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PHARMACOGNOSTICAL STUDY OF AN ENDANGERED MEDICINAL TAXA GYMNEMA TINGENS (Roxb) Wt. & Arn.

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ABSTRACT

India is the country having rich source of medicinal plants used in Indian System of Medicine which include Ayurveda, Siddha, and Unani in additional to traditional medicinal practices. Secondary metabolites of plant products have greater advantages in pharmacological activities with minimal side effect. *Gymnema tingens* (Roxb) Wt. & Arn. is one of the endangered medicinal plants of the genus *Gymnema* belonging to family Asclepiadaceae. It is used in treating the diabetes traditionally. There is a meager scientific data regarding the standardization of this herbal drug. So made an attempt to standardize the botanical through pharmacognostical approach of microscopy, organoleptic study, powder microscopy, physicochemical parameter, phytochemical analysis and fluorescence analysis. Results shows a unique feature like multicellular uniseriate trichomes, druses of calcium oxalate crystal, paracytic stomata, specific characteristic mild odour, bulk density is 0.38g/cc, ash value is 8.5%, specific gravity is 1.24g /cm^3, pH values is 5.6 and aqueous extractive is 3 %. Presence of alkaloid, phytosterol, saponin, flavonoid and tannins. Fluorescence analysis of a drug with different chemical reagent under different wavelength emits a particular colour due to the presence of a particular phytochemical composition. Drug powder with acetone under long wavelength emits saffron colour. The study conclude that the results obtained could be utilized as a reference for setting limits for the reference standards of *Gymnema tingens* botanical to check the quality and purity of the herbal drug. This data can be used by the scientific community to develop a different formulation to serve the society locally to globally.

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INTRODUCTION

India is the country having rich source of medicinal plants used by different system of medicine which include Ayurveda, Siddha, Unani etc., in additional to folklore and ethno medicinal practices. Botanical products and their active secondary metabolites have greater advantages such as very minimal side effect, rich in resource and low price. Therefore, the complementary and alternative approach to screen new effective natural drugs through isolation of phytochemical from botanical sources is imperative [1, 2]. Phytochemical studies of the genus *Gymnema* of the family Asclepiadaceae have led to the isolation and identification of tri terpenoids, triterpenoid saponins, C4-steroids, C21-steroidal glycosides, flavonoids, peptides, polysaccharides, and other phytochemicals [3-9]. All Active principles present in the genus *Gymnema* possess an important pharmacological activity, i.e., an anti-diabetic, anti-hyperlipidemic, antiallergic, and antiviral and lipid lowering effects, cytotoxic and immune stimulatory activities [10-16]. Some triterpenoid saponins from the genus *Gymnema* were found to be attenuate hyperglycemia in traditional herbal medicine, the *Gymnema* species has been well known for the anti-diabetic potential [17]. *Gymnema* tingens (Roxb) Wt. & Arn. a twiner belonging to the family Asclepiadaceae which is very much close to a well-known medicinal plant *Gymnema sylvestris* which is used as an anti-diabetic plant [18-20]. Synonym of *Gymnema tingens* are *Asclepias montana*, *Bidaria montanum* and *Gymnema montanum* and its red list status has been assessed as an endangered globally [21]. *Gymnema tingens* is a folklore medicine used for the treatment of rheumatism and polio [22]. *Gymnema tingens* have triterpenoids, steroids, phenolic glycosides and steroids, phenolic glycosides and several other components [23-25]. Ethanolic extract of *Gymnema tingens* showed a good activity on glucose uptake and have the potential to become a source for new antidiabetic drug discovery. *Gymnema tingens* has potential to become a natural resource for discovering diabetes drugs [26]. Ethanolic extract of *Gymnema tingens* having six phenolic diglycosides namely Gymnetinosides A-F. Compounds of Gymnetinosides A, E and F showed hepatoprotective properties against D-galactosamine induced HL-7702 cell damage [27]. During literature survey it was noticed that there is a meager reference for standardization of this botanical as a drug. There is no research work on pharmacognostical study especially on microscopy of an officinal part of the medicinal plant, organoleptic study, physico-chemical parameters and fluorescence study etc., which are essential parameter for herbal drug standardization. This medicinal plant may become potential natural source in future to treat diabetes. There is a need to study this medicinal plant systematically in scientific way to add to the list of standardized herbal drug to serve the society as an anti-diabetic drug. So, in order fulfil the lacuna pharmacognostic investigation of *Gymnema tingens* is conducted during the course of work to standardize the herbal drug. This is the first report of standardization of this botanical. Objective of this study is to standardize the herbal drug by researching various scientific parameter which are used in pharmacognosy study. So, attempt is made to researching pharmacognostical parameters, which are very much essential before herbal formulation as well as in *vivo* study. This pharmacognostic study play an important role in the standardization of a raw drug, its macro-micro morphological feature, organoleptic study. Powder microscopy, preliminary phytochemical analysis and fluorescence analysis. It is found that the anatomical microscopic details of botanical source helpful to check specific species of the medicinal plants this is the primary and first step and powder microscopy play an important role in the identification and authentication of herbal drug by researching various and specific cell component and cell inclusion. Bulk density value is one of the main parameters when the herbal drug used in the powder form. Ash value indicates presence of inorganic materials that indicates purity of the botanical source. Specific gravity and pH value are specific to the herbal drugs. Fluorescence analysis of herbal drug with different reagent under short and long wave length emits specific colour due to the presence of particular components. This study is contributing towards the fulfil of these parameters by adapting standardized procedure. These pharmacognostical data help to standardize the lesser-known endangered medicinal taxa *Gymnema tingens.*

MATERIALS AND METHODS

Fresh botanical sample was collected from herb garden, Sri Sri College of Ayurvedic Science and Research. Plant specimen was identified by referring floras [28-29]. Fresh stem pieces and leaf material was preserved in FAA preservative for microscopical studies. The sufficient quantity of plant material was shade dried and powdered by using pulverizer and sieved the powder. Coarse and fine powder was stored in air tight container. Preserved plant sample was used for free hand sectioning of stem, petiole and leaf for anatomical studies, safranin and hematoxylin was used to stain the sections and mounted on a glass slide with glycerin [30-31]. The prepared slides were studied under compound microscope for microscopical feature and photographs were taken. Organoleptic study was conducted for texture, odour, colour and taste of the drug powder. Physicochemical parameter assessed for foreign matter, ash value, moisture, extractive values, and specific gravity [32]. Bulk density and for pH value, powder microscopy was carried out by following standard pharmacognostical procedures [33]. Preliminary phytochemical analysis was done by extracting 100 g of shade dried plant powder in the Soxlet apparatus by using distilled water as a solvent. Extract was filtered and collected; volume of filtrate was reduced to 1/10th by evaporation of solvent on a water bath. The dried extract was stored in a sterile container and used for phytochemical analysis [34]. Analysis of stored crude powder was treated with different reagents and observed the colour changes in visible light, short wave length and long wave length, the results are documented [35].

RESULTS

T.S of Stem: TS of stem [Fig. I] shows an epidermis, cortex, vascular tissues and well-developed pith. Epidermis is made up of a single layer of tangentially elongated cell, covered by a thick cuticle and trichome on the epidermal wall. Below the epidermis there are two to three layers of hypodermis which is made up of different size and shape of collenchyma cells followed by a cortex made up of loosely arranged parenchyma cells with intercellular space between the cells. This parenchyma cells are interspersed with patches of sclerenchyma cells. After the cortex, vascular tissues are observed by undifferentiated phloem tissues and a continuous ring of

xylem. At the center of the section there is well developed pith which is made up of parenchyma cells. Cell inclusions are calcium oxalate crystal which is present in the form of druses and a starch granule which are accumulated in the parenchyma cells.

T.S of Petiole and leaf: TS of petiole [Fig. II.A-E] is circular in outline, since it is grooved on dorsal side, it is concave on the grooved side. Epidermal cells having trichome and covered by cuticle. Below the epidermis there is 1-2 layer of collenchymatous hypodermis followed by the ground tissue made up of loosely arranged parenchyma cells. There is a central 'C' shaped vascular bundle and two smaller vascular bundles are present above the larger one.

V.T.S of leaf: VTS of leaf [Fig. II.F-H]at mid rib region showed a convex at upper side and circular at lower side there is prominent one mid rib (Fig. II. F] and two midribs at basal part of the leaf [Fig. II.G] below the dorsal and above the ventral side at the midrib there is supporting collenchyma cells and a parenchymatous ground tissue and prominent central broad vascular bundle. At the laminar region mesophyll tissue is differentiated into double layered palisade parenchyma and multi layered spongy parenchyma below the palisade tissue. Epidermal cells are having trichome both on upper and lower side including midrib region. Epidermal peel [Fig. II.I] shows a scattered paracytic stomata.

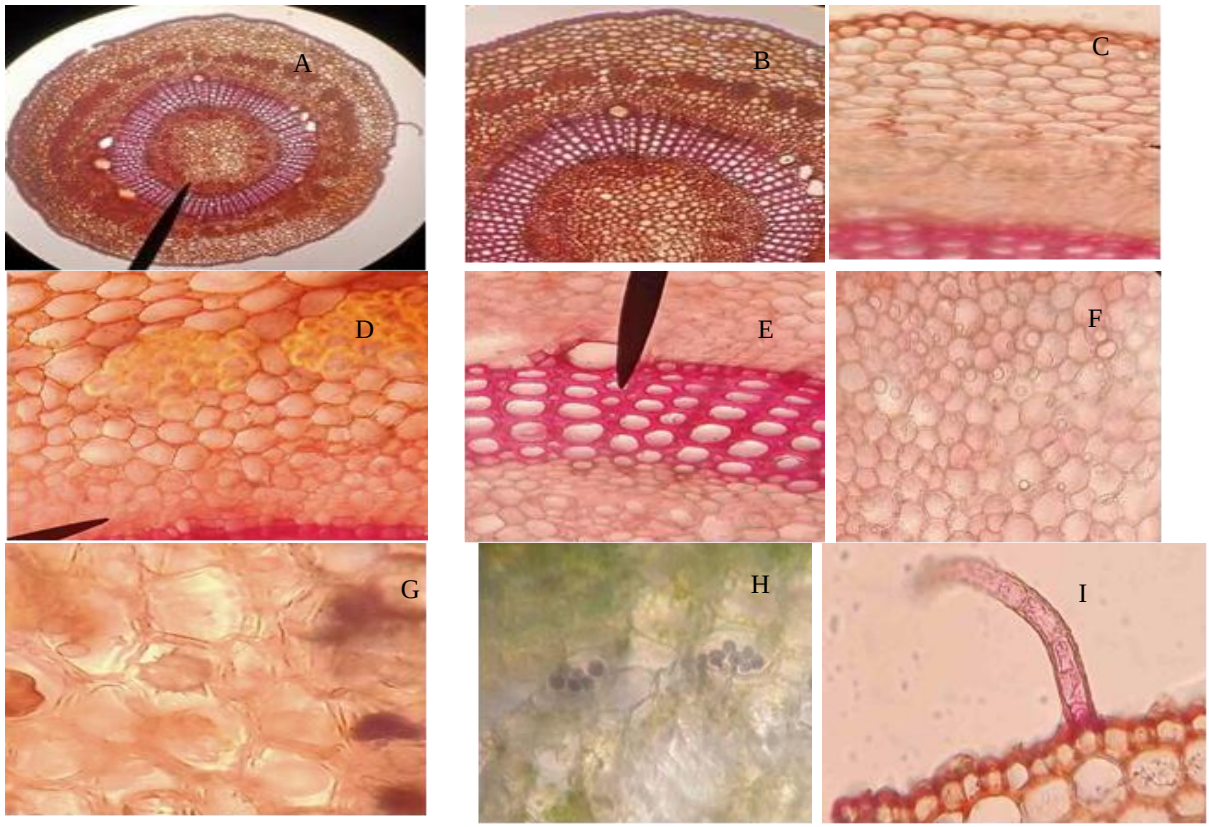

Figure: I. Transverse section of stem

A.t. s of stem, B. t. s of stem portion enlarged, C. Cortex, D. Sclerenchyma patch, E. Xylem, F. Pith, G. Calcium oxalate crystal, H. Starch grains, I. Epidermal trichome

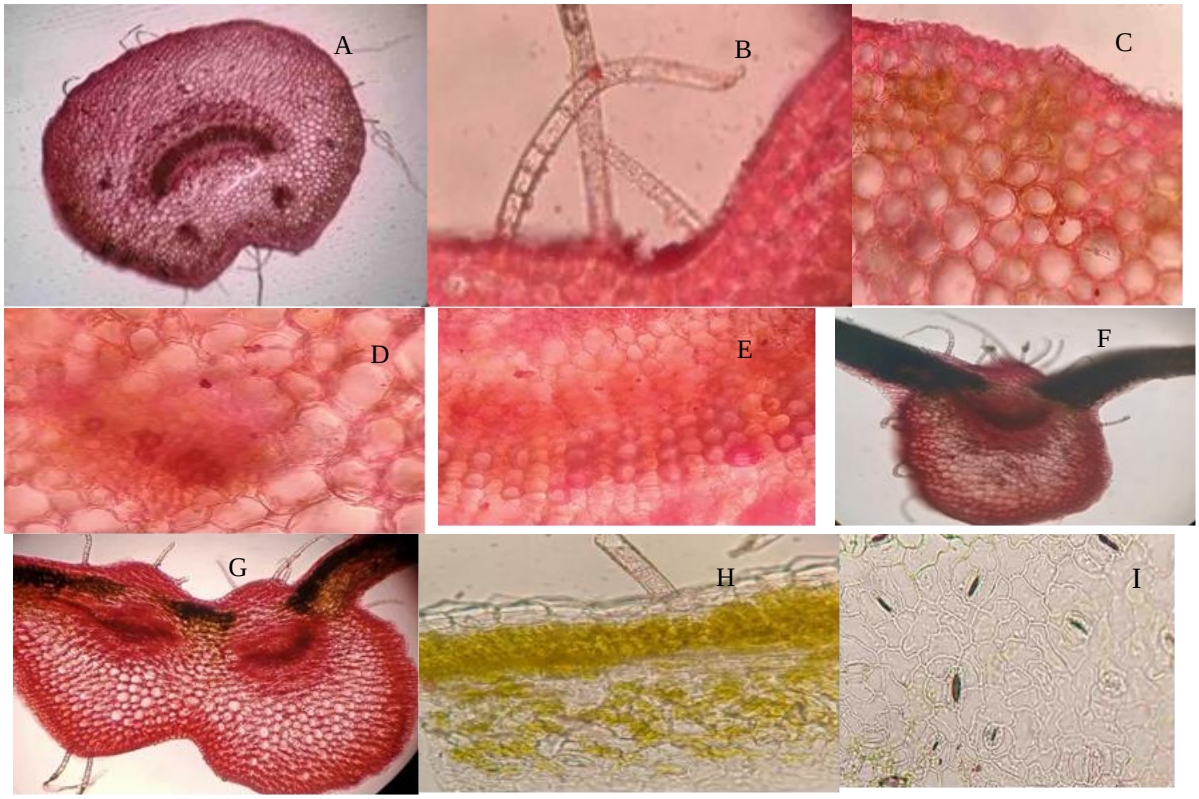

Figure: II. Transverse section of petiole and leaf

A. t. s of Petiole B. Epidermal trichomes C. Enlarged portion of cortex of petiole, D. Vascular tissue in petiole, E. Vascular bundle in petiole, F. v. t. s of midrib, G. v.t.s of midrib, H. v. t. s of lamina, I. Stomata

Organoleptic study: [Tab. I] Whole plant powder was coarse in nature, colour is light yellowish green. There is characteristic mild odour and it has a slightly bitter in taste. The powder microscopy of the drug has the isolated parenchyma cell, elongated whole fibre, annular vessel thickening, chlorophyll pigment present in epidermal cells, stomata, uniseriate trichome, fragment of vessel, elongated columnar palisade parenchyma, epidermal cell, vessel, scleroid stone cell, fragment of parenchyma cell [Fig. III. A-L]

Physico-chemical parameter: [Tab.II] Foreign matter is 0.5 percent, moisture content is 0.37 percent, bulk density is 0.38 gram per cubic centimeter, ash value is 8.5 percent and specific gravity is 1.24 gram per centimeter cube, pH is 5.6 and aqueous extractive value is 3 percent.

Preliminary phytochemical analysis: [Tab.III.] Aqueous extract of crude drug powder showed a presence of alkaloid, phytosterol, carbohydrates, saponin, flavonoid and tannins. Absence of fixed oil and proteins.

Fluorescence analysis: Fluorescence analysis crude powder with different chemical reagents in visible, short wavelength and long wavelength was observed. Powder as such and treated with different reagent like distilled water, concentrated HCl, concentrated HNO_3, acetone, 5 percent Iodine, 5 percent KOH, $FeCl_3$ and NaOH are documented in the table [Tab.IV]

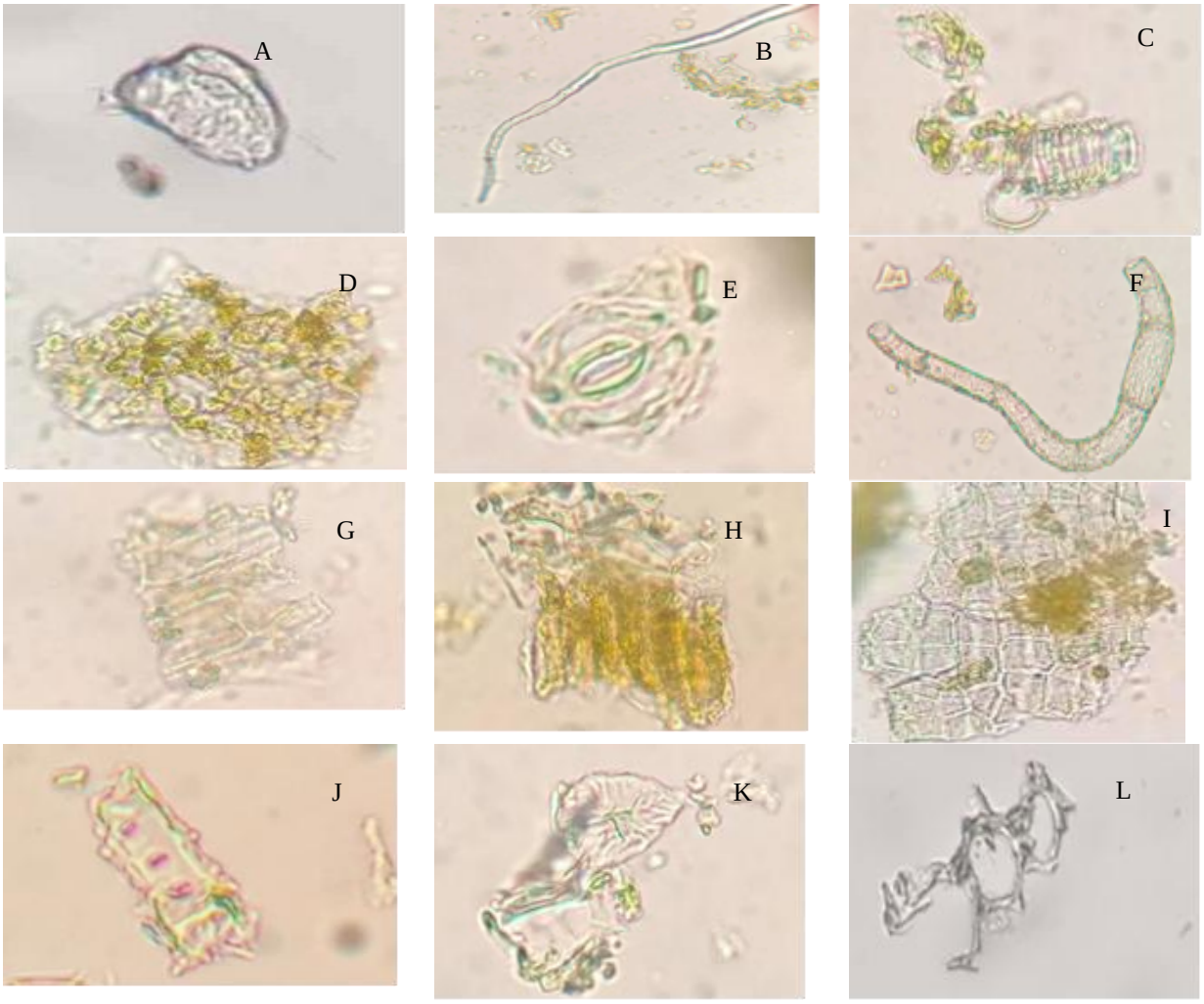

Figure: III. Powder microscopy

A. Isolated parenchyma cell, B. Fiber, C. Vessel thickening, D. Chlorophyll pigment, E. Stomata, F. Trichome, G. Fragment of vessel, H. Palisade parenchyma, I. Epidermal cell, J. Vessel, K. Stone cell, L. Parenchyma cell

Table: I. Organoleptic study of Drug powder.

Sl. No	Nature	Result
1	Texture	Coarse
2	Odour	Characteristic mild odour
3.	Colour	Light yellowish green
4.	Taste	Slightly bitter

Table: II. Physico-chemical parameter

Sl. No	Features	Observation
1	Foreign matter	0.5%
2	Moisture	0.37%
3	Bulk density	0.38g/cc
4	Ash value	8.5%
5	Specific gravity	1.24g/cm^3
6	pH	5.6
7	Extractive values	3%

Table: III. Preliminary phytochemical analysis of aqueous extract.

Sl. No	Phyto constituents	Test	Result
1	Alkaloid	Mayer's test Wagner's test	Positive Positive
2	Phytosterol	Salkowski test Liebermann-Burchard	Positive Positive
3	Carbohydrates	Benedicts test Molisch's test	Positive Positive
4	Proteins	Biuret test Ninhydrin test	Negative Negative
5	Fixed oil	Stain test	Negative
6	Saponin	Froth's test	Positive
7	Flavonoid	Lead acetate test	Positive
8	Tannins	Gelatine test	Positive

Table: IV. Fluorescence study with different chemical reagents in Visible and UV light

Sl. No	Drug powder with reagent	Visible	Short wavelength(254nm)	Long wavelength(366nm)
1	Powder as such	Light yellowish green	Dark green	Dark green
2	Powder +Distilled water	Light brownish green	Dark green	Dark green
3	Powder +Concentrated HCl	Dark brown	Greenish brown	Dark greenish brown
4	Powder +Concentrated HNO_3	Light red	Dark green	Green
5	Powder +Concentrated H_2SO_4	Blackish	Blackish green	Blackish green
6	Powder +Acetone	Yellowish green	Light green	Saffron
7	Powder +5%Iodine	Light yellow	Light green	Light yellowish green
8	Powder +5%KOH	Light yellowish green	Green	Light green
9	Powder +$FeCl_3$	Green	Dark green	Dark green
10	Powder +NaOH	Light yellowish green	Dark green	Light red

DISCUSSION

Microscopy details of stem show a continuous ring of xylem and phloem elements with prominent large xylem vessels arranged irregularly and presence of patches of sclereids [Fig.I-D] in the cortical region and also calcium oxalate crystals present in druse form [Fig.I-G]. Epidermal trichome are multi-cellar but they are uniseriate. Petiole microscopy is having one large vascular bundle and two smaller vascular bundles is unique in nature with epidermal trichomes [Fig.II B]. Microscopy of leaf is having two prominent midribs at the base of leaf blade with paracytic stomata [Fig.II-I]. These characters help for identification and authentication of a species.

Powder microscopy is one of the quality control methods used for powder of a crude herbal drug to study the specific microscopic characters. In this study vessels have annular thickening, specific multicellular uniseriate trichome [Fig. III.F]and stone cell [Fig. III.K].

Organoleptic study is the step towards the standardization of a crude drug that gives sensory feeling about its characters [Tab. I]. Powder of drug having specific mild odour and slightly bitter taste. Physico-chemical parameter such as foreign matter is 0.5percent, indicates the way of harvesting, procuring, packaging and storage of raw drug. Lesser the value increases the physical purity of the drugs. Moisture content is one of the major parameters that is responsible for the deterioration of the drug. In our study it is

0.37percent, lesser the value is always appreciable for stability of the drug. Bulk density is very important in drug process and development to fix the solid doses, it is useful in determine the quantity of powder that can fit in a capsule hopper on a tablet press. This powdered drug has bulk density 0.38 gram per cubic centimeter, this value can be used as a standard. Ash value is 8.5 percent, it is an important parameter in standardization process, specific value that indicates the quality of an herbal drug [Tab. II]. Difference in the value can indicate the level of adulteration, contamination or substitution. Herbal drug powder having its own chemical composition, made up of element, molecule or a compound has its own specific gravity. This drug has 1.24 gram per centimeter cube. So, if, there is any difference in the value that indicates the sample does not contain either the same compound or the same proportion of the compounds as the standard drug. pH value measures the acidity or alkalinity of the drug. Study drug has pH value 5.6, this value directly affects the pharmacological activity which include solubility, absorption and stability. Neutral or higher pH levels bacterial contamination could be high. The herbal drug having lesser pH value has more shelf life than the higher pH value. Extractive value of drug is 3 percent, this value plays a major role in evaluating the crude herbal drug. Lesser the extractive values indicate addition of exhausted material or adulteration or incorrect handling during processing. Phytochemical analysis [Tab.-III] showed a presence of secondary metabolites such as alkaloids, sterols and saponins which are majorly responsible for pharmacological actions.

Fluorescence analysis of an herbal drug is an efficient and precise pharmacognostical tool. Herbal drugs show different colour when subjected to the different chemical reagents under UV light of different wave length [Tab.IV]. Here in this study drug was treated with nine chemical reagent including distilled water and also powder as such. Drug with distilled water under short and long wave length it is dark green in colour. Powder with acetone showed a significant colour difference under visible light it is yellowish green, short wave length it is light green and long wave length it emits saffron colour. The fluorescence colour shows specificity for each chemical compound. It helps for identification and standardization of an herbal drugs from its adulterations.

CONCLUSION

To my knowledge, the present study is the first report of pharmacognostical study of this drug. In the present study it was concluded that the anatomical details including powder microscopy, physicochemical parameters such as the water-soluble extractive value, moisture content, bulk density, ash values and organoleptic characters and fluorescence results can be efficiently used for standardization of herbal drugs. The results obtained from the study could be utilized as a reference for setting limits for the reference standards for the quality control and quality assurance of drug *Gymnema tingens.* Further quantification of secondary metabolites and isolations and purification of individual components are recommended before animal and clinical study. Since it is an endangered taxa genetic conservation of this species is highly recommended before extinct.

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CONFLICT OF INTEREST

The author declared no conflict of interest

ABBREVIATION

T.S: Transverse section
V.T.S: Vertical Transverse Section
UV: Ultra violet
%: Percentage
CC: Cubic centimeter
g: Gram
CM^3 : Cubic centimeter
Fig: Figure
Tab: Table

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