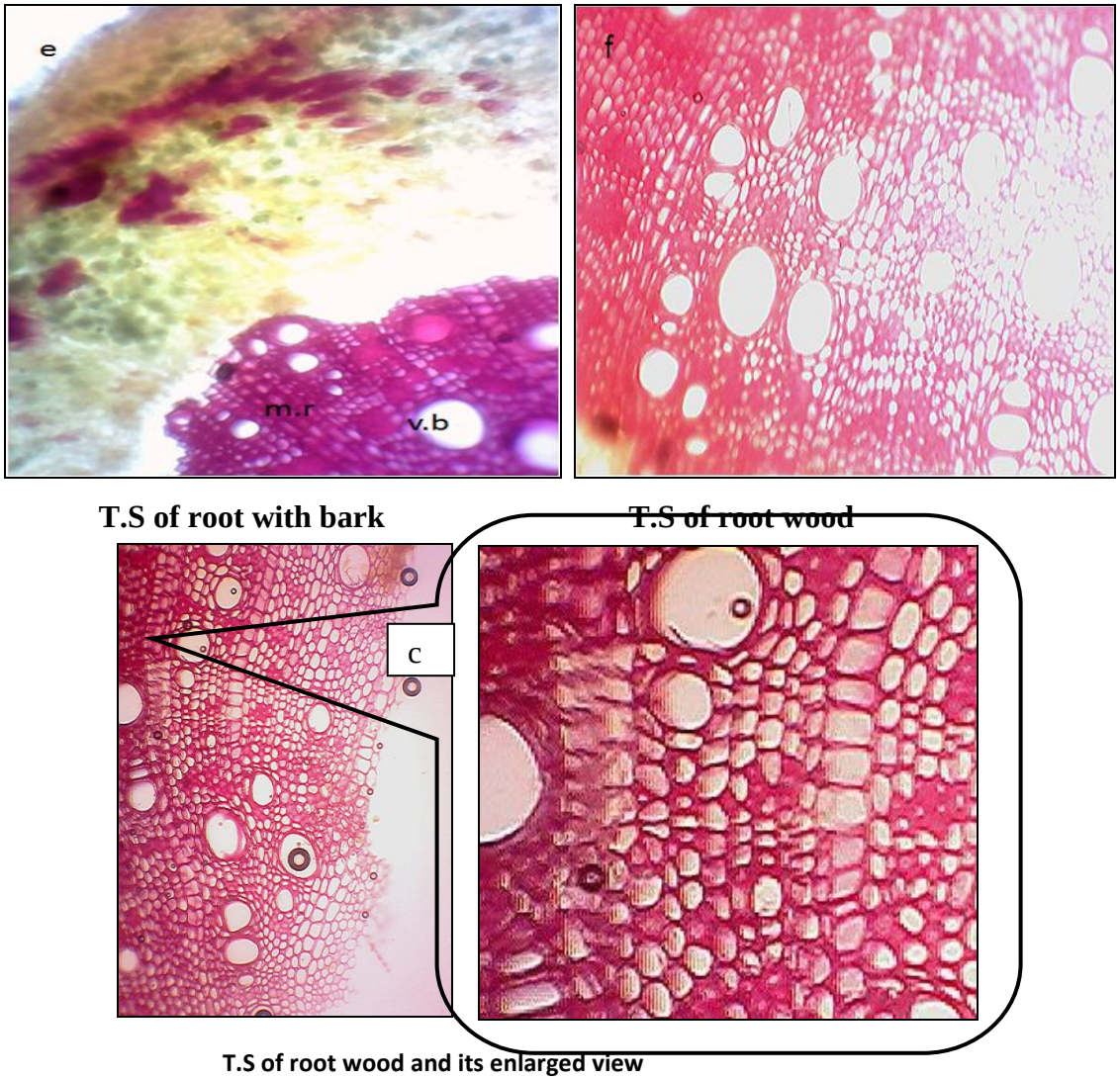

Fig: 4. Intact Microscopy of N. nimmoniana root wood and root bark (a) T.S showing cork, cortex, , pericyclic fibre , vb-vascular bundles and m.r- medullary rays (b) T.S showing xylem , phloem and medullary rays (c) T.S showing xylem , phloem and medullary rays enlarged view

Root: The transverse section of mature root presents a circular outline with following important tissue from periphery to centre.

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Pericycle: multilayered lignified scherenchymatous cells, forming a continuous circle of arches. Vascular bundle: open and collateral.consists of:

Phloem: appears as cap over the metaxylem

Xylem: wedge shaped patches separated by multiseriate medullary rays. Large number of lignified pitted xylem vessels. Xylem vessels and xylem parenchyma are prominent.

Medullary rays: appear like spokes of wheel. Thin walled parenchymatous cells packed with starch grains.

Table 2: SUCCESSIVE EXTRACTIVE VALUES

State	Solvent	Extractive values (% w/w)		
		Leaf	Stem	Root
Sirsi (ka)	Pet. Ether	0.299 ± 0.001	0.808±0.01	0.55±0.01
	Chloroform	1.33 ± 0.05	1.156±0.02	1.32±0.08
	Acetone	2.98 ± 0.02	2.91±0.01	2.56±0.02
	Methanol	15.43 ± 0.03	13.80±0.01	13.6±0.04
	Water	35.63± 0.05	35.09±0.11	32.24±0.09
Hassan (ka)	Pet. Ether	0.253 ± 0.01	0.762±0.01	0.40±0.05
	Chloroform	1.40 ± 0.01	1.194±0.008	1.29±0.01
	Acetone	3.49 ± 0.07	2.507±0.02	3.04±0.07
	Methanol	15.94±0.04	14.01±0.01	14.59±0.04
	Water	34.94±0.01	34.85±0.16	32.40±0.04
Satara (MH)	Pet. Ether	0.195±0.005	0.63±0.001	0.29±0.004
	Chloroform	1.35±0.004	1.18±0.04	1.37±0.04
	Acetone	2.88±0.02	3.14±0.05	2.54±0.07
	Methanol	14.55±0.02	13.57±0.07	13.92±0.05
	Water	35.19±0.04	32.67±0.13	33.37±0.07
Ooty (TN)	Pet. Ether	0.298±0.01	0.757±0.04	0.29±0.004
	Chloroform	0.256±0.01	1.27±0.02	1.18±0.01
	Acetone	3.44±0.04	3.004±0.03	2.96±0.01
	Methanol	16.24±0.08	13.45±0.11	14.19±0.02
	Water	35.16±0.05	30.94±0.07	32.51±0.06

Determination of extractive value for successive extraction of solvent in *Nothapodytes nimmoniana* plant of different geographical region

*Values are in mean± Standard deviation, where n=3.

The mean values of different extractive have been indicated in the Table 2. Successive extractive have higher values in case of water that indicated the presence of polar compounds in all parts.

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Table 3: INDIVIDUAL EXTRACTIVE VALUES

State	Solvent	Extractive values (% w/w)		
		Leaf	Stem	Root
Sirsi (Ka)	ESEV	0.299 ± 0.001	0.808±0.01	0.55±0.01
	ASEV	15.53±0.11	13.96±0.06	13.79±0.22
	WSEV	36.24±0.07	35.17±0.04	33.25±0.14
Hassan (Ka)	ESEV	0.253 ± 0.01	0.762±0.01	0.40±0.02
	ASEV	16.26±0.02	15.65±0.03	14.89±0.08
	WSEV	36.70±0.07	36.59±0.04	35.09±0.04
Satara (MH)	ESEV	0.195±0.005	0.63±0.001	0.29±0.004
	ASEV	14.57±0.01	13.54±0.07	14.09±0.09
	WSEV	36.16±0.08	33.65±0.11	34.61±0.10
Ooty (TN)	ESEV	0.298±0.01	0.757±0.04	0.29±0.004
	ASEV	17.03±0.03	15.15±0.08	15.10±0.10
	WSEV	35.78±0.02	32.19±0.06	34.48±0.10

Individual extractive values of *Nothapodytes nimmoniana* plant of different geographical region
*Values are in mean± Standard deviation, where n=3. Ether soluble extractive value, ESEV Alcohol soluble extractive value Water-soluble extractive value

Table 4: ASH VALUES

State	ash parameter	Ash values(% w/w)		
		Leaf	Stem	Root
Sirsi (ka)	Total ash	12.92±0.07	2.5±0.05	6.85±0.07
	Water soluble ash	0.50±0.01	1.29±0.005	1.45±0.05
	Acid insoluble ash	0.50±0.01	0.05±0.01	0.05±0.01
Hassan (ka)	Total ash	13.02±0.07	2.49±0.004	5.69±0.05
	Water soluble ash	0.49±0.01	1.26±0.002	1.65±0.02
	Acid insoluble ash	0.50±0.005	0.05±0.01	0.06±0.03
Satara (MH)	Total ash	12.55±0.34	2.51±0.005	4.47±0.04
	Water soluble ash	0.59±0.01	1.05±0.05	1.3±0.05
	Acid insoluble ash	0.52±0.06	0.06±0.01	0.049±0.003
Ooty (TN)	Total ash	13.63±0.47	3.02±0.08	7.17±0.06
	Water soluble ash	0.69±0.01	1.32±0.04	1.34±0.10
	Acid insoluble ash	0.44±0.01	0.07±0.01	0.057±0.005

Ash value determination of *N. nimmoniana* plant of different geographical region
*Values are in mean± Standard deviation, where n=3.

Table 5: FOREIGN ORGANIC MATTER

State	% foreign organic matter		
	Leaf	Stem	Root
Sirsi	4.25±0.03	1.00±0.04	1.59±0.10
Hassan (Ka)	2.62±0.05	0.53±0.02	1.26±0.05
Satara (MH)	1.74±0.01	0.62±0.01	1.33±0.09
Ooty (TN)	1.58±0.02	0.46±0.05	1.69±0.04

*Values are in mean± Standard deviation, where n=3.

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Total ash, water soluble ash, acid in soluble ash of *N.nimmoniana* collected from different geographical sources comparise in table no.4. Total ash value was relatively higher due to the high content of carbonates, phosphates, silicates and silica. Foreign matters were also comprised in table no.5. Low value of foreign matter indicated first hand collection of plant from Satara (MH), Sirsi (KA), Hassan (KA) and Ooty (TN), India. Losses on drying were comprised in table no.6. account of loss of water and volatile chemicals. The pHs of aqueous extract were comprise in table no.7. Phytochemical screening was undertaken for the identification of different type of chemical constituents present in the leaves, stem and root parts of plants. Screening of all extracts indicate the presence of all major phytoconstituents i.e., alkaloids, amines, flavonoids, carbohydrates, phenolic, saponins but resins were absent (table no.8)

Table 6: LOSS ON DRYING

State	% LOD of different parts		
	Leaf	Stem	Root
Sirsi	8.05	7.96	8.01
Hassan	8.59	8.98	8.08
Satara	8.34	8.82	7.96
Ooty	8.90	8.32	8.15

Results of loss on drying in *Nothapodytes nimmoniana*

Table 7: PH DETERMINATION

State	Concentration of the solution	pH of the Solution		
		Leaf	Stem	Root
Sirsi	1% solution	6.6	6.8	6.7
	10 % solution	6.0	5.8	5.9
Hassan	1% solution	6.6	6.6	6.6
	10 % solution	5.7	6.0	6.0
Satara	1% solution	6.4	6.4	6.9
	10 % solution	5.8	5.9	6.2
Ooty	1% solution	6.6	6.5	6.8
	10 % solution	5.7	5.8	6.1

Table 8: Phytochemical screening in Leaves, Stem & Root of *N.nimmoniana*

Phytochemical screening has been done to find out presence or absence of various secondary metabolites in Leaves, Stem & Root of *Nothapodytes nimmoniana*.

S.No.	Plant Constituents	*N.nimmoniana* Leaves	*N.nimmoniana* Stem	*N.nimmoniana* Root
1.	**Alkaloids tests**	+	+	+
2.	**Glycosides**	-	-	+
3.	**Tannins**	+	+	+
4.	**Carbohydrates**	+	+	+
5.	**Proteins**	+	+	+
6.	**Saponins**	+	+	+
7.	**Resins**	-	-	-

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8.	**Flavonoids**	-	+	-
9.	**Phenols**	+	+	+
10.	**Coumarins**	+	-	-
11.	**Phytosterols**	+	+	+
12.	**Terpenoids**	+	+	-

Where (+) = presence of Phytoconstituents , **(-) =** absent of the Phytoconstituents.

Table 9: Finger printing of *N.nimmoniana* in different samples.

Sample No	Region name	Number of peaks	Corresponding Rf values
1.	Camptothecin	1	0.60
2.	Hassan Stem bark	4	0.50, 0.60, 0.70, 0.80
3.	Sirsi Stem bark	4	0.08, 0.50, 0.61, 0.70
4.	Satara stem bark	3	0.51, 0.60, 0.70
5.	Hassan stem wood	3	0.53, 0.61, 0.71
6.	Sirsi stem wood	2	0.60, 0.71
7.	Satara stem wood	3	0.54, 0.62, 0.71
8.	Sirsi root bark	4	0.36, 0.48, 0.59, 0.70
9.	Satara root bark	3	0.49, 0.61, 0.71
10.	Sirsi root wood	4	0.38, 0.50, 0.60, 0.71
11.	Satara root wood	2	0.61, 0.72
12.	Hassan leaf	4	0.51, 0.61, 0.72, 0.84
13.	Sirsi leaf	4	0.12, 0.51, 0.62, 0.71
14.	Satara leaf	3	0.52, 0.60, 0.72
15.	Ooty root wood	4	0.50, 0.60, 0.70, 0.80
16.	Ooty root bark	4	0.51, 0.61, 0.70, 0.82
17.	Ooty stem bark	4	0.51, 0.60, 0.70, 0.82
18.	Ooty stem wood	2	0.61, 0.71
19.	Ooty leaf	3	0.51, 0.62, 0.70

Table10: Powdered drug reaction of *N.nimmoniana*. aerial parts with different chemicals

Chemical treatments	Observation (L)	Observation (S)	Observation (R)
Powder as such	Green	Greenish yellow	Brownish yellow
Picric acid	Green	Light green	Yellowish brown
Ammonia solution	Yellowish green	Light yellowish green	Brownish green
1 N NAOH	Green	Green	light brownish green
Methanol	Green	Light green	light brown
Conc.H_2SO_4	Black	Black	Blackish brown
Conc.HNO_3	Green	Light green	Light brown
Conc.HCL	Green	Light green	Light brown
5% iodine solution	Greenish black	Black	Black

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TLC fingerprints of all parts of plant were developed and represented in Table no.9. The solvent system i.e., Ethyl acetate: Acetone (4:1) was worked out on hit and trial basis and gave best resolution of spots without overlapping and was applicable to all extracts.

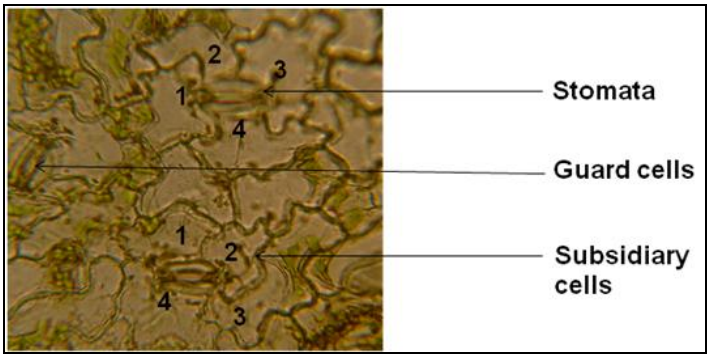

Fig 5: Surface preparation of *N.nimmoniana* leaf.

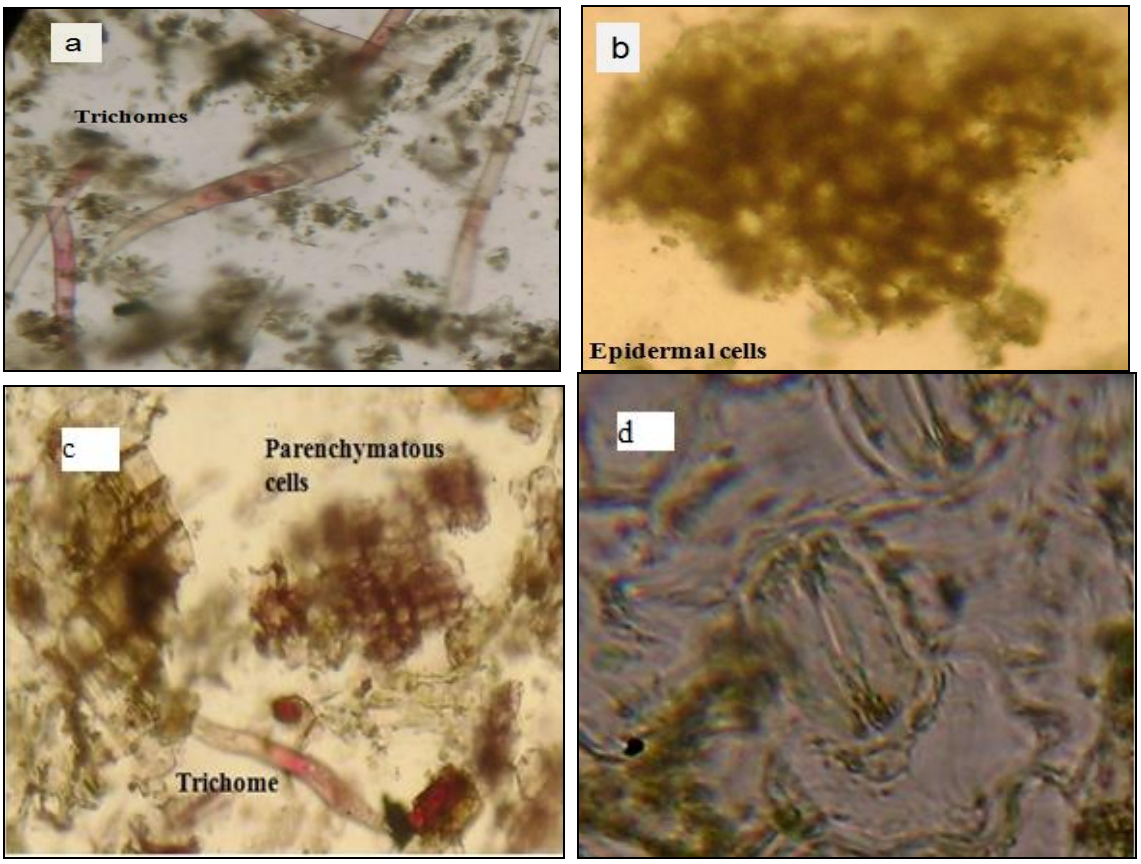

Fig 6: Powder microscopy of *N.nimmoniana* leaf (a) covering trichomes (b) epidermal cells (c) parenchymatous cells along with trichome (d) anomocytic stomata

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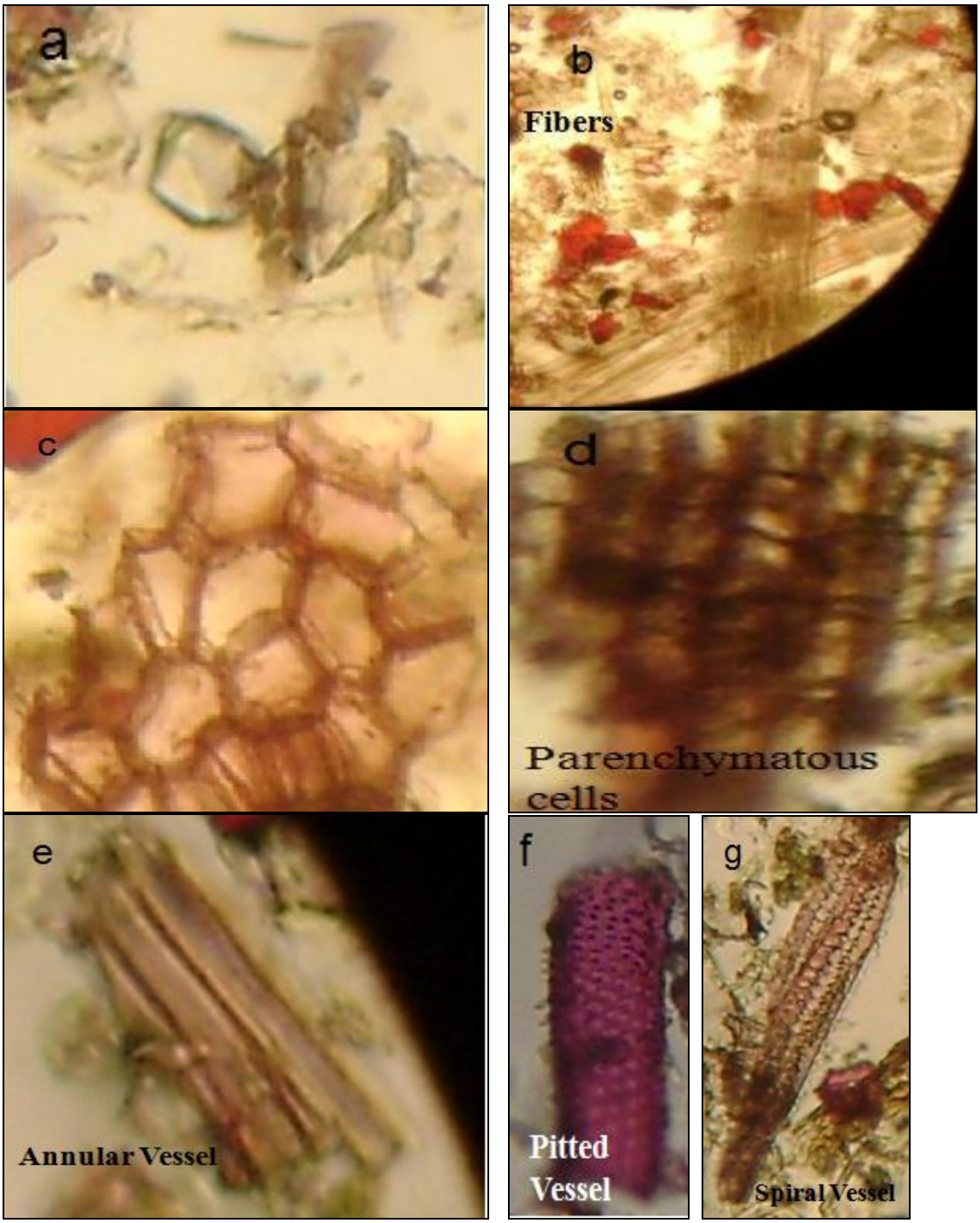

Fig 7: Powder microscopy of *N.nimmoniana* stem (a) calcium oxalate crystal (b) fibers (c) cork cells (d) parenchymatous cells (e) annular vessel (f) pitted vessel (g) spiral vessel

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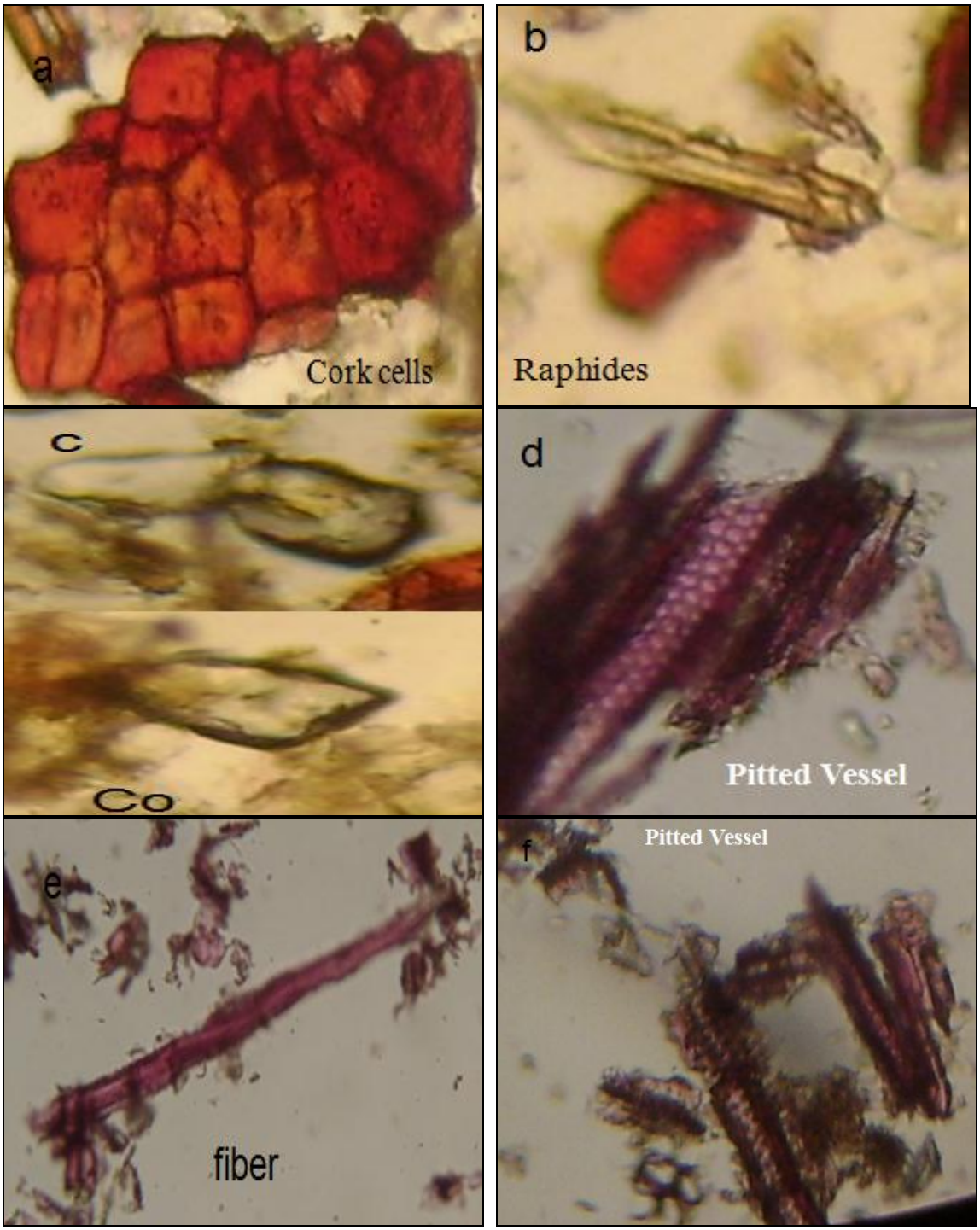

Fig 8: Powder microscopy of *N.nimmoniana* root (a) cork cells (b) raphides (c) calcium oxalate crystal (d) pitted vessel (e) fiber (f) pitted vessel

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CONCLUSION

The leaves,stem and root parts of N.nimmonia collected from different geographical sources have been subjected to pharmacognostic standardization including phytochemical screening. The existing knowledge regarding happy tree (Amrut) may be increased by present investigation and may be quite useful for the quality control of various formulations containing CPT. The main highlight of the present work is that it will be helpful to ascertain the correct identity of *N.nimmonian*a for which there is adultrant available in the drug market for taken more profit. The preliminary standardization studies of all parts of *N.nimmonian*a are underway in our herbal drug laboratory. The current report will also help researchers and scientists design strategies for resolving cases of misidentification of plant material.

REFERENCES

[1] Fulzele DP, Satdive RK, Pol BB. Planta Med 2001; 67: 150-2.
[2] Ravi kumar k & Ved D.100 Red listed Medicinal plants of conservation concern in south India 2000; 261-263.
[3] Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT and Sim GA. J Am Chem Soc 1966; 88:3888–3890.
[4] Gunasekera SP, Badawi MM, Cordell GA, Farnsworth NR and Chitnis M. J Nat Prod 1979; 42: 475-477.
[5] Arisawa M, Gunasekera SP, Cordell GA and Farnsworth NR. Planta Med 1981; 43: 404–407.
[6] Govindachari TR and Viswanathan N. Phytochem 1972; 11: 3529–3531.
[7] Tafur S, Nelson JD and DeLong DC. Lloydia 1976; 39: 261–262.
[8] Aimi N, Hoshino H, Nishimura M, Sakai S and Haginiwa J. Tetrahedron Lett 1990; 31: 5169–5172.
[9] Suhas S, Ramesha BT, Ravikanth G, Rajesh P Gunaga, Vasudeva R, Ganeshaiah KN and Uma Shaanker R. Curr Sci 2007; 92: 8.
[10] Govindachari TR and Viswanathan N. Indian J Chem 1972; 10: 453-454.
[11] Govindachari TR and Viswanathan N. Phytochem 1994; 36:65-66.
[12] Wall ME, Wani MC, Nicholas AW, Manikumar G, Tele C, Moore L, Truesdale A. Leather P and Besterman JM. J Med Chem 1993; 36: 2689-2701.
[13] Kunimoto T, Nitta K, Tanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miyasaka T and Mutai M. Cancer Res 1987; 47: 5944.
[14] Sawada S, Nokata K, Furuta T, Yokokura T and Miyasaka T. Chem Pharm Bull (Tokyo) 1991; 39: 2574-2580.
[15] Kingsbury WD, Boehm JC, Jakas DR, Holden KG, Hecht SM, Gallagher G, Caranfa MJ, McCabe FL, Faucette LF, Johnson RK and Hertzberg RP. J Med Chem 1991; 34: 98.
[16] Lilenbaum RC et al. J Clin Oncol 1995; 13: 2230–2237.
[17] Romanelli SP, Perego G, Pratesi N, Carenini M and Tortoreto Zunino F. Cancer Chemother Pharmacol 1998; 41: 385–390.
[18] Vladu B et al. Mol Pharmacol 2000; 57: 243– 251.

[19] Priel E, Showalter SD & Blair DG. AIDS Research and Human, Retroviruses 1991; 7: 65–72.
[20] Li CJ, Wang C, Pardee AB, J Bio chem 1994; 269: 7051-7054.
[21] Bodley AF, Cumming JN and Shapiro TA. Biochem Pharmacol 1998; 55: 709.
[22] Bendixen C, Thomsen B, Alsner J and Westergaard O. Biochemistry 1990; 29: 5613-5619.
[23] Kumar RN, Vishwanathan H, Suresh T and Mohan PS. Fitoterapia 2002; 73: 734–36.
[24] Sheeja E, Edwin E, Dhanbal SP and Suresh B. I J Pharm Sci 2005; 67: 251—253.
[25] Zhang X I and Bao Juchen et al CN. 1, 045 266 (CI Co7D39 261 12 Sep. 1990, C: A. 114 1647607v 1991).
[26] Ved DK. Trade in medicinal plants – the state of our ignorance. Amruth, 1997; 1: 2–8.
[27] Vasudeva R, Kazi GN. Curr Sci 2002; 83: 9-10.
[28] Van Hangel AJ, Harkes HP, Wichers HJ, Hesselink RGM & Buitelaar RM. Plant Cell Tiss Org Cult 1992; 28: 11–18 .
[29] Ciddi V and Shuler ML. Biotech Lett 2000; 22: 129–132.
[30] Fulzele DP, Satdive RK, Pol BB. Planta Med 2001; 67: 150-2.
[31] Thengane SR, Kulkarni DK, Shrikhande VA, Joshi SP, Sonawane KB and Krishnamurthy K V. Plant Cell Tissue Organ Cult 2003; 72: 247–251.
[32] Lorence A and Craig LN. Phytochem 2004; 65: 2731–2841.
[33] Padmanabhan BV , Chandrashekar M, Ramesha BT, Hombe Gowda, Rajesh P, Gunaga S, Suhas R, Vasudeva KN, Ganeshaiah and Uma Shaanker R. Cur Sci 2006; 90: 95–100.
[34] Raskin I. Trends Biotechnol 2002; 12: 522–531.
[35] Evans WC, Trease and Evans Pharmacognosy. 14th Edn., WB Saunders, London, 1997; ISBN: 0-7020-1899-6.
[36] Singh MP, Dey S. Indian Medicinal Plants. (Satish Serial Publ. House, Delhi, 2005).