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Pharmacognostic Studies on *Rumex vesicarius*

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ABSTRACT

Rumex vesicarius is a branched succulent herb which belongs to the family Polygonaceae and is distributed in India. The whole plant is medicinally important and cures several diseases. Therefore in this context the detailed pharmacognostic study of various parts like leaf,stem,petiole and root has been carried out with the aim to establish its Pharmacognostical standards. The parameters selected were microscopical studies, proximate analysis, fluorescence analysis and preliminary phytochemical screening including thin layer chromatographic analysis.The microscopical studies of leaf,stem and root revealed the presence of sessile glandular trichomes, spiral and scalariform xylem vessels.In physico-chemical evaluation the ash values and extractive values were studied. Fluorescence analysis performed showed the wide range of fluorescence colours for the crude powder as well as the extracts. The powder of R.vesicarius was successively extracted with hexane,benzene,chloroform,ethylacetate,ethanol and water for the identification of the best solvent. Preliminary phytochemical screening was carried out for all the extracts and maximum chemical constituents was observed in the ethanolic extract. The inorganic elementary analysis performed revealed the presence of sodium,chloride and iron.The TLC studies performed produced fluorescent spots for the solvent systems selected for flavonoids,steroids and anthraquinones .

Keywords: Scalariform , Fluorescence studies, adulterants , flavonoids, sessile glandular trichomes.

INTRODUCTION

Standardisation of a drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical,physical observations. Standardisation and evaluation of herbal drugs mainly done by two method they are organoleptic and botanical characters. In the organoleptic studies like colour,odour,tast and texture where as in botanical microscopic and macroscopic characters are

observed[1,2].*Rumex* the ancient Latin name for the docks or sorrels. *vesicarius*, vesica, a bladder; from the inflated pods following the flowers on these herbs.It is a pale ,green ,dichotomously branched,succulent herb.Leaves are fleshy, sour, alternate, elliptic-ovate,broadly ovate,entire,acute (or) obtuse,cordate at base,long petiole.Flowers are white,monoecious. Fruits are nutlets,seeds are erect and trigonous [3] .Several C-glycosides,Flavonoids and Anthraquinones are known to be constituents of this plant. The folklore claims that the plant is a potent diuretic, astringent, carminative, stomachic and tonic[3,4,5]. Anti-bacterial and Anti-oxidant activities of *Rumex vesicarius* was performed.[6,7] So far there is no pharmacognostic report so this paper is aimed to report the pharmacognostic and phytochemical studies.

MATERIALS AND METHODS

Rumex vesicarius Plants were collected from local market in Nalgonda. It was identified and authenticated by Prof. A. Lakshma Reddy, Retired Professor, Dept. of Botany, Nagarjuna Govt. College (Autonomous) Nalgonda. The plant herbarium was prepared and deposited in the Dept. of Pharmacognosy for further reference. The plant was identified as *Rumex vesicarius* Linn. (Polygonaceae) under the voucher no: NCOPNLG/ph'cog/2010-2011/034.

Instruments Used

Micro senior precision rotary microtome (latest Spencer 820 type), Sisco muffle furnace (3003137),Rotary vacuum evaporator,Hot air oven.

Chemicals and Reagents

All the chemicals and reagents like chloral hydrate, phloroglucinol, Hexane, Benzene, Chloroform, Ethylacetate, Ethanol, used were of analytical grade.

Anatomical studies:

Transverse Section of Leaf, Stem and Root:[8-11]

Microtome Section was done for leaf,stem and root to obtain a thin section. The sections were stained with phluroglucinol and hydrochloric acid in the ratio 1:1. Photo micrographs of different magnifications were taken to study the anatomical features.

Powder Microscopy:[8,9]

Shade dried Leaf, Stem and root was powdered with the help of an electric grinder till a fine powder was obtained. This fine powder was subjected to powder microscopy studies, as per standard procedures mentioned.

Measurement of Cell Structure and Content:[8-10]

The length and width of phloem fibres,Length and Width of Trichomes, Width of Xylem Vessels, Diameter of Calcium oxalate Crystals and diameter of the starch grains were measured using stage micrometer and the eyepiece micrometer by standard methods.

Determination of Physico Chemical Parameters:[8,12,13]

Total ash, acid insoluble ash, water soluble ash, crude fiber content, moisture content, alcohol soluble extractive value, water soluble extractive value, chloroform soluble extractive value and petroleum ether soluble extractive values of Aerial parts of *Rumex vesicarius* were determined as per standard procedures

Leaf Constants:[8,17]
The stomatal number, stomatal index, vein islet number and vein termination number were determined as per standard references.

Determination of Fluorescence Analysis:[12-15]
Powdered Leaf, Stem and root was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents.

Extraction
The collected aerial parts of the plant were washed and dried under the shade. Around 25 g of the coarsely powdered aerial parts of the plant was packed in a soxhlet apparatus and exhaustively extracted with the solvents of increasing polarity. The extract so obtained was concentrated under vacuum using rotary vacuum evaporator and dried in dessicator until use.

Preliminary Phtyochemical Screening[8,13,15-19]
The extract so obtained were subjected to various chemical tests as per the procedure mentioned in the standard reference books to determine the nature of chemical constituents present the in the plant .

Thin Layer Chromatography Analysis[20,21]
Thin layer chromatography (TLC) is a chromatography technique used to separate mixtures of chemical compounds. It is the most basic method of confirming the presence of a phytochemical compound. The conditions maintained for performing the TLC analysis of the root are as mentioned below:
Adsorbent: Precoated silical gel plates
Solvent systems:
STEROIDS: Benzene:Chloroform (3:7).
ANTHRAQUINONES: Ethyl acetate:Methanol:Water (100:13.5:10)
FLAVONOIDS: Benzene:Pyridine:Formic acid (72:18:10)
Toulene:Dioxan:Glacial acetic acid (90:25:4)
Chloroform:Methanol (9.3:0.6)

Detection by U.V at long wavelength

RESULTS AND DISCUSSION

ANATOMY OF THE LEAF: The leaf has dorsi-ventral orientation.

- **Upper epidermis**:Upper epidermis was single layer of thin walled closely arranged cells and covered externally by a layer of cuticle. The upper epidermis was not a continous layer but slight projection is seen in the centre .
- **Lower epidermis**:Lower epidermis was single layer of parenchymatous cells which is interrupted by the stomata.The type of stomata is Anisocytic type of stomata.
- **Mesophyll**:The region between the upper and lower epidermis is constituted by mesophyll.The mesophyll was formed of chlorenchymatous cells and is the seat of photosynthesis.Mesophyll was differentiated in to upper palisade parenchyma and lower spongy parenchyma.Palisade parenchyma consists of two or three layers of compactly arranged cells in perpendicular to the surface.Spongy parenchyma consists of loosely arranged cells having air filled spaces in between. The chloroplast are most abundant in Palisade cells than spongy parenchyma.

• **Vascular bundles**:Vascular bundles ranges from 2 to 4 in number.The pholem lies towards the lower epidermis and the xylem lies towards the upper epidermis.

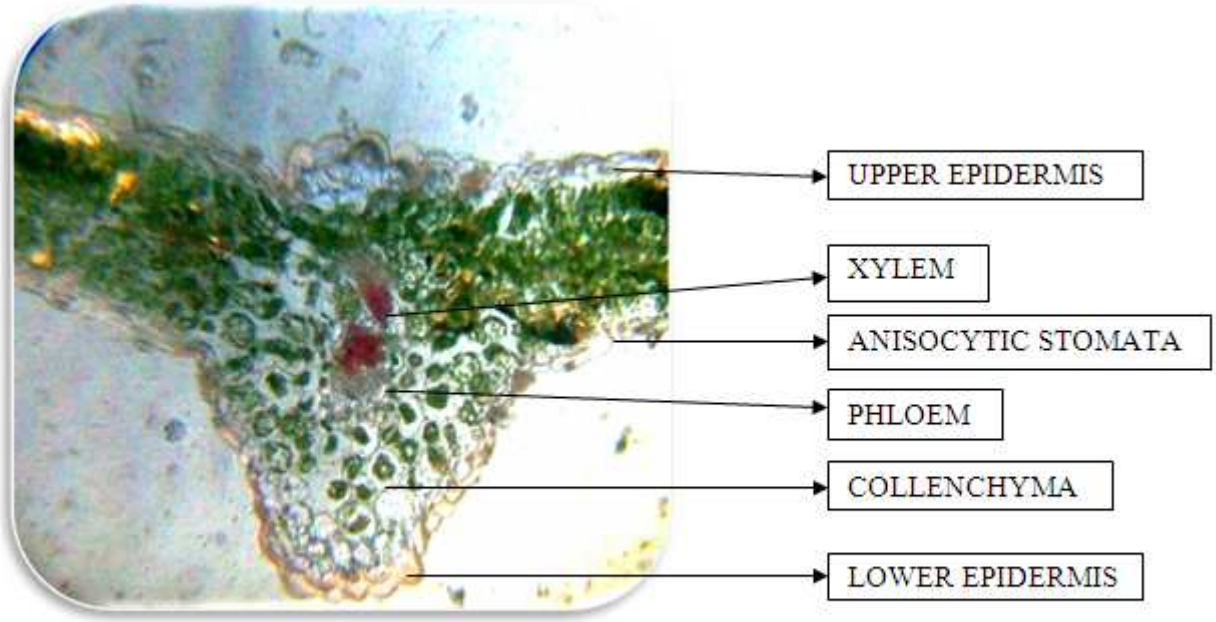

Fig 1:-T.S of *Rumex vesicarius* Leaf.

ANATOMY OF THE STEM:

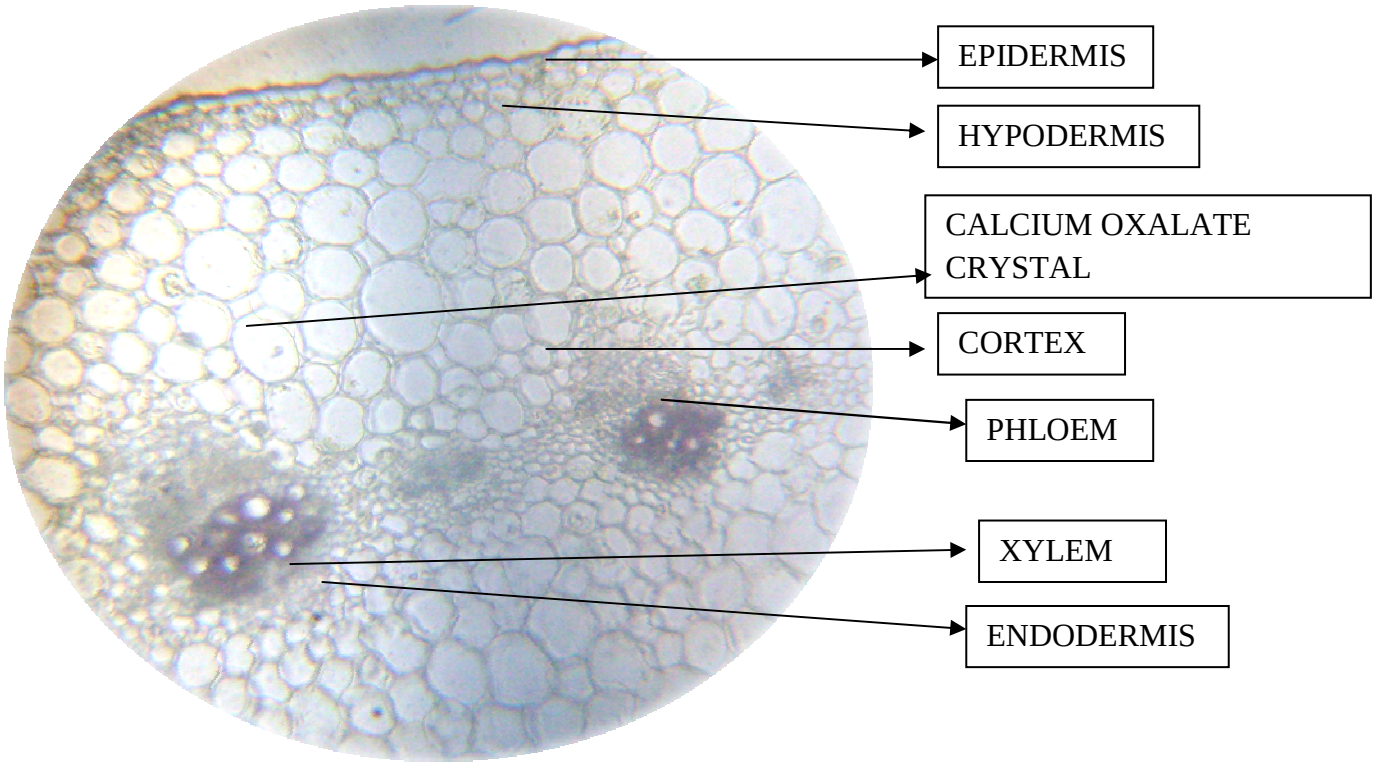

Fig 2:- T.S of *Rumex vesicarius* Stem

- **Epidermis**:Epidermis was outer most layerof compactly arranged cells. The cells are elongated and rectangular and covered externally by cuticle. And the unicelluar trichomes was seen on the epidermis region.
- **Hypodermis**:The hypodermis was inner to the epidermis and is formed of a few layers of dead,thick walled sclerenchymatous cells.
- **Cortex**:The entire region inner to the hypodermis is called Cortex.It is formed of thin-walled parenchymatous cells and having the large intercellular spaces in between them.The cortex consists of a calcim oxalate crystals
- **Vascular bundles**:They are arranged as collateral and conjoint.Pholem towards the epidermis and xylem towards the centre of ground tissue.And Pholem and Xylem are differentiated by the endodermis and the cambium.

ANATOMY OF PETIOLE:

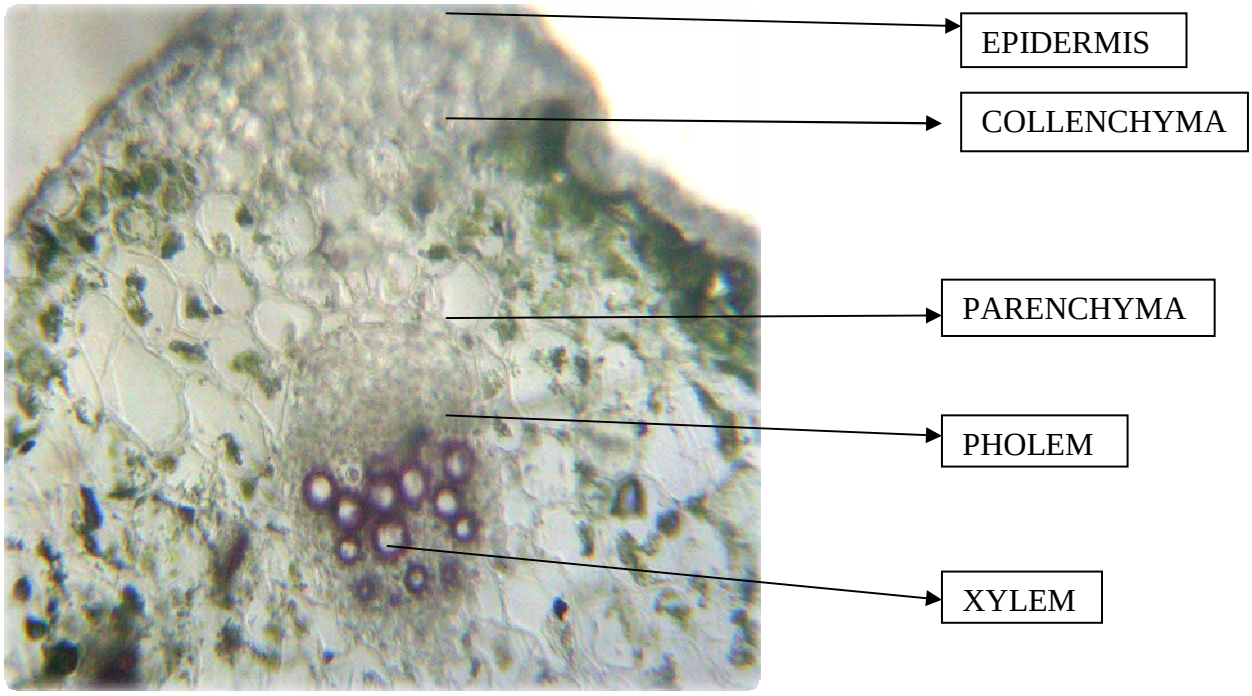

Fig 3:- T.S of *Rumex vesicarius* Petiole

- **Epidermis**:It was the outer layer of the petiole and consists of oval or nearly circular cells of a single layer which are thickened and slightly cutinized upon their exterior surface, and presenting a fringed apperance.
- **Collenchyma**:They are arranged in several layers or thick angled cells underlyng the epidermis.
- **Parenchyma**:Under the collenchymas there are a few layered parenchymatous cells.They had a clear intercellular spaces.
- **Vascular bundles**:They are arranged in a collateral manner. They are in the form of v-shape. Pholem and xylem is in the single bundle.

ANATOMY OF THE ROOT:

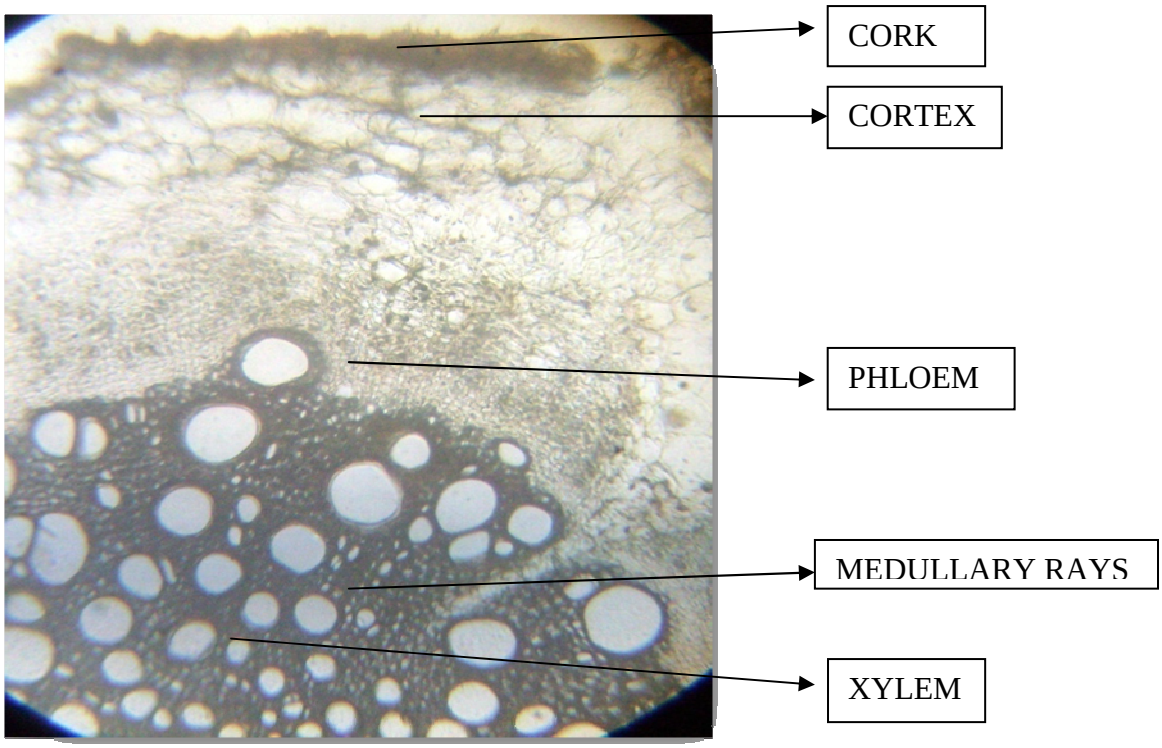

Fig 4:- T.S of the *Rumex vesicarius* Root

- **Cork**:It was the outer most region of the root and is one cell thick in nature.The cells are thin walled on the surface.
- **Cortex**:The region inner to Cork was formed of several layers of loosely arranged thin walled parenchymatous cells.
- **Pholem**: Below the Cortex pholem is present .pholem consists of sieve tubes and pholem fibres.
- **Xylem**:It is present in the middle region and xylem consists of Xylem vessels ,Xylem parenchyma and xylem fibres.They are radially arranged.and the medullary rays are also present in the xylem..Xylem vessels are in different radii.Xylem bundles are present alternately on different radii number of each bundle ranges from 2 to 6 and the condition is called tetrarch.and pith is usually absent.The diameter of the vessels in the xylem ranges from 113.6-28.4µ.

POWDER MICRSCOPY OF LEAF:

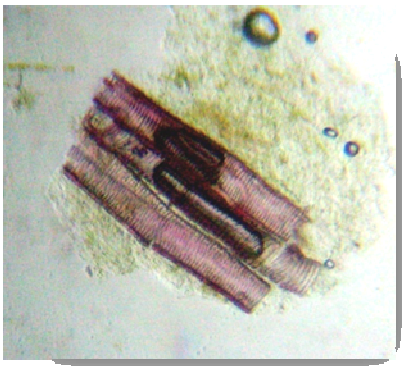

Fig 5:-SPIRAL XYLEM VESSEL

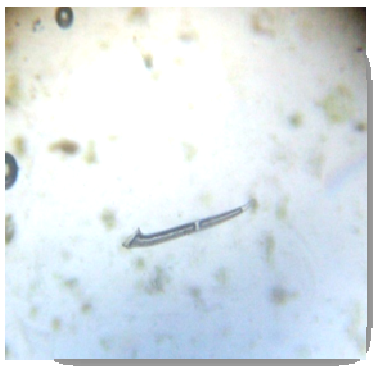

Fig 6:-UNICELLUAR TRICHOME

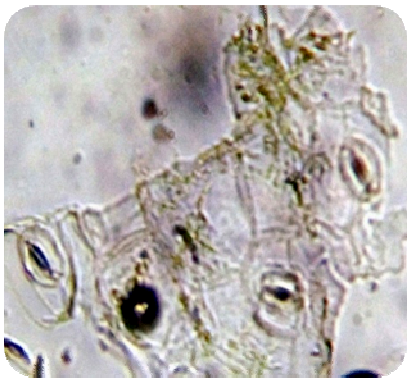

Fig 7:-ANISOCYTIC STOMATA WITH EPIDERMAL CELLS

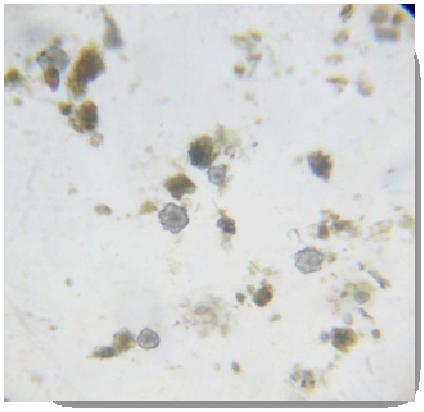

Fig 8:-SESSILE GLANDULAR TRICHOME

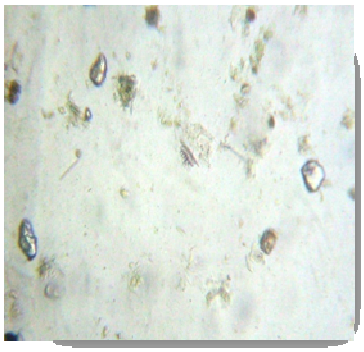

Fig 9:-CALCIUM OXALATE CRYSTAL

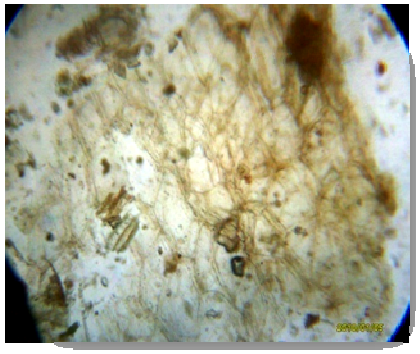

Fig 10:-PARENCHYMA

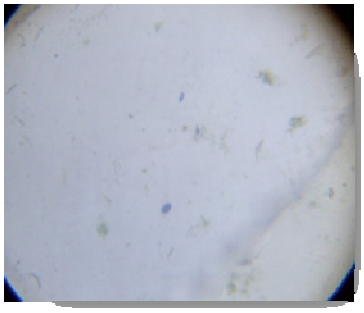

Fig 11:-STRACH GRAIN

POWDER MICROSCOPY OF STEM:

Fig 12:-NON-LIGNIFIED PHLOEM FIBRE

Fig 13:-UNICELLULAR TRICHOME

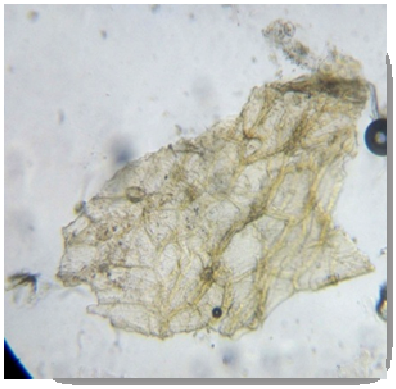

Fig 14:-PARENCHYMA

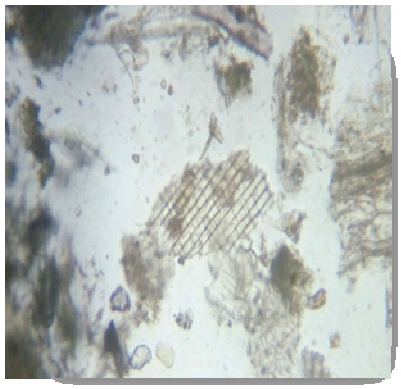

Fig 15:-BUNDLE OF PHLOEM FIBRES

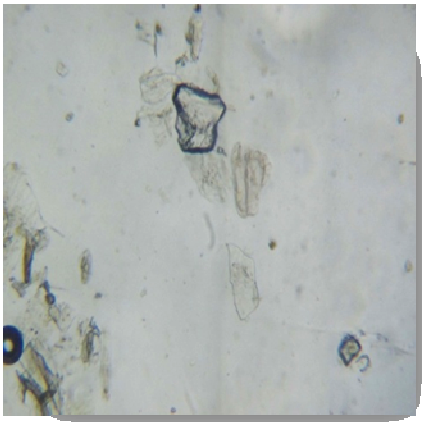

Fig 16:-CALCIUM OXALATE CRYSTAL

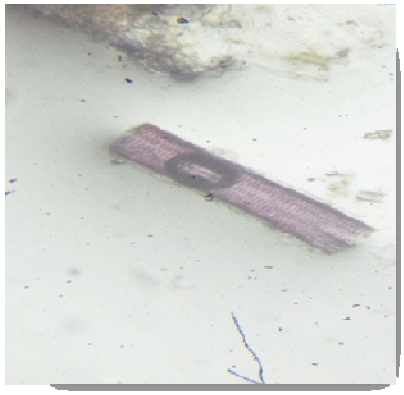

Fig 17:-XYLEM VESSEL

POWDER ANALYSIS FOR ROOT:

Fig 18:-SCALARIFORM XYLEM VESSEL

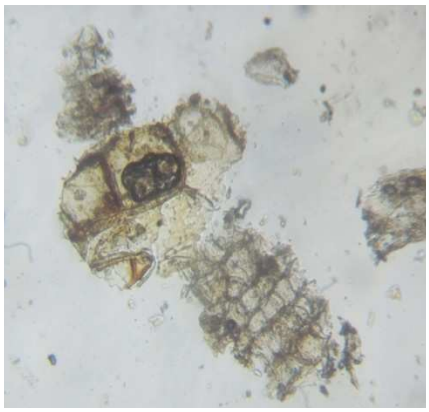

Fig 19:-CORK CELL

MEASUREMENTS:

This helps in identification of adulteration. The results obtained are tabulated in Table-1,2,3.

TABLE 1:-QUANTITATIVE MICROSCOPY OF LEAF

PARAMETERS	LENGTH µm	WIDTH µm
Trichome	37.5-217.35-462.5	12.5-32.5-62.5
Calcium oxalate crystal	-	25-41.25-62.5
Xylem vessel	-	25-38.125-75
Starch grain	-	12.5-36.25-62.5

TABLE 2:-QUANTITATIVE MICROSCOPY OF STEM

PARAMETERS	LENGTH µm	WIDTH µm
Trichome	125-318.12-1587.5	12.5-28.12-50
Fibre	125-406.8-850	12.5-30-50
Xylem vessel	-	25-52.12-150
Calcium oxalate crystal	-	12.5-51.25-100

TABLE 3:-QUANTITATIVE MICROSCOPY OF ROOT

PARAMETER	WIDTH µm
Xylem vessel	25-47.5-100

Determination of physico chemical properties:

The physico chemical properties help to estimate the amount of impurities like soil and particle present in the drug. It also helps to assess the calculi salts present in the drug sample. Ash values were used to detect the presence of any siliceous contamination and presence of any water soluble salts .Alcohol and water soluble extractive values indicate the presence of the adulterants, faulty processing and poor quality of the drug. While petroleum ether soluble extractive value indicates percent of lipid content present in the crude drug.. Crude fibre content is useful technique for differentiation of similar drugsThe results obtained for the proximate analysis are tabulated in Table-4.

TABLE 4:-PHYSICO-CHEMICAL ANALYSIS OF AERIAL PARTS

PARAMETERS	VALUES IN % W/W
Total ash	19.03
Acid insoluble ash	0.044
Water soluble ash	6.83
Sulphated ash	14.6
Water soluble Extractive	120
Ethanol soluble Extractive	51.40
Chloroform soluble Extractive	4.80
Pet-ether soluble Extractive	10.4
Moisture content	6.6
Crude Fibre Content	31.3

Determination of leaf constants

The stomatal number, stomatal index, vein islet &termination nos. obtained are tabulated in Table-5

TABLE 5:-LEAF CONSTANTS OF *RUMEX VESICARIUS*

PARAMETERS	UPPER SURFACE	LOWER SURFACE
Stomatal number	12/sq.mm	15/sq.mm
Stomatal index	24	27.7
Vein Islet number	9	
Vein Termination number	8	

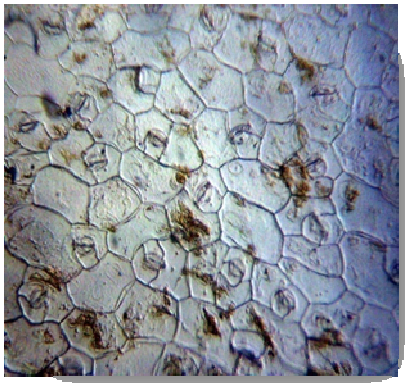

Fig 20:-ANISOCYTIC STOMATA

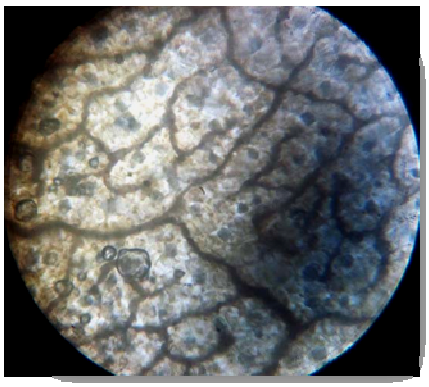

Fig 21:-VEIN ISLET

TABLE 6:- FLUROSCENCE ANALYSIS FOR LEAF POWDER

DRUG+REAGENT	DAY LIGHT	SHORT U.V 245nm	LONG U.V 365nm
Drug+HNO_3	Yellowish Brown	Light Green	Dark Green
Drug+H_2SO_4	Brownish Yellow	Dark Green	Fluroscent Green
Drug+NH_3	Light Green	Fluroscent Green	Dark Green
Drug+HCL	Pale Green	Fluroscent Green	Black
Drug+Methanol	Light Yellow	Light Green	Blackish Green
Drug+$Fecl_3$	Yellow	Fluroscent Green	Dark Green
Drug+5%NaOH	Yellow	Fluroscent Green	Green
Drug+5%KOH	Yellow	Fluroscent Green	Dark Green
Drug+1%KOH	Yellowish Green	Fluroscent Green	Black
Drug+50%H_2SO_4	Pale Green	Pale Green	Dark Green
Drug+50%HNO_3	Brown	Fluroscent Green	Dark Green
Drug+Methanolic NaOH	Yellowish Brown	Brown	Yellow

Determination of fluorescence analysis

Fluorescence analysis is a tool to determine the kind of chemical nature of the drug. The fluorescence obtained in short wavelength, long wave length and day light in table 6,7,8.

TABLE 7:-FLUORESCENCE ANALYSIS FOR STEM POWDER

DRUG+REAGENT	DAY LIGHT	SHORT U.V 245nm	LONG U.V 365nm
Drug+HNO_3	Yellowish Brown	Fluorescent Green	Black
Drug+H_2SO_4	Brownish Green	Light Green	Dark Green
Drug+NH_3	Yellow	Light Green	Dark Green
Drug+HCL	Pale Yellow	Pale Green	Black
Drug+Methanol	Pale Brown	Light Green	Yellow
Drug+$Fecl_3$	Yellowish Green	Fluorescent Green	Black
Drug+5%NaOH	Pale Brown	Light Green	Dark Green
Drug+5%KOH	Yellow	Fluorescent Green	Dark Green
Drug+1%KOH	Pale Yellow	Pale Green	Dark Green
Drug+50%H_2SO_4	Pale Brown	Pale Green	Black
Drug+50%HNO_3	Pale Yellow	Pale Green	Black
Drug+Methanolic NaOH	Pale Yellow	Greenish Yellow	Yellow

TABLE 8:-FLUORESCENCE ANALYSIS FOR ROOT POWDER

DRUG+REAGENT	DAY LIGHT	SHORT U.V 245nm	LONG U.V 365nm
Drug+HNO_3	Yellow	Light Green	Black
Drug+H_2SO_4	Brown	Light Green	Dark Green
Drug+NH_3	Reddish Brown	PaleGreen	Light Green
Drug+HCL	Pale Yellow	Pale Green	Black
Drug+Methanol	Pale Brown	Pale Greenish Yellow	Brownish Black
Drug+$Fecl_3$	Yellow	Fluorescent Green	Dark Green
Drug+5%NaOH	Brown	Pale Brown	Brown
Drug+5%KOH	Brown	PaleGreen	Dark Green
Drug+1%KOH	Brown	Pale Yellow	Dark Green
Drug+50%H_2SO_4	Brownish Black	Pale Green	Light Brown
Drug+50%HNO_3	Pale Brown	Pale Green	Brownish Black
Drug+Methanolic NaOH	Dark Brown	Brownish Black	Black

TABLE-9:-FLUORESCENCE ANALYSIS OF EXTRACTS

EXTRACTS	LONG WAVELENGTH	SHORT WAVELENGTH	DAYLIGHT
Hexane extract	Black	Green	Reddish Brown
Benzene extract	Black	Green	Reddish Brown
Chloroform extract	Black	Fluorescent Green	Brown
Ethyl acetate extract	Black	Fluorescent Green	Reddish Brown
Ethanol extract	Black	Dark Brown	Brown
Aqueous extract	Brown	Greenish Brown	Reddish Brown

EXTRACTION

- Extraction was carried out by the soxhaltion based on the increasing order of polarity as follows:

TABLE 10:-NATURE AND %YIELD OF DIFFERENT EXTRACTS

EXTRACTS	COLOUR	CONSISTENCY	%W/W
Hexane	Greenish Black	Oily	3.46
Benzene	Greenish Yellow	Gummy	3.04
Chloroform	Greenish Yellow	Oily	2.092
Ethylacetate	Greenish Black	Waxy	1.824
Ethanol	Reddish Green	Oily	12.604
Water	Brown	Amophorous	26.092

Preliminary phytochemical screening:

Chemical test helps in the confirmation of the chemical nature of the active principles present in the plant extract. The results of the chemical tests are tabulated in Table-10.

TABLE 11:-PRELIMINARY PHYTOCHEMICAL SCREENING OF *RUMEX VESICARIUS*

TEST	HEXANE	BENZENE	CHLOROFOM	ETHYLACETAE	ETHANOL	WATER
Carbohydrates	-	-	+	-	-	+
Proteins	-	-	-	-	-	-
Aminoacids	-	-	-	-	-	-
Fats and Oils	+	+	+	-	-	-
Steroids	+	+	-	-	-	-
Glycosides	+	+	+	+	+	-
Anthraquinone	+	+	+	+	+	+
Flavonoids	-	-	-	+	+	-
Alkaloids	-	-	-	-	-	-
Saponins	-	-	-	-	+	+
Tannins and PhenolsCompounds	-	-	-	-	-	-

TABLE 12:-TEST FOR INORGANIC ELEMENTS OF THE POWDER

Test	OBSERVATION
Test For Calcium	-
Test For Magnesium	-
Test For Sodium	+
Test For Potassium	-
Test For Iron	+
Test For Sulphate	+
Test For Chloride	+
Test For Phosphate	+
Test For Carbonate	-
Test For Nitrate	-

TABLE 13:- TLC ANALYSIS OF *RUMEX VESICARIUS*

PHYTOCONSTITUENTS	SOLVENT SYSTEMS	RF VALUES	COLOUR OF SPOTS
Steriods	Benzene:chloroform (3:7)	0.4	Orange
		0.3	Orange
		0.1	Orange
Anthraquinones	Ethylacetate:methanol:water(100:13:5:10)	0.66	Brown
		0.55	Brown
	Benzene:Pyridine:Formic acid	0.4	Yellow fluorescence
	(72:18:10)	0.75	Orange
		0.65	Orange
		0.82	Yellow fluorescence
		0.95	Yellow fluorescence

Flavonoids			
	Toulene:Dioxan:Glacial acetic acid	0.93	Orange
	(90:25:4)	0.81	Orange
	chloroform:Methanol (9.3:0.6)	0.84	Yellow fluorescence
		0.25	Yellow fluorescence

CONCLUSION

Rumex vesicarius is widely cultivated in India for its culinary purposes. The microscopical studies, physico chemical parameters,fluorescence analysis and chemical tests performed will guide in the proper identification of the plant species from other species of *Rumex* as well as help in authentication of the purity of the plant.By above all these parameters we can build up a suitable plant profile.

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