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Pharmacognostic and phytochemical investigations of roots of *Hibiscus micranthus* Linn

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ABSTRACT

The objective of the present investigation was determine the Pharmacognostical, physicochemical and phytochemical parameters as standardization markers for *Hibiscus micranthus* Linn.,. The macroscopic and microscopic characters, physical constant values, extractive values, ash values, micro chemical analysis and fluorescence analysis were performed. The presence of lignified, thick wall, libiform type of fibres with pointed tips & vessel elements with oblique perforations plates having short, pointed tails, calcium oxalate druses are abundant in the phloem and periderm as seen in the powder/transverse sections of root were the distinguishing microscopic features and can be used as anatomical markers. Chemomicroscopic characters present included lignin, starch, suberin, mucilage, cellulose, protein bodies and calcium oxalate crystals. Physical constants performed were loss on drying, ash content, acid insoluble ash and water soluble ash. Extractive values in pet. ether (60-80^{o}C), chloroform, alcohol and hydroalcoholic were determined. Fluorescence studies of the powder were carried in ordinary light and UV light with various solvents. Phytochemical screening of successive extracts showed positive reactions for steroids, flavonoids, carbohydrates, phenols and tannins. The fingerprints of the hydroalcoholic extract were obtained by HPTLC technique in three best mobile phase solvent systems. The flavonoid content of hydroalcoholic extract was determined by colorimetric method. Chemical profiling of hydroalcoholic extract was also performed by GC-MS analysis. Further a HPLC method with photodiode array detector was followed to quantify rutin in hydroalcoholic extract of *Hibiscus micranthus* (HEHM). The present study provides details to characterize the Pharmacognostical, physicochemical and phytochemical parameters. An accurate and rapid HPLC quantification method has also been developed for quality control determination of rutin from *Hibiscus micranthus* roots.

Key words: *Hibiscus micranthus,* Pharmacognostical analysis, Chemical profiling, HPTLC, HPLC, GC-MS.

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INTRODUCTION

Human population in countries around the world has been using plants from thousands of years for treating various ailments of humans & animals. This traditional knowledge about the plants can be transferred to several generations only by proper documentation of their botanical, physicochemical, phytochemical characters and along with their medicinal uses in the form of monographs. The monograph of these plants are prepared according to the WHO guidelines and presented as herbal pharmacopoeia. These guidelines enable to identify, authenticate, detect adulterants and standardize the plant material.

Hibiscus micranthus is a shrubby, erect, branched, slender and stellately hairy plant. It is widely distributed in hotter parts of India, Ceylon, Saudi Arabia and tropical Africa. In India, the plant is known by different vernacular names in different regions as Chalabharate in telugu, sittamutti in tamil, chanakbhindo in gujrati and as okder in Sanskrit. Traditionally the plant is considered a valuable febrifuge in india, Ceylon, Saudi Arabia and tropical Africa [1]. In India certain parts of Gujarat, the fruits and flowers of this plant is used as hypoglycemic agent [2]. The plant has been scientifically validated for its antipyretic, anti-inflammatory, hematological effects [3], antimicrobial, antiviral, antitumor [4], female antifertility, viralizing[5] and anabolizing[6] activities. Few compounds like Phenolic acids, flavonoids, β-sitosterol, alkanes, fatty alcohols and acids have been reported [7]. Upon literature survey, it was revealed that, no work as been reported on its pharmacognostic diagnostic features and chemical analysis of root parts.

MATERIALS

Plant material: The whole plant parts were collected in bharat institute of technology, mangalpally, Ibrahimpatnam & were authenticated by Taxonomist Jayaraman at the National Institute of Herbal science, Chennai, India. In order to ensure the sample used was from the same source throughout the experiment, the sample was collected in sufficient quantities at a time.The plant *Hibiscus micranthus Linn.,* was washed thoroughly with running tap water, followed by rinsing with distilled water and then leaves, stem & roots were separated and cut into small pieces. The leaves and stems were shade dried at room temperature, while roots were dried in oven at 45^0c for two weeks. The dried parts of the plant were powdered in mill to a mesh size of 150 and stored in an air tight container till further use.

Chemicals and equipments: All the chemicals used in the study were of analytical grade (SD fine chemicals pvt ltd. Mumbai) obtained from the central store house of the institution. Rutin was obtained from lobei chem. Pvt ltd Mumbai. Microtome (secor, India) UV spectrophotometer 1801 shizadzu, Muffle furnace (Biotechnics, India) , Nikon camera, HPLC (waters), HPTLC (Camag, Switzerland), GCMS shimadzu.

METHODS

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Pharmacognostic Studies

Macroscopic

The following macroscopic characters for the fresh roots were noted with the help of organs of sense: size and shape, color, odor and taste whether herbaceous or woody, upright or creeping, smooth or ridged, hairs present or not if so whether of the glandular or covering form[8].

Microscopy

Plant Collection and preparation for anatomical studies: The plant specimens for the anatomical study were collected from Bharat institute of technology, Mangalpally, Ibrahimpatnam. Care was taken to select healthy plants and normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin-5ml + Acetic acid-5ml + 70% ethyl alcohol- 90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol [9]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning: The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. dewaxing of the sections was by customary procedure [10]. The sections were stained with Toluidine blue. Glycerin mounted temporary preparations were made for macerated/cleared materials [11].

Powder microscopy: Powdered material of root part was cleared with sodium hydroxide and mounted in glycerin medium after staining. Different cell component were studied and measured [11].

Histo-chemical tests: Examination of the powder for starch grains, lignin, mucilage, calcium oxalate crystals, cutin and suberin were carried out using standard techniques [11].

Photomicrographs: Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Descriptive terms of the anatomical features are as given in the standard anatomy books [12].

Physicochemical studies

The Loss on drying, total ash, acid insoluble, water soluble ash and successive soxhlet extractives values were assayed according to standard Indian pharmacopoeia methods. For

fluorescence analysis of the powder sample it was treated with different chemical reagents to observe various colour reactions in ordinary and UV light [13-15].

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as tannins, phenols, steroids, carbohydrates, proteins, alkaloids, saponins, anthracene derivatives, flavonoid glycosides, and cyanogenetic glycosides[16-19].

Extraction

The powdered roots were exhaustively extracted with 70% Hydroalcohol for 1 week in a soxhlet extractor. The collected extracts were filtered and evaporated under vacuum, yielded thick red residue. The residues was dried and stored in air tight container for further use.

HPTLC Fingerprinting Analysis

The TLC fingerprint profile of Hydroalcoholic Extract of roots of *Hibiscus micranthus* was carried out by HPTLC technique[21].

Preparation of sample: 5 gms of *H. micranthus* root powder sample was extracted with 25 ml 70 % ethanol for 8 h under reflux, filtered the extract and repeated the process thrice. Pooled the filtered extracts and evaporated to dryness. Dissolve the residue in 50 ml 70 % methanol. Aliquot of the extract was taken for TLC analysis.

Chromatographic development: Apply 10 µl to the chromatographic plate using a suitable applicator and place the plate in twin trough chamber, add mobile phase in one trough and plate in another. Allow the plate to equilibrate for about 20 minutes, and develop the plate to 8 cm. Remove the plate from the chamber and dry in air.

Colour development: Plates were derivatized with anisaldehyde-sulphuric acid and Lieberman burchard reagent.

Thin Layer Chromatographic conditions:

Stationary phase: Silica Gel 60 F254, **Solvent front:** 7 cm, **Detection:** 254 nm and 366 nm. **Instrument used:** High Performance Thin Layer Chromatography (CAMAG, Switzerland). **Applicator:** Linomat V, **Derivatisation: 1. Anisaldehyde-sulphuric acid reagent, 2. Liebermann-Burchard reagent**
Mobile Phase – I: Ethyl acetate: methanol – glacial acetic Acid (10: 1.35:1)
Mobile phase – II: Chloroform – methanol (9.9:0.1)
Mobile phase – III: Ethyl acetate: formic acid – glacial acetic acid – water (10:1.1:1.1:2.6)

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Spectroscopy analysis of hydroalcoholic extract of root of Hibiscus micranthus for total flavonol content

The total flavonol content of the extract was determined by aluminum chloride colorimetric method[20].

Preparation of Standard: The standard curve was prepared using rutin with methanol as solvent.. The total flavonol content of the extract was obtained using the standard curve.

Preparation of Sample solution: The sample solution of Hydroalcoholic extract of root of *Hibiscus micranthus* 1mg/ml was prepared using methanol as solvent. The solution is passed through a vacuum filter containing whatman filter paper of pore size 0.45µ to get particulate free sample.

Procedure: Hydroalcoholic extract of root of Hibiscus micranthus extract/standard rutin (1 ml) was mixed with 2 ml of methanol, 1 ml of 10% aluminum chloride, 1 ml of 1M potassium acetate and 1 ml of distilled water. The mixture was incubated at room temperature for 30 min. Blank sample was prepared by omitting the standard/HEHM extract. The absorbance of the mixture was measured at 415 nm with a Shimadzu UV-1801 spectrophotometer**.**

HPLC analysis of hydroalcoholic extract of roots of *Hibiscus micranthus*

Preparation of Sample solution: 100 mg of the Hydroalcoholic extract of roots of *Hibiscus micranthus* was dissolved in methanol and suitably diluted to get a concentration of 10µg/ml. The solution was subjected to sonication for degassing and later passed through a vacuum filter containing whatman filter paper of pore size 0.45µ to get clear sample solution.

Preparation of standard solution: The procedure followed was same as that of sample solution, except the standard used was rutin 10µg/ml.

Testing procedure: Test solution and standard solution are subjected to HPLC separately.

HPLC operating conditions: Shimadzu chromatographic system with two LC-10AT VP pumps, variable wavelength programmable UV–vis detector SPD-10A, VP CTO, -10 AS VP column oven (Shimadzu) A reversed phase C18 column (25 cm×4.6mm i.d., particle size 5 _m; YMC, IMC, Wilmington, NC, 28403, U.S.A.) and the HPLC system was monitored by software “Class-VP series version 5.03 (Shimadzu)”. Mobile Phase: Methanol: 2% acetic acid in water (70:30), Flow rate: 1ml/minute, Injection volume: 20 µl, Detection: 264nm

Characterization of Hydroalcoholic extract of roots of *Hibiscus micranthus* by GCMS analysis

Sample Preparation: 200mg of the sample was dissolved in 1ml of the n-hexane. The mixture was sonicated for 15 minutes. 3 µl of the test solution was directly injected into the system.

Chromatographic analysis: The samples were analyzed using Shimadzu GC-MS-QP2010 Plus apparatus equipped with quadrapole detector and split injection system. The GC was fitted with a ZP-624 capillary column (30mm x 1.4 mm, film thickness 0.25µm). The temperature programmed was as follows: injector temperature 220^0c , initial oven temperature at 120^0c for 2 minutes, then rise to 250^0 at the rate of 10^0c per minute at 250^0c for 25 minutes, transfer line temperature 220^0c. Helium was used as carrier gas at 35.6 Kpa pressure with flow 2.5 ml/min and electronic pressure control on. The EM voltage was 952.9 V with lower and upper mass limits set at 30& 350 m/z. Samples were solved in n-hexane and injected automatically. MS spectra of separated compounds were compared with one from Wiley 7 Nist 05 mass spectral database. The identity of the spectra above 95% was needed for the identification of compounds.

RESULTS AND DISCUSSION

Pharmacognostic Studies

Macroscopical study revealed the roots are generally cylindrical, up to 1 cm thick, woody, upright, outer surface smooth in young stems and rough in old stems, greenish externally, yellowish internally, fracture splintery , taste astringent and slightly bitter, odour without any characteristic aroma, agreeable[16].

Microscopical characters:The periderm is wide, comprising of 10-15 layers of narrow suberised tabular phellem cells. The periderm zone is nearly 150 µm wide. The secondary phloem is 200 µm wide. It has three or four, discontinous tangential blocks of fibres. In the outer portion, occurs a narrow portion of crushed phloem and in the inner part, the phloem is intact.(fig1). Secondary xylem is dense and solid cylinder of fibres and vessels. Vessels are diffuse in distribution. They are either solitary(fig 2) or as radial multiples.The solitary vessels are in clusters and the multiple vessels are in radial or oblique lines. The diameter of the vessels ranges from 50-150 µm. Xylem fibres are thick walled with wide lumen. Xylem rays are wide and have thick lignified walls. They have dense accumulation of starch grains . The calcium oxalate druses are abundant in the phloem and periderm. In the phloem and periderm, the druses are diffuse and densely crowded in the collapsed zone(fig 3) . They are 70 µm wide.

Root powder analysis (fig. 4 & 5) revealed the presence of Fibres and vessel elements abundant in the powder. The fibres are libriform type with lignified thick walls and pointed tips. They are 500-650 µm long. The vessel elements are cylindrical and elongated. They have perforations plate which may be horizontal or oblique. The vessel elements with oblique perforations plates have short, pointed tails. The lateral wall pits elliptical, multiseriate and alternate. The vessel elements are 200 µm long and 40 µm wide.

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Physicochemical studies

Physical constants like Ash values, Extractive values and Loss on Drying at 110^{o} C were determined and results are shown in **Table No. 1.** The behavior of powdered drug in different solutions towards ordinary and UV light were observed and the results are recorded in **Table No. 2.** The Preliminary Phytochemical tests of different extracts were performed, identified with using specific reagents and results are shown in **Table No. 3.** HPTLC fingerprinting studies was carried out & R_f values are measured and tabulated in **Table No. 4.**

Histo chemical tests

Cytochemical reactions were obtained using toluidine blue a polychromatic stain. 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. whereas IKI (blue for starch)

Extraction: The hydroalcoholic extract obtained was thick red residue and yield was found to be 1.2%

HPTLC fingerprinting studies was carried out & R_f values are recorded and tabulated in **Table No. 4.**

Spectroscopy analysis of Hydroalcoholic extract of *Hibiscus micranthus* for total flavonol content was determined by using standard curve prepared by using rutin. The linearity was found in the range of 10-to100 µg/ml. The total flavonol content was expressed as rutin equivalent in % w/w of the extract. The flavonoidal content was found to be 1.24mg/100gm of the extract.

HPLC analysis of Hibiscus micranthus extract : The rutin content of the 70% methanolic extract of the roots was determined by HPLC method. The analysis was performed by the injection of 20µl of extract on a lichrospher 100RP-18(5µm) column (250 x 4 mm), elution using mobile phase as methanol and 2% acetic acid in water (70:30) with runtime of 10min and detection by UV detector at 355nm. Rutin eluted at 3.691 min and the peak area was compared to the standard. The rutin content of the root was found to be 0.64 % w/w of air dried extract.

GC-MS ANALYSIS: The results of GC-MS analysis are presented in table-5. A total of sixty three compounds (above 95%) were identified from the Hydroalcoholic extract of roots of *H. micranthus Linn.,*. The identified compounds were found to be Fatty acid esters (15) were more in number followed by fatty acids(13), hydrocarbons, aldehydes, alcohols were equal in number (8), , phenols (4), ketones (4) and amides(3) respectively.

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CONCLUSION

The pharmacognostic and phyto-chemical investigations of the *Hibiscus micranthus L*. root has been carried out for the first time. These parameters could serve in the identification and preparation of a monograph on this medicinal plant.

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Table 1: Physico-chemical parameters *of Hibiscus micranthus*

Parameters	% constituents
% LOD at 110°C	1-2.0 %
% Ash content	4.8%
% Acid insoluble ash	0.5%
% water soluble ash	4.3%
% Extractive values Pet. Ether (60-80°C) Chloroform Alcohol Water	 1.2 1.1 1.2 2.10

Table 2: Fluorescence studies of *Hibiscus micranthus*

Sl. No.	Treatment	Ordinary light	UV 254 nm
1.	Powder as such	Pale Yellowish	Yellowish fluorescence
2.	Powder + concentrated Hydrochloric acid	Light Brown	No change
3.	Powder + concentrated sulphuric acid	Deep reddish brown	No change
4.	Powder + concentrated nitric acid	Orange	No change
5.	Powder + 10% sodium hydroxide	Orange red	Dark green colour
6.	Powder + glacial acetic acid	Yellowish green	Pale yellow
7.	Powder + chloroform	Greenish yellow	Greenish fluorescence
8.	Powder +Distilled water	Pale yellow	Pale yellow fluorescence

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Table 3: Results of phytochemical screenings of successive extracts of roots of *H. micranthus*

Constituents	Pet ether	chloroform	alcohol	water
Steroid	**+++**	**+++**	-------	-------
Triterpenoid	-------	-------	-------	-------
Flavonoid	-------	-------	**+++**	-------
Phenols	-------	-------	**+++**	-------
Tannins	-------	-------	**+++**	-------
Alkaloids	-------	-------	-------	-------
Saponins	-------	-------	-------	-------
Sugars	-------	-------	-------	**+++**
proteins	-------	-------	-------	**+++**
+++ Present ----- Absent				

Table 4: Rf values of the HPTLC fingerprint of *Hibiscus micranthus* in mobile phase-I/II/III

Table. 1 Rf values of the HPTLC fingerprint of *Hibiscus micranthus* in mobile phase-I					
Extract	**Amount applied (µg/spot**	Rf values			
		(*H. micranthus* root extract)			
Alcohol: water (70:30)	1000	**UV 254 nm**	**UV 366 nm**	**After derivatisation with anisaldehydesulfuric acid reagent under visible light**	**After derivatisation with anisaldehydesulfuric acid reagent under UV 366 nm**
		0.64, 0.71 (dark)	0.69 (paleyellow)	0.38,0.67 (pink)	0.08 (blue quenching), 0.62,0.88 (pink)
Rf values of the HPTLC fingerprint of *Hibiscus micranthus* in mobile phase-II					
Extract	**Amount applied (µg/spot)**	Rf values			
		(*H. micranthus* root extract)			

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Alcohol: water (70:30)	1000	**UV 254 nm**	**UV 366 nm**	**After derivatisation with anisaldehydesulfuric acid reagent under visible light**	**After derivatisation with anisaldehydesulfuric acid reagent under UV 366 nm**
		0.36, 0.45 0.79 (dark)	------	0.36,0.53,0.64 (grey)	0.13 (blue), 0.17 (light blue), 0.37,0.46,0.53,0.65 (blue)

Rf values of the HPTLC fingerprint of *Hibiscus micranthus* in mobile phase-III

Extract	**Amount applied (µg/spot)**	Rf values			
		(*H. micranthus root* extract)			
Alcohol: water (70:30)	1000	**UV 254 nm**	**UV 366 nm**	**After derivatisation with Anisaldehydesulfuric acid reagent under visible light**	**After derivatisation with anisaldehydesulfuric acid reagent under UV 366nm**
		0.71 (dark)	0.12, 0.29, (blue)	0.12 (yellow)	0.12 (light blue), 0.20, 0.28 (blue)

Table 5. Volatile compounds from methanolic extract of roots of *H. micranthus Linn* .as detected by GC-MS

peak	Retention Time	Compound	% matching with Wiley library
1	0.92	Ethyl-D5 ethyl ether	96
2	1.82	Nitromethane-D3	93
3	3.83	Cyclohexan, 1,2,3,4,5,6-Hexadeutero	77
4	4.16	2-Propenoic acid, ethyl ester	98
5	4.54	Propane, 2-methoxy-2-propoxy-	83
6	5.15	Phospholane	70
7	5.41	Methanol	91
8	7.10	3-Furanmethanol	96
9	7.52	Hexanoic acid	90
10	8.37	Undecane	99
11	8.94	Butyl carbamate	96
12	9.18	Octanoic acid	92
13	9.65	Benzoic acid	95
14	9.76	Nonanoic acid	45
15	9.92	1-Dodecanol	97
16	10.43	2-Methoxy-4-Vinylphenol	99
17	10.54	Phenol, 2,6-dimethoxy	96
18	10.72	3,3-Dimethoxyhexane	97
19	10.81	Cyclohexanecarboxylic acid	98
20	10.91	1-Butoxy-1-ethoxyethane	97
21	11.06	1,3-Propanediol, 2-methyl-2 propyl	95

22	11.24	Benzaldehyde, 3-hydroxy-4-methoxy-	99
23	11.38	Phenol, 2-methoxy-5-(1-propenyl)-	98
24	11.55	2-Propenoic acid, 3-Phenyl-	97
25	11.77	Octanoic acid, 8-Hydroxy-	97
26	11.82	Octanedioic acid, dimethyl ester	97
27	12.25	Suberic acid monomethyl ester	98
28	12.37	9-Octadecenoic acid	98
29	12.45	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	44
30	12.51	Dodecanoic acid	99
31	12.77	1,2,4,6 Di-o-isopropylidene-L-sorbopyranose	100
32	12.86	Octanedioic acid	100
33	12.99	Cyclohexanepropanoic acid, 2-oxo-, methyl ester	97
34	13.10	1,6-Anhydro-beta-D-Glucopyranose	82
35	13.18	Benzoic acid, 4-hydroxy-3-methoxy-	100
36	13.28	Benzophenone	97
37	13.36	Methanone, diphenyl	98
38	13.49	Phenol, 3,4,5 trimethoxy-	97
39	13.69	Undec-10-ynoic acid	98
40	13.97	3-methyl-2-butenoic acid, cyclohexyl ester	49
41	14.11	1,5-Pentanediol	95
42	14.32	2-Amino-5-methylamino-1,3,4-thiadiazole	97
43	14.79	Trans-caryophyllene	99
44	15.07	Eicosanoic acid	99
45	15.14	Ethyl-1-thio-beta-d-glucopyranoside	98
46	15.29	(1,1'-Bicyclopropyl)-2-octanoic acid, 2'-hexyl-,methyl ester	96
47	15.55	Benzenemethanol, 4-(1-(3,5-dimethoxyphenyl)ethoxy)-3-methoxy-	99
48	15.71	3-(p-hydroxy-m-methoxyphenyl)-2-propenal	97
49	15.82	2-Butenal, 3-methyl-, dibutylhydrazone	70
50	16.01	Pentadecanoic acid	98
51	16.14	Ethyl-1-thio-beta-d-glucopyranoside	98
52	16.30	1,2-Benzenedicarboxylic acid, dibutyl ester	93
53	16.49	Benzaldehyde, 2,3,4-trimethoxy-	98
54	16.66	3-pyridinecarboxylic acid, 1,6-dihydro-4-hydroxy-2-methyl-6-oxo, ethyl ester	98
55	16.74	9-Octadecenoic acid	96
56	16.95	Acetamide, 2,2,2-trifluoro-N-(2-hydroxy-2(4-hydroxy-3-methoxyphenyl)ethyl)	98
57	17.37	1,2-Benzenedicarboxylic acid, dibutyl ester	97
58	17.66	9,12,15-Octadecatrienoic acid, methyl ester	97
59	18.68	1,10-Dimethyl-2-methylene-trans-decalin	98
60	18.97	1-Methyl-1-n-decyloxy-1-silacyclobutane	97
61	19.36	Cyclopropanebutyric acid-2[(2-nonylcyclopropyl)methyl]-, methyl ester	97

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62	19.60	9,12,15-Octadecatrienoic acid, methyl ester	99
63	19.88	7-Hexadecyn-1-ol	99
64	20.31	7-Hexadecyn-1-ol	96
65	21.25	Octadecanoic acid, methyl ester	98
66	21.60	2-nitrovanilin-o-acetate	99
67	22.32	9-Octadecynoic acid, methyl ester	98
68	22.78	6-Tetradecanesulfonic acid, butyl ester	99
69	23.06	Terpinyl formate	98
70	23.26	Eicosanoic acid	98
71	23.65	7-Hexadecyn-1-ol	99
72	24.26	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester	93
73	24.58	Methyl 8-oxohexadecanoate	99
74	24.71	Tetrapentacontan, 1,5-Dibromo-	99
75	24.93	Octadecane, 1-chloro	89
76	27.11	Z,Z-6,2,8-Heptatriactontadien-2-one	98
77	27.99	6,11-Hexadecadien-1-ol	85
78	28.56	5-(2,3,5,6-Tetradeuterioocta-2,5 dien-1-yl)-4,5-dideuteriotetrahydrofuran-2-one	97
79	32.05	Tetrapentacontan, 1,5-Dibromo-	95

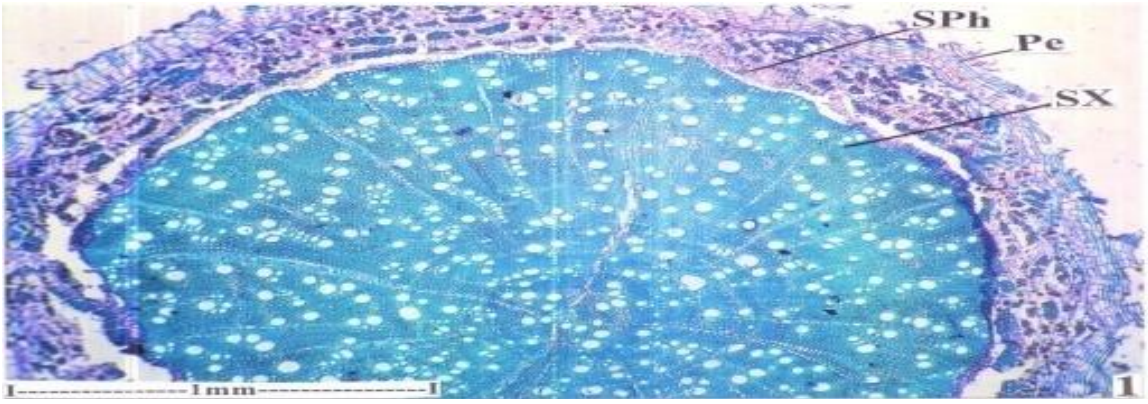

Fig: 1 T.S. of root – a sector enlarged

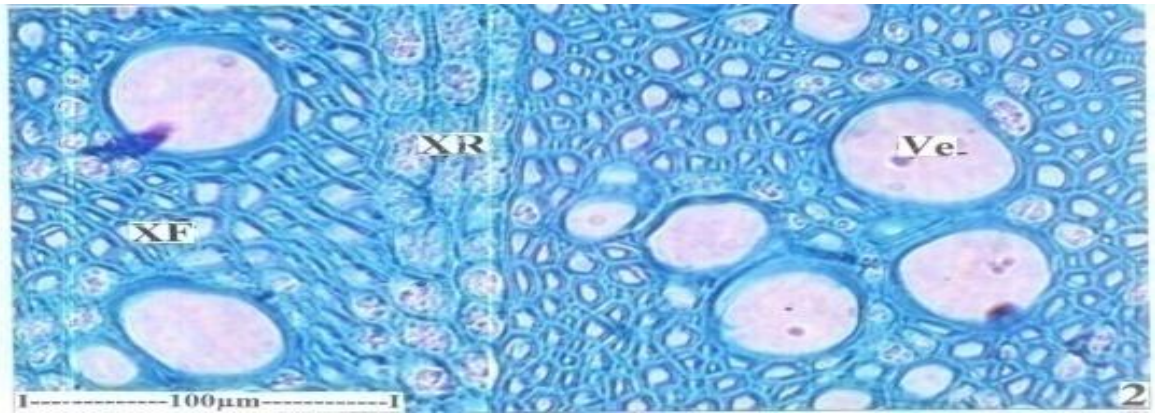

Fig: 2 T.S of root- secondary xylem enlarged

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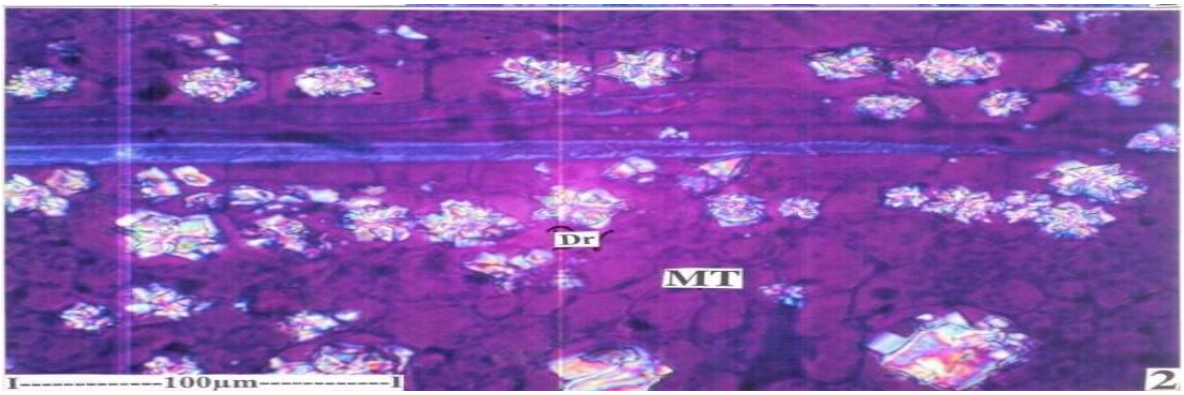

Fig: 3 Druses in paradermal section

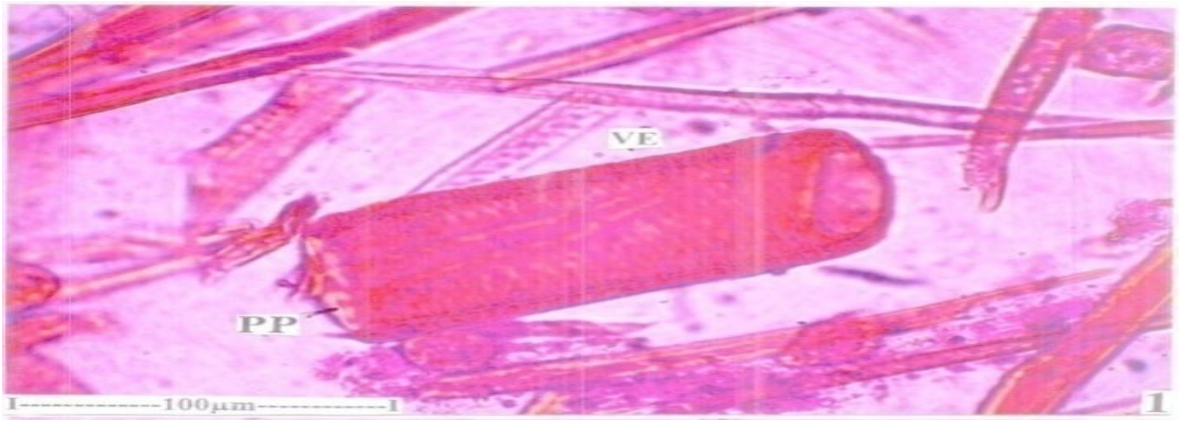

Fig: 4 Vessel element and fibre

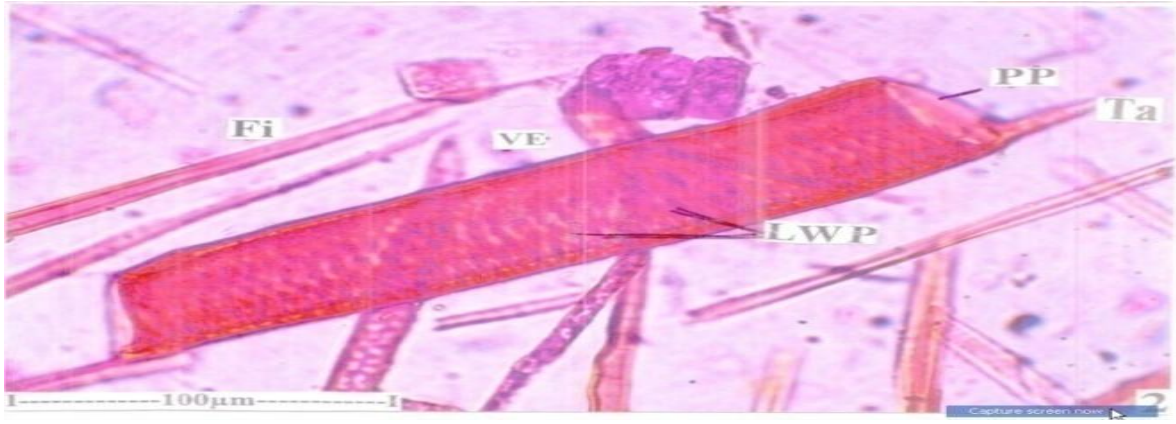

Fig: 5 Tailed vessel element showing lateral wall pits and fibres

Abbreviations: VE- vessel, PP-perforation plate, LWP-Lateral wall pits, Fi-Fibre, Ta-Tail
Ph: Phloem, PhS: Secondary phloem, SX: Secondary xylem, DR: Druses, Pi: Pith, Pe: periderm Ve: Vessels, XF: Xylem fibres, XR: Xylem rays

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