Original Article

Pharmacognostic & Phyto-chemical study of *Ikshu* root (*Saccharum officinarum* Linn.)

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Abstract:-

Ikshu Mool (root of *Saccharum officinarum* Linn.) is one of the easily available medicine mentioned as diuretic in Ayurvedic literature. Pharmacognostic and phytochemical studies of *Ikshu Mool* has not been done yet, hence this study was undertaken to find out pharmacognostic nature and phytoconstitute of *Ikshu Mool*. Phytochemical study showed presence of steroids, alkaloids, reducing sugar, absence of proteins, monosaccharides and pentose sugar in both alcohol and water-soluble extracts. Hexose sugar, phenolic compounds and tanines were found only in alcohol soluble extractives.In HPTLC the ingredients separated in aqueous extract were more in number than alcohol soluble extract because of high extractive value in water. Pharcognostic study showed various structures in *Ikshu* root such as epidermis hypodermal layer, epidermal cells, vascular bundles, metaxylem elements, protoxylem elements, fibres etc. Analytical study showed moisture content (11.63%), total ash (9.67%), acid soluble ash (7.58%) & insoluble ash (2.09%), water soluble (6.88%) & insoluble ash (2.79%),Sulphated Ash value(7.58%) within API standards which shows standard quality of the drug. The aqueous & alcohol extractive values were 7.08%, 4.215%, respectively.

Key Words:-*Ikshumool,* Pharmacognostic & Phyto-chemical study

Introduction:-

Nature has been a source of medicinal agents for thousands of years. The widespread use of herbal remedies and healthcare preparations has been described in ancient texts like the Vedas and the Bible. In fact, plants produce a diverse array of bio-active molecules, making them a rich source of diverse type of medicines [1]. Thus, natural products with pharmacological or biological activities still play a very important role in medicine [2-3]. World Health Organization (WHO) described plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [4].

Susruta explains the importance to learn other field of science. Vagbhata has also stressed that science should always adapt to the change of the generation. *Ikshu* is one of the ingredient of *Tranapanchamoola Gana* (group of 5 herbs) explained by Sushruta, and classified on the basis of *Mahabhootadhikya*, on their effect on *Tridosha*, their pharmacological action and therapeutic efficacy. *Ikshu* have properties like pittahara, mutrala and also used in the management of *Mutrakrichchra* and *Raktapitta*. Hence

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by considering its therapeutic importance and pharmaceutical application in various *Kalpana.* It is essential to understand Pharmacognostical and Physico-chemical property of *Ikshu*.

Materials & Methods:

Pharmacognostical Evaluation:

Raw drugs were identified and authenticated by the Pharmacognosy department. The identification was carried out based on the morphological features, organoleptic features and powder microscopy of the individual drugs. The microphotographs were also taken under the microscope.

2. Macroscopic Study:

The macroscopic study refers to the physical evaluation of the drug in terms of size, shape, surface, fracture, etc. The sample was subjected to the macroscopic study with the help of simple microscope and magnifying glass.

3. Method of microscopic examination of root: Prepare transverse or longitudinal section of root. Cut the sections with razor moisten the surface of the root with glycerol solution remove the section with brush and place them on the slide. The sections treated with various reagents before examining.

4. Methods for powder analysis

Powder preparation- All the samples powdered in pulverizer to size of coarse powder (Passing through 60 No mesh). Then all the samples subjected for Macro and Microscopic examination.

Powder macroscopy- The samples tested for organoleptic characters like Colour, Appearance, Taste, Odor and Fineness.

Powder microscopy:

Examination for Lignin- Moisten the powder with an alcoholic solution of Phloroglucinol and allow standing until nearly dry add concentrated Hydrochloric acid. Apply a cover glass and examine. Note the presence or absence of lignified vessels, fibers, parenchyma, sclerieds or hairs.

5.Methods for determination of foreign matter:

Weight 100-500 gm of the drug sample to be examined or minimum quantity prescribed in the monograph, and spread it out in a thin layer. The foreign matter should be detected by inspection with the unaided eye or by the use of lens 5x: separate and weigh it and calculate the percentage.

6.Physico-chemical evaluation:- Tests such as Moisture estimation, ash value estimation, determination of sulphated ash, determination of acid soluble and insoluble Ash, water soluble and insoluble ash, extractive values, Alcohol extract from root powder and Aqueous extract (Water extract) were conducted according pharmacopoeia standards of India.[5]

7.Preliminary phytochemical screening:

Both, aqueous and alcohol extracts were subjected for qualitative preliminary phytochemical screening as given below.

Test for reducing sugars

Benedict's test:

Mixed equal volume of Benedict's reagent and test solution in the test tube and heated on water bath for 5 minutes. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Test for monosacchrides:

Barfoed's test:

Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. on water bath and cool it. Red precipitate is observed

Test for pentose sugars:

Bial's Orcinol test: To boiling Bial's reagent add few drops of test solution. Green or purple coloration appears.

Test for hexosugaras:

Selwinoff's test: Heat 3 ml Selwinoff's reagent and 1ml. test solution on water bath for 1-2 min. Red colored is formed.

Test for proteins:

Million's test:

Heat 3 ml Million's reagent 1ml.test solution.

Tests for steroids:

Salkowski reaction:

To 2 ml of extract, add 2 ml of chloroform and 2 ml concentrated H_2SO_4. Shake well. Chloroform layer appears. Red and acid layer shows greenish yellow fluorescence

Test for alkaloids:

Wagner's Test: To 2-3 ml of filtrate with few drops of Wagner's Reagent shows reddish brown ppt.

Tests for tannins and phenolic compounds:

To 2 3 ml of aqueous or alcoholic extracts, add few drops of following reagents: 5% $FeCl_3$ solution

deep blue-black colour.

Tests for Inorganic Elements:

The ash of drug was taken in a test tube and 50% HCl v/v or 50% H No3 v/v was added to. Keep for 1 hr. filter and with filtrate performed following tests.

8.Methods of high performance thin layer chromatography:

H.P.T.L.C. is very useful qualitative analysis method; it combines the art of chromatography with quickness at a moderate cost. It is a major advancement of TLC principle with short time duration and better resolution. It needs a high concentrated solution, as very less amount of sample need to be applied. For normal phase chromatography using silica gel pre-coated Plates solvents should be non-polar of volatile type. For reversed phase chromatography usually polar solvents are used for dissolving the sample. Layer of H.P.T.L.C. are available in the form of pre coats silica gel of very fine particle size is widely used as adsorbent. Plates were produced from 4 to 5 mm silica gel with an inert binder to form a 200mm layer. Plates of 20x20cms are 5x7.5cms is used. Silica gel F254 having a pore size of 6 mm with fluorescent indicator is a coat material. The difference between T.L.C. and H.P.T.L.C. plates is particle size of coated material, which is 5 to 20 mm of T.L.C. and 4 to 8 mm for H.P.T.L.C. The plated were cleaned by methanol. The size of the sample spot applied must not exceed 1mm in diameter. There are different techniques for the spotting of sample; one of them is self-loading Capillary in which small volume of samples may be applied to the plate. Surface using platinum- iridium tubing fused into the end of a length of glass tubing.

The linear development method is most familiar technique in H.P.T.L.C. here the plate is placed vertically in solvent system in a suitable container. The solvent is usually fed by capillary action and chromatogram can be developed from the both sides. Circular development, anti-circular device and multiple development are some of others methods which are used for chromatographic development. Immediately after the development is completed,

The plated are removed from the chamber and dried to remove the mobile phase. Generally, detection can be known by iodine vapor in iodine chamber. The H.P.T.L.C. equipmentis supplied with computer and data recording and storing devices. The development of H.P.T.L.C. plates scanned at selected UV regions wavelength by the instruments and the detected spots seen on computers in the form of peaks. The scanner converts bond into peak and peak heights or area is related to the concentration of the substance on the spot. The peak heights and the area under the spot are measured by the instrument and are recorded as percent on the printer.

Discussion:

*Charaka*mentioned that root collected in summer (*Grishmarutu*) wherein Raj Nighantuadvocated *Shishirarutu* for collection of root. Foreign matter is nil in respective collected sample, because of personally collected from natural habitat and maximum precaution was taken to avoid foreign matter (physical impurity) like mud, sand, grass etc. Root micro and macroscopic descriptions are as per Ayurvedic Pharmacopeia of India. The epidermis is uniseriate and continuous. It is 10 µm thick, the walls of the epidermal cells are thick and the cuticle is heavily deposited on the

Table No- 1: Root microscopy of *Ikshu*

Characters	*Ikshu* Root
Cork	Single layered epidermis consisting of 2-3 rows of thin, rectangular cells.
Cortex	Outer cortex -2or 3 layers of thick-walled polygonal to circular, sclerenchymatous cells filled with dark brown blackish pigment. Inner cortex-composed of large parechymatous cells.
Vascular bundle	Xylem and phloem forms an equal number of separate bundles arrange in a ring, center occupied by large pith

outer walls .The epidermal cells are squares in shape and have cell contents. Liner to the epidermis is a hypodermal layer; the hypodermal cells are similar to the epidermal cells in shape and size; but the cell walls of the hypodermis are thin and the cells are hyaline. (Fig.3) The vascular bundles are numerous and diffuse in distribution. The size of the vascular bundles varies in size and shape from the outer to the central zone. The outer vascular bundles are more or less circular in shape.(Fig. 4)

Outer vascular bundlesare collateral and closed. They are 150 µm in tangential plane. They have two narrow metaxylem elements and small protoxylem elements. Protoxylem lacuna is lacking the metaxylem elements are 30 60 µm in diameter : Phloem mass is 20 µm in width. The vascular bundle is surrounded by a sheath of fibres ; the fibres have thick walls and wide lumen .The central vascular bundles are larger in size; they are 150 µm in tangential plane. They have two metaxylem elements, one or two intact protoxylem elements and generally a protoxylem lacuna. The metaxylem elements are 110 µm in diameter; Theprotoxylem elements are 20µm in diameter; the protoxylem elements are 50 µm in diameter. The phloem mass is 150 µm. wide. The vascular bundles are surrounded by this sheath of fibers; they have thicker walls and narrow lumen .When the vascular bundles are viewed under the polarized light microscope, the xylem elements and the bundle sheath fibres appear bright under dark back ground. This indicates that these elements have lignified walls.

The powder of the culm shows two types of elements. Fibers(Fig. 5) are long cells with tapering pointed ends. Some of the fibers are narrow, thick walled and narrow lumened.

They are 1.25 to 2mm long; 8µm thick. Some other fibers are wider, shunter and wide

Table No-2: Physico-chemical analysis of *Ikshu* root

	***Ikshu* Root**	**API Standard**
Foreign matter	Nil	Not more than 2 %
Loss on Drying	11.63%	NA
Total Ash value	9.67%	Not more than 8 %
Sulphated Ash value	7.58%	NA
Acid insoluble Ash	7.58%	Not more than 5 %
Water soluble Ash	9.49%	NA

Table No-3: Extractive values of *Ikshu* root

Sl.No.	**Solvents**	***Ikshu* Root Extractive values**	**API Standard**
01	Water	7.08%	Not less than 7 %
02	Ethanol	4.215%	Not less than 4 %

Table No-4: Test for inorganic components in *Ikshu* root

Sl.No.	**Test**	**Test drug**
01	Test for Iron: a) Test soln. + Ammonium thiocynate	+
02	Test for Calcium: a) Test soln. + Ammonia + Potassium ferocynaide	+
03	Test for Chlorides: a) Test soln. + $AgNO_3$	+
04	Test for sodium: a) Test soln. +Potassium pyroantimonate	+
05	Test for Potassium: a) Test soln. + Sodium cobalt nitrate	+

Table No-5: Preliminary phytochemical screening of *Ikshu* root

Sr. no	**Test**	**Test drug (Aqueous Extract)**	**Test drug (Alcoholic Extract)**
01	Test for monosaccharide's a)Barfoed's test	-ve	-ve
02	Test for pentose sugars a) Sol ^{n}. + HCl+ Crystals of Phloroglucenol	-ve	-ve
03	Test for Reducing Sugars a) 3ml Sol ^{n}. + few drops of Iodine (Blue colour appears)	+ve	+ve
04	Test for Hexos sugars: a)Selvinoff's test	-ve	+ve
05	Test for Steroids: a) Salkowski reagent	+ve	+ve
06	Test for Proteins: a) Million's test	-ve	-ve
07	Test for Alkaloids: a)Wagner's Reagent	+ve	+ve
08	Test for Phenolic compounds and Tannin	-ve	+ve

lumened. The wide fibres are up to 700µm long and 12 µm wide.

Vessel elements (Fig. 6) are wide long and cylindrical cells. They have wide, circular opening at the ends, this opening are called perforation plate. The vessel elements are 270 to 400µm long. The lateral walls have dense, minute pits.

The fluorescence analysis of the stem of *Saccharum officinarum* powder was observed in day/visible light and UV light. The results are tabulated.(Table 2) Extractive value of crude drug is useful for their evaluation especially when the constituents of drug cannot be readily estimated by any other means. All the parameters as moisture content (11.63%), total ash (9.67%), acid soluble ash (7.58%) & insoluble ash (2.09%), water soluble (6.88%) & insoluble ash (2.79%),Sulphated Ash value(7.58%) within API standards which shows standard quality of the drug. The aqueous & alcohol extractive values were 7.08%, 4.215%, respectively. It shows more ingredients extracted in the water as compared to

Fig. 1 :Leaves with Racemes

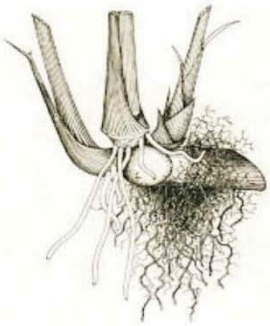

Fig. 2 :Collection of Roots

Fig. 3 : Dried Collected Roots

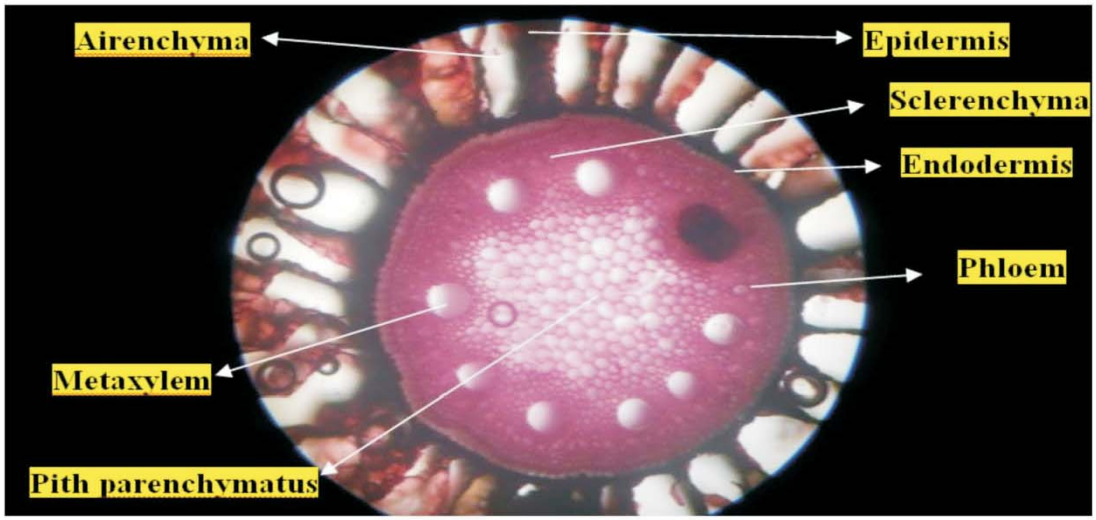

Fig. 4 : T.S. of Ikshu Root Structure

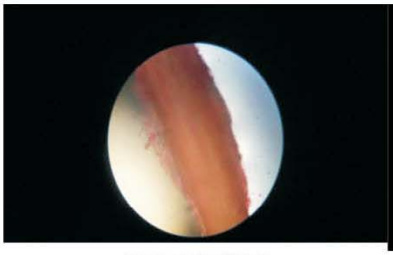

Fig. 5 : L.S. of Root

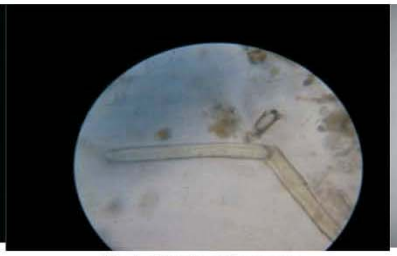

Fig. 6 : Powder Microscopy

Fig. 7 : Aqueous extract

Fig. 8 : Alcoholic extract

Fig. 9 : Alcoholic extract

Fig. 10 : Aqueous extract

Fig. 11: Image @254 nm after development

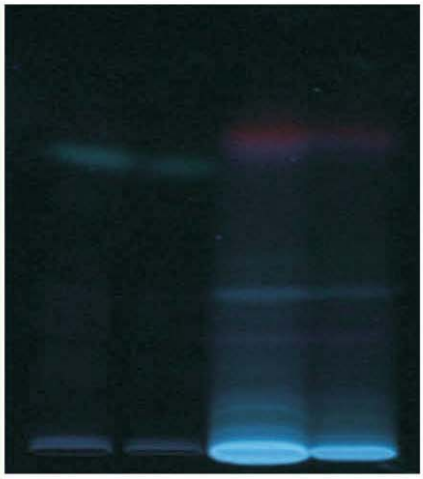

Fig. 12: Image @366 nm after development

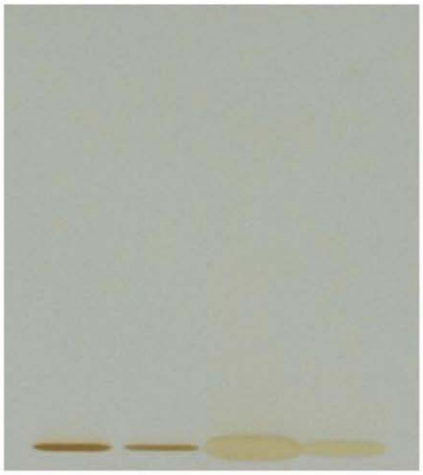

Fig. 13: Image @visible region afterdevelopment

SL No	SAMPLE	Rf VALUES
1	Aqueous Extract	0.03, 0.07, 0.11, 0.18, 0.25, 0.34, 0.44, 0.64, 0.79, 0.83
2	Alcoholic Extract	0.25, 0.41, 0.77, 0.88.

Table No-7:- HPTLC analysis profile of *Ikshu* root at Rf value 366 nm

SL No	SAMPLE	Rf VALUES
1	Aqueous Extract	0.11, 0.38, 0.77
2	Alcoholic Extract	0.73

Table No-8:- HPTLC analysis profile of *Ikshu* root at Rf value 580 nm

SL No	SAMPLE	Rf VALUES
1	Aqueous Extract	0.04, 0.17, 0.21, 0.25, 0.31,0.40,0.54,0.59,0.66,0.72,0.79,0.88
2	Alcoholic Extract	0.05,0.20,0.33,0.42,0.53,0.62,0.68,0.87.

alcohol. In Ayurvedic formulations also most of the water soluble ingredients are used for clinical purpose. pH values of *Kwatha* is 5.5 , it shows sample is acidic in nature. Preliminary Phyto-chemical tests shows presence of Steroids, alkaloids reducing sugar present in both Alcoholic and Aqueous extract but absence of proteins, monosaccharides, pentose sugars in both the extracts. Preliminary Phyto-chemical tests showed presence of hexose sugars, Phenolic compounds and tannins only in alcoholic extract but absent in aqueous extract. The presence of hexose sugar, reducing sugar shows sweet taste of root. Only one ingredient is separated at same RF at 0.25 in both the extracts .Otherwise all the ingredients separated in aqueous as well as alcoholic are at different rf. The ingredient separated in aqueous are more as compared to alcohol because of high extractive value in water.

Conclusions:

The critical review Ayurvedic literature shows that very few references found regarding Nephro-toxicity. As per description and ParyayiNamani mentioned in the Ayurvedic literature regarding *Ikshu*, botanically it is *Saccharumofficinarum* Lin. Analytical study of *Ikshumool*a concludes that the tested drug is of Ayurvedic Pharmacopeia Standard. The organoleptic study shows that rasa of *Ikshumool*a is Madhur.

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