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PRELIMINARY PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF KUSHA (IMPERATA CYLINDRICA BEAUV)

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ABSTRACT:

Backgroud: *Kusha* (*Imperata cylindrica* Beauv.) is distributed throughout the tropical and temperate regions. It is common in India, tropical Africa, southern Europe, Afghanistan, Ceylon, Malaya, Java, China, Japan and Australia. According to Ayurveda its roots are used in conditions such as *Mootrakruchchra*¸ *Ashmari, Raktapitta, Pitaprakopa,* etc.

Aim: To investigate preliminary pharmacognostical and phytochemical parameters of plant drug.

Materials and Method: Identification of the plant was done as per the standard guidelines given in the floras. Preliminary physico-chemical and phytochemical screening was done and after achieving the idea of phyto constituents group, quantitative test of sugar content and volatile oil content and thin layer chromatography studies were carried out for different organic solvent extracts.

Results: The presence of air cavities in the root and stolon of *Kusha* indicates that the plant is mesophytic. Aqueous extracts showed the presence of alkaloids, flavonoids, triterpenoids, tannins, carbohydrates and sugars.

Conclusion: The findings of the study will be helpful in the identification of *Kusha* plant**.**

Keywords: *Kusha*, *Imperata Cylindrica* Beauv., Diuretic, Sugar.

INTRODUCTION:

Kusha (*Imperata cylindrica* Beauv.) is distributed throughout the tropical and temperate regions. It is common in India, tropical Africa, southern Europe, Afghanistan, Ceylon, Malaya, Java, China, Japan and Australia. In India, it is found throughout the hotter parts, often in damp and weedy places, both in plains and hills, ascending upto 2,300 m in the Himalayas. It is frequent along river banks, cultivated lands and on black cotton soil. The plant is known by the names of *Ulu*, Thatch grass, Cogon grass, *Kusa*, *Vidulam*,[1] etc. in different parts of India and is one of the constituents of *Trinapanchmoola*[2].

MATERIALS AND METHODOLOGY

Literary Review

Kusha has been quoted under medicinal drugs since the *Vedic* era[3]. Though a very useful and easily available drug, it has been confused with *Darbha* as both are used as synonyms for each other and quoted together usually under the name of *Darbhayugma*[4] and *Darbhadway*[5]. Despite being used as synonyms their botanical appearance has been described as two different plants in almost all the *Nighantus*, which has led to botanical confusion about the source plants. In many contemporary botanical texts *Kusha* has sometimes been quoted as *Desmostachya bipinnata* Stapf. and at some places as *Imperata cylindrica* Beauv.

The rhizomes are used in China as restorative, tonic and antipyretic[6]. It is also used as an antidote for snake bite as well

as for various ailments such as fever, piles, etc.[7]. The rhizomes contain flavonoids, sinensitin, eupatorin, tetra-O-mythylscutellarein and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, together with two novel lignans, graminone A ($C_{20}H_{20}O_7$) and B ($C_{21}H_{22}O_8$). A sesquiterpenoid, cylindrene and two biphenyl ether compounds, cylindrol A and B are also reported. The other compounds reported are arundoin, fernenol, imperarene, 5-hydroxy-2-(2-phenylethyl)chromone, 5-hydroxy-2-styrylchromone[8].

In the present study, the focus has been on identification of the source plant via macroscopic and microscopic study as well as physico chemical analysis.

Collection of Sample: *Kusha* (*Imperata cylindrica* Beauv.) (Family - Poaceae)[9] was collected from Sasoi region of Jamnagar district and authenticated by Shri A.P.G. Pillai, (OSD), PGT-SFC cell I.P.G.T. & R.A., Gujarat Ayurved University. The rhizomatous roots were made into small pieces, shade dried, pulverized to fine powder (mesh number 80) and stored in airtight glass container for experimental purposes.

Preparation of Herbarium:

Plant sample of *Imperata cylindrica* Beauv. was identified and authenticated with the help of The Flora of Gujarat State[10] and The Flora of Maharashtra State[11]. Herbarium was prepared and deposited in the herbarium section of the Institute.

Preparation of wet sample:

Freshly collected and thoroughly washed plant sample was kept in a glass bottle containing solution of formalin-aceto-alcohol (FAA).

Microscopy

a) Root, Stolon and Leaf: Transverse sections of root, stolon and leaf were taken and photomicrography was done after proper mounting and staining.

b) Powder microscopy: Powder of drug was studied microscopically and microscopic characters were photographed by using Canon digital camera attached to Zeiss microscope.

Phytochemical and physico-chemical analysis : Dried rhizomatous powder was used for analysis of physic-chemical parameters such as loss on drying, ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH, particle consistency and phytochemical tests for alkaloids, tannins, triterpinoids, carbohydrate, flavanoids, saponin and glycosides as well as sugar content and volatile oil content.

Phytochemical screening: Preliminary phytochemical screening was done and after achieving the idea of phytoconstituents group, Alkaloids, Tannins, Triterpenoids, Carbohydrate, Flavonoids, Saponin, Glycoside; quantitative test of Sugar content and Volatile oil content.

TLC fingerprinting profiling

TLC glass plates were prepared using silica gel-G (E- Merck) and were activated at 110^{o}C for 30 minutes. Petroleum ether, chloroform and ethanol, all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material. All the solvents employed as mobile phase for thin layer chromatography were of AR grade.

• Preparation of sample:-

1gm of powder was extracted with 10ml methanol by warming, it was filtered and the solvent was concentrated to 5ml.This solution was used for spotting.

Track – A: *Imperata cylindrica* Beauv.

• Stationary phase-
Silica gel G
• Mobile phase-
(1) Chloroform: Toluene: Isopropyl alcohol: Acetic acid: Water (22:8:1:0.5:1)
(2) Hexane : Diethyl ether : Acetic acid (7 : 1.5 : 0.1)
• Detection-
For mobile phase 1:
a. Long UV (366nm)
b. Short UV (254nm)
c. Iodine chamber
d. Spraying with 5% methanol-sulphuric acid and heating up to 110°C. for 10 minutes.

For mobile phase 2:
a. Long UV (366nm)
b. Short UV (254nm)
c. On spraying with LB (Liebermann – Burchard reagent) and heating for 10 minutes at 110°C.

The volume of both the Samples was made equal with methanol and then same quantity was spotted on the TLC plate. For development, the plate after spotting was kept in a chamber saturated with the solvent system. The plate after development was viewed under long and short UV, then placed in Iodine chamber for 10 minutes and finally sprayed with 5% methanol-sulphuric acid and observed for spots for the first phase. While for the second phase the plates were observed under long and short UV as well as after being sprayed with LB reagent for the spots.

OBSERVATION AND RESULTS

Macroscopic evaluation

Root and stolon

The roots are fibrous, up to 2 mm. in diameter, about 6-9 cm long, almost cylindrical, arising from the nodes of stolons; surface uneven, with fine wrinkles, light yellow to light brown in colour; fracture and fibrous. (Figure 1) and (Figure 2).

Leaf : The leaves are linear, flat, tapering from the middle to finely acuminate point, smooth above and scaberulous beneath, loose, glabrous, leaf blades with a noticeably off-center whitish mid-vein and scabrous margins. (Figure 3) and (Figure 4).**Microscopic evaluation**

T.S. of ROOT

Epiblema: Outermost region made up of two layered epidermii, thin walled, hexagonal, covered with thin cuticle and root hairs. (Figure 5)

Exodermis: Thick walled, 2-3 layered, compactly arranged, lignified and sclerenchymatous.(Figure 5 and Figure 6)

Cortex: Made up of several layers and can be distinguished into three regions; outer region 2-3 layered, thin walled, oval-circular parenchymatous, central single layered aerenchymatous with large air cavities; and the innermost 3-5 layered, parenchymatous, smaller in size, at few places single or in groups of 2-5 thick walled pitted parenchyma or stone cells are scattered. (Figure 6)

Endodermis: Innermost layer of cortex, single layered, oval-rectangular characterized by the presence of Casparian strips, passage cells opposite to protoxylem. (Figure 6 and Figure 7)

Pericycle: Single layered, thick walled, oval-hexagonal, lignified. (Figure 6)

Vascular Bundle: Vascular tissue 5-6 in number, polyarch with alternate strands of xylem and phloem; xylem exarch consisting vessels and fibers; phloem parenchymatous.(Figure 7)

Pith: Central most region, thin walled, made of oval-circular parenchymatous cells.(Figure 7)

T.S. of STOLON

Epidermis: The outermost region is single layered, thin walled oval, pentagonal-hexagonal covered with thin cuticle. (Figure8)

Hypodermis: 2-3 layered, closely packed, thick walled, lignified, sclerenchymatous. (Figure 8)

Cortex: Covering half the region of total section, thin walled oval-circular, parenchymatous consisting of starch grains, alternating with wide lacuna and air cavities in the centre. Outer vascular bundles are very few in number, poorly developed. Vascular bundles are collateral, endarch and closed. (Figure8)

Endodermis: Consisting of single layered, barrel shaped, oval-rectangular cells. (Figure 8 and Figure 10)

Pericycle: Composed of 3-5 layered, thick walled, lignified, and sclerenchymatous. (Figure 9)

Vascular Bundle: Typical monocotyledonous, polyarch, collateral, scattered throughout the stele region, surrounded by ground tissue of parenchyma, vascular ring covered with 2-3 layered sclerenchymatous sheath. (Figure 9 and Figure 10)

T.S. of LEAF: Transverse section of this typical iso-bilateral shows the following structures:

Epidermis: Both upper and lower epidermis are made up of single layered, thin walled, oval to rectangular cells, covered with thin cuticle and simple hooked shaped trichomes; lower cells are somewhat bigger than the upper. Large number of stomata (not much sunken) on both the layers with bulliform cells. (Figure 11)

Mesophyll: Palisade cells are chlorenchymatous, mostly located at both the sides of vascular bundles. Parenchymatous cells are spongy, thin walled, oval-circular or rectangular with intercellular space. Less number of cells occurs between upper and lower epidermis. The cells are iso-diametric with chloroplasts and compactly arranged with very few intercellular spaces. (Figure 12) (Figure 13)

Vascular Bundle: The median portion of the lamina is thickened or bulging on the lower side having numerous collateral and closed vascular bundles arranged in a parallel series, the other part consisting of several small and big vascular bundles surrounded by many layered sclerenchymatous sheath. In some cases the sheath is connected with upper and lower epidermis by groups of sclerenchymatous fibers. Large number of collenchymas cells on both the lower and upper epidermis. (Figure 14)

Stomata: Graminaceous, on both the surfaces, in between the veins made up of rectangular and wavy margined beaded parenchymatous cells, two rows of alternately arranged stomata. (Figure 15)

Powder microscopy:

The powder of root exhibits fragments of simple trichomes, thin walled parenchyma cells, lignified sclerenchymatous fibers, pitted parenchyma, pitted vessels, stone cells, yellowish colouring material, lignified xylem vessels, simple and compound starch grains without hilum. (Figure 16, 17, 18 and 19)

Physico-chemical content : Powder shows loss of drying at 110^{o}C is 3.4 % w/w, ash value 9.3 % w/w, acid insoluble ash 1% w/w, water soluble extractive 22.6 % w/w, alcohol soluble extractive 20.3 % w/w and pH 5.64. The particle size consistency above 60 mesh is 31.52% w/w; between 60-85 mesh it is 27.75% w/w; between 85-120 mesh it is 25.77%

w/w and below 120 mesh it is 12.79% w/w. (Table 1)

Table 1. Physico-Chemical Parameters Of *Imperata Cylindrica* Beauv. Root Powder Samples

Sr.	Parameters	Results
1	Loss on drying	3.4 % w/w
2	Ash value	9.3 % w/w
3	Acid insoluble ash	1 % w/w
4	Water soluble extractive	22.6 % w/w
5	Alcohol soluble extractive	20.3 % w/w
6	pH value	5.64
7	Particle consistency	
	A. above 60 mesh	31.52 % w/w
	B. between 60-85 mesh	27.75 % w/w
	C. between 85-120 mesh	25.77 % w/w
	D. below 120 mesh	12.79 % w/w

Preliminary phytochemical screening: Preliminary phytochemical screening of Root powder of *Imperata cylindrica* Beauv. was performed to have an idea of the phyto constituents group present in the plant part as reported in Table 2. Aqueous extract of root showed the presence of alkaloids, tannins, triterpenoids, carbohydrates, flavonoids and glycosides.

Table 2 Preliminary Phytochemical Screening Of *Imperata Cylindrica* Beauv. Root Powder

Sr. No.	Tests	Reagents	Results
1	Alkaloids	Wagner's reagent	+ve
		Dragendorff's reagent	+ve
2	Tannins	Lead acetate	+ve
		Gelatin test	+ve
		Dil. HNO_3	-ve
3	Triterpenoids	Leibermann-Burchard reagent	+ve
4	Carbohydrate	Fehling's test	+ve
		Tollen's reagent	-ve
5	Flavonoids	Shinoda test	+ve
		Sodium hydroxide test	+ve
6	Saponins	Foam test	-ve
5	Glycoside	Legal test	-ve
		Keller Killiani	+ve

+ve – Positive -ve – Negative

Quantitative Test: The samples were quantitatively tested for the estimation of Sugar content and Volatile oil content from the alcohol extract and dry powder of the sample. On applying the spectrophotometric method and observing the absorbance of the sample at 490

nm it was seen that the sugar content was 2.54 µg/mg. Volatile oil content was observed in trace amounts. (Table 3)

Table 3 Result Of Sugar Estimation Of Alcohol Extract Of *Imperata Cylindrica* Beauv. Root Powder

Sr.	Parameter	Method used	Result
1	Sugar	Spectrophotometric	2.54 µg/mg
2	Volatile oil	Clevenger Apparatus	Trace

DISCUSSION:

Macroscopic study: The root and stolon of *Imperata cylindrica* are fibrous, up to 2 mm. in diameter, about 6-9 cm long, almost cylindrical, arising from the nodes of stolons; surface uneven, with fine wrinkles, light yellow to light brown in colour; fracture and fibrous.

The leaves of *Imperata cylindrica* are linear, flat, tapering from the middle to finely acuminate point, smooth above and scaberulous beneath, loose, glabrous, leaf blades with a noticeably off-center whitish mid-vein and scabrous margins

Microscopic Study:

Table 4 Showing the Characteristics Of *Imperata Cylindrica Beauv.*

Sr.	Part	Component	*D. bipinnata*
1.	***Root***	Epiblema	Hexagonal cells
		Root hairs	Less in number
		Exodermis	2-3 layered, lignified sclerenchymatous.
		Cortex	Air cavities small in size.
		Passage cells	Present
		Casparian thickenings	More in number.
		Pericycle	Single layered, oval to hexagonal shaped cells.
		Vascular Bundles	5-6 vascular bundles.
		Conjunctive tissue	Less in number and occupies less area. Located mostly in the periphery of the vascular bundles.
2.	***Stolon***	Epidermis	Pentagonal to hexagonal cells. Presence of some hairs.
		Hypodermis	2-3 layered.
		Cortex	Outer vascular bundles are very few in number and poorly developed. Cortex has intercellular spaces like air cavities.
		Pericycle	3-5 layered.
		Vascular Bundles	-
3.	***Leaf***	Epidermis	Large number of not much

			sunken stomata and presence of bulliform cells.
		Mesophyll	Less number of cells occurs between upper and lower epidermis. Cells are iso-diametric.
		Vascular Bundle	1. Vascular bundles of nearly same size except the mid-rib region. 2. Median portion of lamina is bulging on the lower side.
		Stomata	Alternately arranged stomata.

Table 4 gives the characteristics of the plant. The presence of air cavities in the root and stolon of *Imperata cylindrica* indicates that the plant is mesophytic.

On comparing these observations with the classical referred plant, *Imperata cylindrica* can be considered as the source plant of Kusha, as Kusha is quoted to be found in Anupa Desha or Sadharan Desha where the water table is comparatively high (A.H. Ka 6/1,2)[12].

Powder microscopy: Powder microscopy of the plant reveals that simple and compound starch grains, stone cells, lignified sclerenchymatous fibres, pitted parenchyma, pitted vessels, and yellow coloured material was found. *Imperata cylindrica* powder consisted of starch grains without hilum.(Figure 16,17,18,19)

Physico-Chemical Parameters: The ash value indicates the inorganic load of a drug. The water soluble extractive value of Sample (22.6% w/w) indicates that the load of polar components.The alcohol soluble extractive value which is indicative of the load of non-polar components of Sample (20.3% w/w). The pH is 5.64 which shows that the drug is slightly acidic. The overall particle size consistency of the sample suggests that the uniformity of therapeutic dose is medium. The acid insoluble ash suggest the amount of silica in the drug which can be present due to a number of reasons such as improper washing being one of them.

Phytochemical Analysis: The qualitative tests conducted by using different reagents for Alkaloids, Tannins, Triterpenoids, Carbohydrates especially Sugars, Flavonoids and Sugar part of Glycosides gave positive results were considered to be present in the sample. Qualitative test for Saponins was negative for the sample.

Quantitative Analysis: Volatile oil was found to be in trace amounts in the sample. Qualitatively and quantitatively sugar was found to be present in the sample. As sugar is considered to be diuretic, the plant with higher sugar content can be supposed to cause more diuresis. *Imperata cylindrica* has sugar which suggests that therapeutically it can prove to be an effective diuretic.

TLC Study: The TLC pattern of the sample showed that in mobile phase 1 under long UV 6 spots at R_f value 0.8, 0.27, 0.37, 0.46, 0.57 and 0.79 were found. (Fig.20). While on exposure to iodine vapours, 4 spots of R_f value 0.10, 0.34, 0.41 and 0.51 were found (Fig.21). On spraying with 5% methanol-sulphuric acid,

5 spots of R_f value of 0.35, 0.41, 0.48, 0.56 and 0.64 were found(Fig.22).

Under long UV showed 3 spots with R_f values of 0.04 0.10 and 0.16 (Fig.23), while on spraying with LB reagent and subjecting to heat one spot was observed with R_f value of 0.12 (Fig.24).

CONCLUSION: Pharmacognostic and preliminary phytochemical investigation included with TLC fingerprint profile of the plant showed some unique diagnostic characters, which could be helpful to identify the plant of *Kusha*.

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Figure 1: Whole plant *I. cylindrica*

Figure 2: Dry whitish root of *I. cylindrica*

Figure 3: Lower surface of leaf

Figure 4: Upper surface of leaf with white mid rib

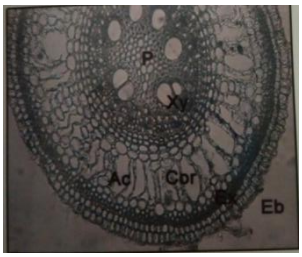

Figure 5: Transverse section of root, Eb-epiblema, Ex-exodermis, Ac-air cavities, Cor-ortex, Xy-xylem, P-pith

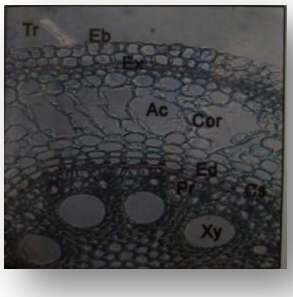

Figure 6: Arrangement of Ed-endodermis, Tr-trichomes, Ex-exodermis, Ac-air cavities, Cor-cortex, Cp-casparian strips, Pr-pericycle, Xy-xylem

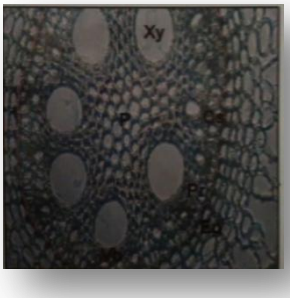

Figure 7: Ed-endodermis, Cp-casparian strips, Pr-pericycle, Vb-vascular bundle, Xy-xylem, P-pith

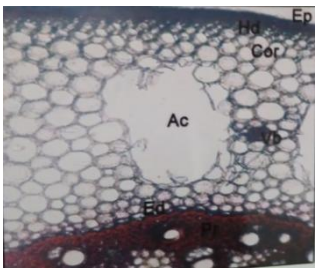

Figure 8: Transverse section of Stolon, Ep-epiblema, Ac-air cavity, Hd-hypodermis, Vb-vascular bundle, Cor-cortex, Ed-endodermis, Pr-pericycle,

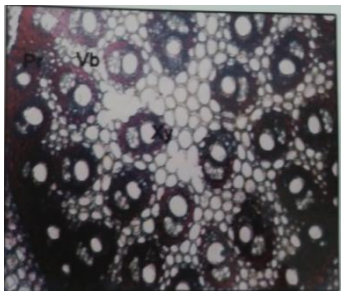

Figure 9: Pr-pericycle, , Vb-vascular bundle, Xy-xylem,

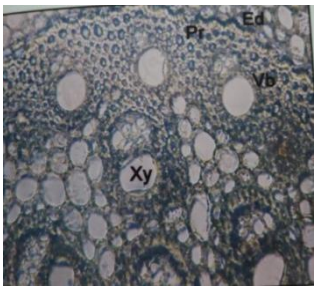

Figure 10: Arrangement of Ed-endodermis, Pr-parenchyma, Vb-vascular bundle, Xy-xylem

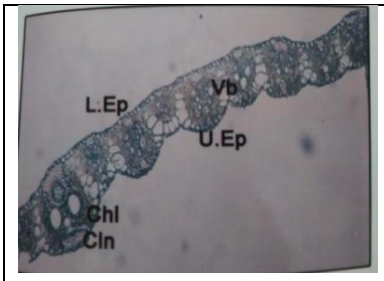

Figure 11: Transverse section of leaf, L.Ep-lower epidermis, Cln-collenchyma, Chl-chlorenchyma, U.Ep-upper epiblema

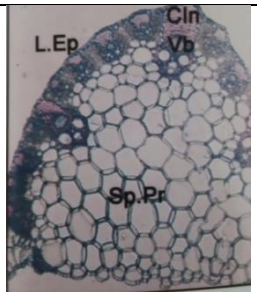

Figure 12: Mid-rib region L.Ep-lower epidermis,Cln-collenchyma, Vb-vascular bundle

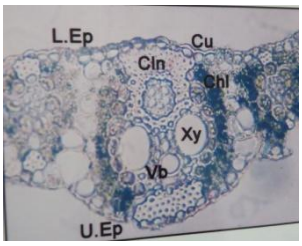

Figure 13:U.Ep-Upper epidermis, L.Ep-lower epidermis, Chl- Chlorenchyma, Cln-collenchyma , Xy-xylem, Vb-vascular bundle, Cu-cuticle

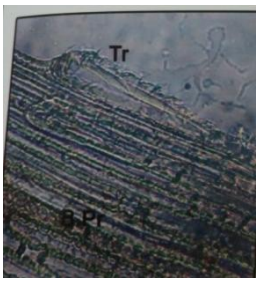

Figure 14: Tr-Trichome, B.Pr-beaded parenchyma

Figure 15: B.Pr-beaded parenchyma and St-stomata

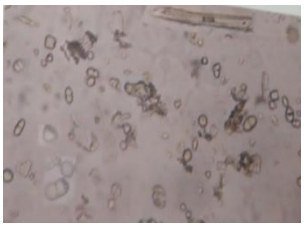

Figure 16: Simple, compound, cup shaped starch grains, trichomes and yellow colouring material in root powder

Figure 17: Stone cells

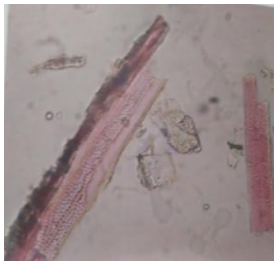

Figure 18: Pitted vessels

Figure 19: Xylem fiber and septed fiber

Figure 20: Track B – Rf 0.44, 0.52, 0.79

Figure 21: Track B – Rf 0.10, 0.52

Figure 22: Track B – Rf 0.24, 0.32, 0.41, 0.43, 0.46

Figure 23: Track B – Rf 0.04, 0.10, 0.16

Figure 24: Track B – Rf 0.12