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Pharmacognostical studies of *Gymnema sylvestre* Leaves with reference to Estimation of Gymnemic acid by RP-HPLC Method

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Abstract

Gymnema sylvestre commonly known as gurmar belongs to family Asclepiadaceae is used for its distinct property as sugar destroyer and is a reputed herb in the Ayurvedic system of medicine. The phytoconstituents responsible for sweet suppression activity includes triterpene saponins known as gymnemic acids, gymnemasaponins, and a polypeptide, gurmarin. The herb exhibits a broad range of therapeutic effects as an effective natural remedy for diabetes, besides being used for arthritis, diuretic, anemia, osteoporosis, hypercholesterolemia, cardiopathy, asthma, constipation, microbial infections, indigestion, and anti-inflammatory. In the present paper pharmacognostical profile of leaves of *Gymnema sylvestre* were reported. Also, Gymnemic acid which is a major constituent of the plant was estimated using HPLC.

Key-words: *Gymnema sylvestre*, Gymnemic acid, HPLC

Introduction

Medicinal plants are a source of great economic value in the Indian subcontinent and India has one of the richest plant medical traditions in the world. India has a tradition that is of remarkable contemporary relevance for ensuring health security to the millions. Nature has bestowed on India a rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity[1]. India has 2.4% of world's area with 8% of global biodiversity. It is one of the 12 mega-diversity hot-spot regions of the world. There are about 45,000 plant species in India, with concentrated hotspots in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island. It is difficult to estimate the number of medicinal and aromatic plants present worldwide; the fact remains true that India with rich biodiversity ranks first in percent flora, which contains active medicinal ingredient and 20-44% of all the plants found in India are used for medicinal purpose[2].

*Gymnema sylvestre*is an indigenous herb, belonging to the class dicotyledonous of the family Asclepiadaceae. The plant is a good source of a large number of bioactive substances[3]. It has deep roots in history, being one of the major botanicals used in Ayurvedic system of medicine to treat conditions ranging from diabetes, malaria, to snakebites[4]. The herb is cultivated worldwide and also known as Chigengteng or Australian Cow plant, *Waldschlinae* in German, periploca of the woods in English and gurmar in Hindi[5]. The present paper deals with evaluation pharmacognostical profile of the selected plant.

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Material and Methods

Collection and Authentication of plant material

The air-dried leaves of *Gymnema sylvestre* were purchased from the local market (Khari Baoli),Delhi, India and the drug was authenticated by the Pharmacognosist in Institute of Professional Studies College of Pharmacy, Gwalior (M.P.).

Pharmacognostic evaluation[6-9]

Materials

Compound microscope, stage micrometer, camera lucida, glass slide, cover slip, watch glass, Leica DMLS microscope attached with Leitz MPS 32 camera, silica crucible, ash less filter paper (Qualigenswhatman filter paper 615A), Petri dish, UV apparatus, stopper conical flask, magnetic stirrer, alcohol (95%), chloroform water, chloral hydrate solution, phloroglucinol, hydrochloric acid, glycerin, sodium hydroxide, pet ether, acetone, benzene, chloroform, methanol.

Anatomical study

Free hand sections of the fresh parts of the all the plants were boiled with chloral hydrate to remove all the coloring matter and then carefully stained with phloroglucinol and HCl (1:1). The sections were transferred to mountant (glycerin) on a slide and a cover slip was placed.

Powder analysis

The dried and powders all the plants selected were passed through sieve no. 60 and used for powder analysis. Macroscopic characters of the powders were observed. Then the powder was examined microscopically by mounting

Physico-chemical Evaluation

Determination of foreign matter

The plant material was accurately weighed (500g) and spread it out in a thin layer. Sample was inspected with the use of 6x lens and separated the foreign matter manually. Weighed and the percentage of foreign matter with reference to the air dried drug was determined.

Determination of ash values

The ash values, following ignition of medicinal plant materials is determined by four different methods, which measures total ash, acid in soluble ash, water-soluble ash and sulphated ash.

Total ash

The finely ground and dried plant material was accurately weighed (4g) and placed in a previously ignited and tarred crucible (silica). The material was spread in an even layer and ignited by gradually increasing the temperature to 500-600°C until it is white, indicating absence of carbon. It was then cooled in a desiccator and weighed. If carbon- free ash cannot be obtained in this manner, the crucible was cooled and moistened the residue with about 2ml of distilled water or a saturated solution of ammonium nitrate. Dried on a water bath then on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The percentage of ash with reference to the air dried drug was calculated**.**

Acid-insoluble ash

To the crucible containing the total ash, 25 ml of hydrochloric acid (Dilute 260ml of hydrochloric acid with sufficient water to produce 1000ml) was added. It was covered with a watch glass and boiled gently for 5 minute. The watch glass was rinsed with 5ml of hot water and the liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a vacuum desiccator for 30 min. and weighed without delay. The percentage of ash with reference to the air dried drug was calculated.

Water-soluble ash

To the crucible containing the total ash, 25 ml of distilled water was added and boiled for 5 min. The insoluble matter was collected in a sintered glass crucible or on an ash less filter paper. Washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450^0C and the ash obtained was weighed. The weight of this residue was subtracted from the weight of total ash. The percentage of ash with reference to air dried drug was calculated.

Sulphated ash

A silica crucible was heated to redness for 10 minutes, allowed to cool in desiccators and weighed. 1 g of the substance being examined was transferred to the crucible and again the crucible and its contents were weighed accurately. Then the crucible was ignited gently in a Muffle furnace until the substance was thoroughly charred. The

residue was cooled and moistened with 1 ml of concentrated sulphuric acid and heated gently until white fumes were no longer evolved and then ignited at 800 ± 25ºC until all black particles had disappeared. The ignition was conducted in aplaceprotected from air currents. Thecrucible was allowed to cool and heated again after addition of few drops of sulphuric acid. It was ignited as before and allowed to cool and weighed. Operation was repeated until two successive weighing that did not differ by more than 0.5 mg.

Determination of loss on drying

The finely ground and dried plant material was accurately weighed (3g) and placed in a dried and tarred flat weighing bottle. The sample was dried in an oven at 100-105°C. The operation was repeated until two consecutive weighing that did not differ by more than 5mg. The loss of weight in mg per g of air-dried material was calculated.

Determination of swelling index

Coarsely powdered and air dried material was accurately weighed (1g). The plant material was transferred to a 25 ml glass-stoppered measuring cylinder and then added 25 ml of water to the measuring cylinder. Mixture was shaken thoroughly for every 10 minutes till 1 hour. Allowedit to stand for 3 hours at room temperature and volume was measured in ml occupied by the plant material, including any sticky mucilage. Mean value of the individual determinations related to 1 g of plant material was calculated.

Determination of foaming index

Coarsely powdered and air dried material was accurately weighed (1.0g). The plant material was transferred to a 500 ml conical flask which already had 100 ml of boiled water. Moderate boiling was maintained for 30 minutes. Cooled and filtered it into a 100 ml volumetric flask. Sufficient water was added to dilute to volume. Decoction was poured into 10 stoppered test-tubes in successive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml. The volume of the liquid was adjusted in each tube with water to 10ml. Tubes were stoppered and shaken them in a lengthwise motion for 15 seconds. Tubes were allowed to stand for 15 minutes. Then the height of foam was measured.

Phytochemical Evaluation

Extraction of plant material

Preparation of extracts: Thirty one hundred grams of shadow dried whole plant powder was extracted with 80% methanol by using soxhlet apparatus. The methanol extract was partitioned between petroleum ether and water (6:1) using separating funnel. This mixture was thoroughly mixed for 15min and after 6 h the petroleum ether fraction (PE) was collected. The aqueous layer was further fractionated with chloroform (CF), ethyl acetate (EA) and then with ethanol by with a slight modification. The solvent was removed by concentrated *in vacuo* in a rotary evaporator and dried in vaccum desiccators**.** The dried extracts were stored in refrigerator until further studies. The yield was calculated and the extracts were subjected to further analysis and all assays were done in triplicates.

Qualitative phytochemical screening

Preliminary phytochemical screening of the extracts of Gymnema sylvestre was carried out for various plantconstituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins and tannins by using standard procedures.

Results and Discussion

The epicarpic cells are rectangular in shape and their outer and radial walls are highly cuticularized. In surface view the epicarpic cells appear polygonal in outline with thick walls. Anomoytic type of stomata are found to be present, but rare. Collateral fibro vascular bundles are scattered throughout the inner mesocarp. Pitted and helical tracheids with tapering ends are seen. At places in the phloem, large cavities filled with crystal mass are present. The results of powder characters are Colour: Buff green; Odour: Indistinct; Taste: Bitter. Microscopical characters of the powder include paracytic stomata, uniseriate multicellular covering trichomes, (usually collapsed trichomes), reticulated wood elements and lingo cellulose fibers.

The plant material of the both herbs were extracted and fractionated, the highest yield was obtained from the ethyl acetate fraction and water fraction. Least yield was obtained by Chloroform fraction. Percentage yield (w/w) of ethyl acetate

fraction of *Gymnemasylvestre,* were found to be 6.67 whereas Chloroform fractions were 0.081 respectively.

Results of preliminary phytochemical screening are given in Table 3. It was found that *Gymnema sylvestre* contains mainly sterols and lactones in pet ether fraction; flavonoids, glycosides, carbohydrates and lactones in butanol fraction, ethyl acetate, water fraction and water extract.

Estimation of Gymnemic acid in *Gymnema sylvestre* methanol extract was carried out by RP-HPLC method using the optimized chromatographic conditions. The calibration curve of standard Gymnemic acid is shown in Fig. 5. A typical chromatogram of Gymnemic acid is shown in Fig 6. Detection was done at 255 nm. The retention time of Gymnemic acid standard was found to be 2.237 min. The retention time of Gymnemic acid in the methanol extract of *Gymnema sylvestre* was found to be 2.268 min. The peak area ratios of standard and sample solutions were calculated. The assay procedure was repeated for six times and the mean peak area and mean peak area ratios of standards were calculated. The chromatogram of the methanol extract of *Gymnema sylvestre*is shown in Fig. 7. The percentage of Gymnemic acid in the methanol extract of *Gymnema sylvestre*was found to be 0.0424% (w/w) as shown in table 4.

The linearity of the method was determined at five concentration levels ranging from 0.1 to 1 µg/ml. The calibration curve was constructed by plotting peak area against concentration of drugs. The slope and intercept value for calibration curve were Y = 3.77e+004X-1.43e+003 and R^2 = 0.996. The results show an excellent correlation between peak area and concentration of Gymnemic acid within the concentration range indicated above.

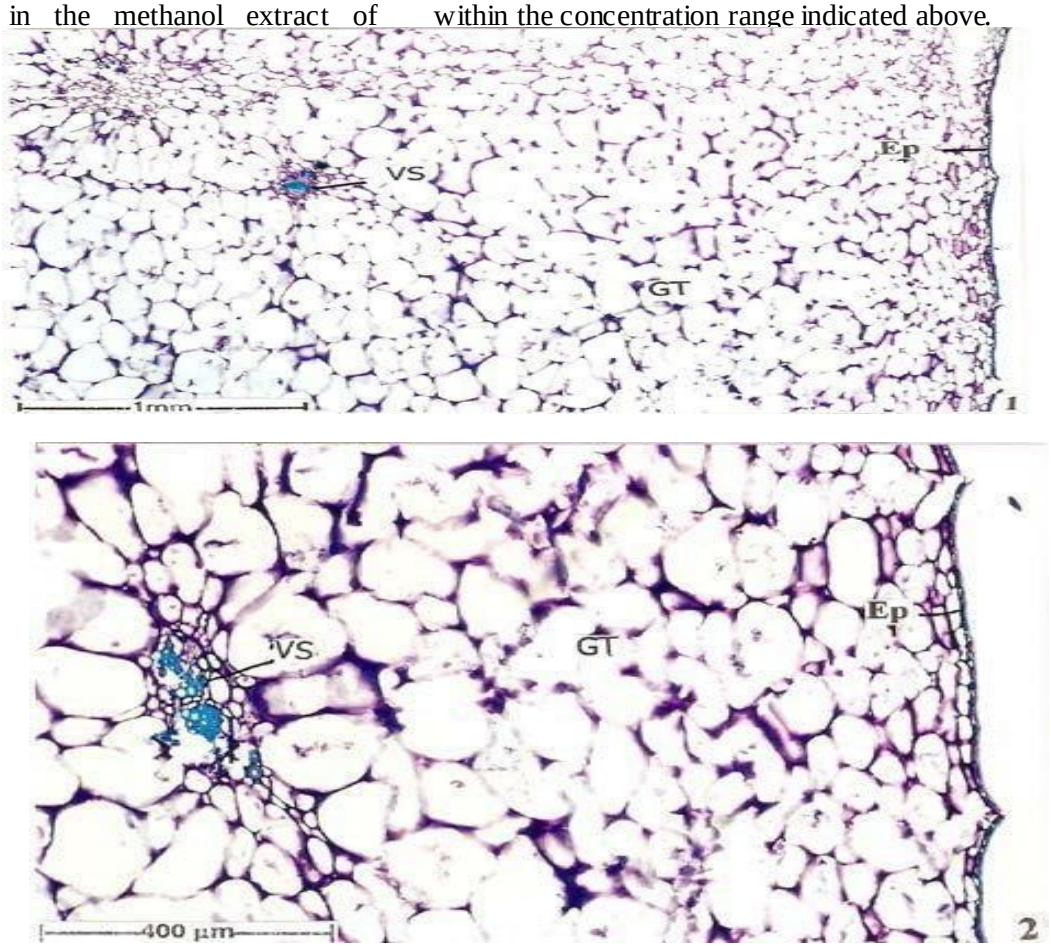

Fig. 1: Anatomy of the Pericarp

(1) Enlarged pericarp tissues(2) Ep: Epidermis; GT:Ground Tissue; VS: Vascular Strand

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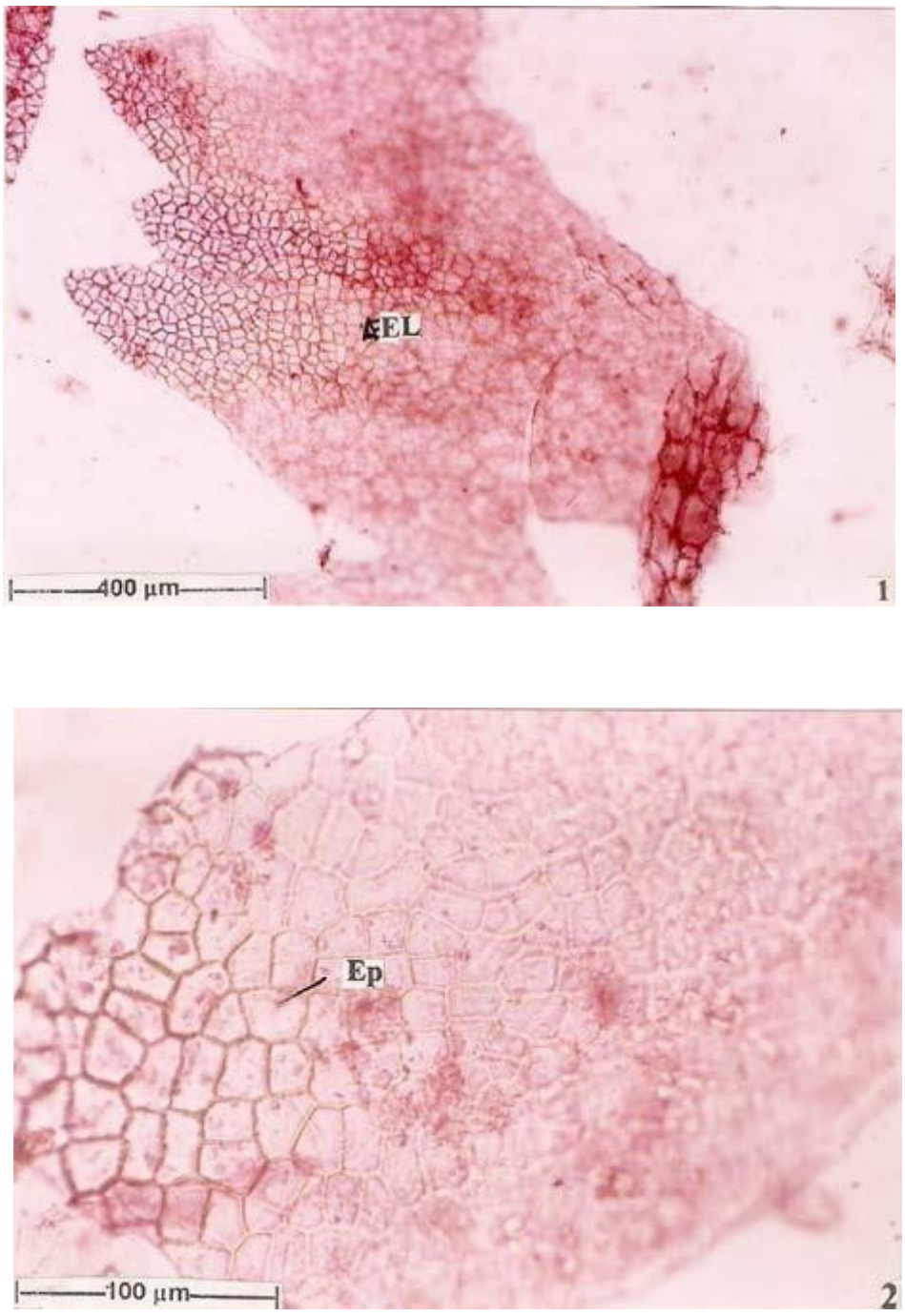

Fig. 2: Fragment of the Epicarp of *Gymnema sylvestre*
Cells under low magnification (2.1) and enlarged cells showing cell wall structure (2.2) Ep: Epidermal cells

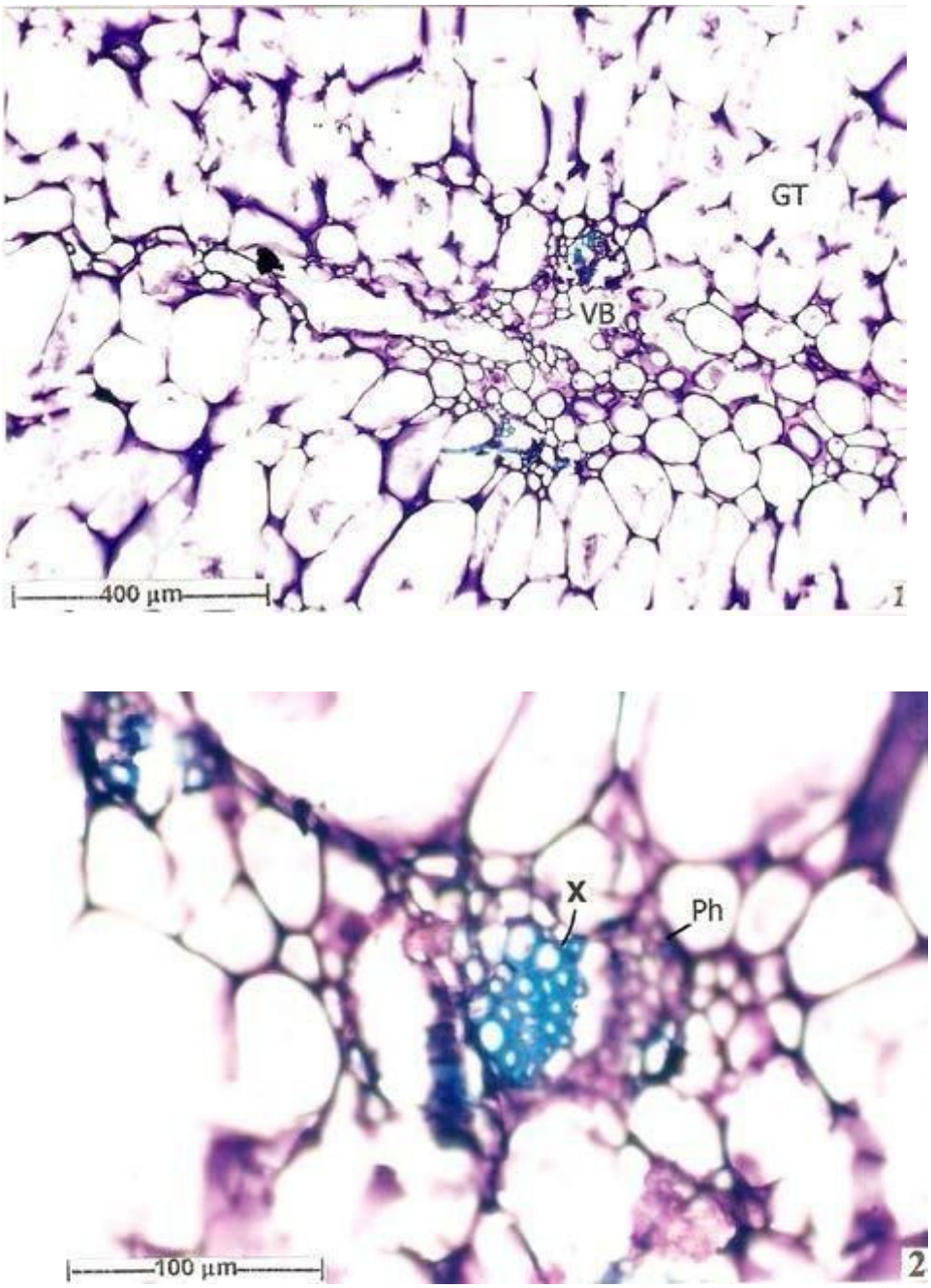

Fig. 3: Structure of the Vascular Strands in the mesocarp of *Gymnema sylvestre* Vascular strand and the ground tissue (3.1) and enlarged vascular strand showing xylem and phloem (3.2)
GT: Ground Tissue; Ph: Phloem; VB: Vascular Bundle; X:Xylem

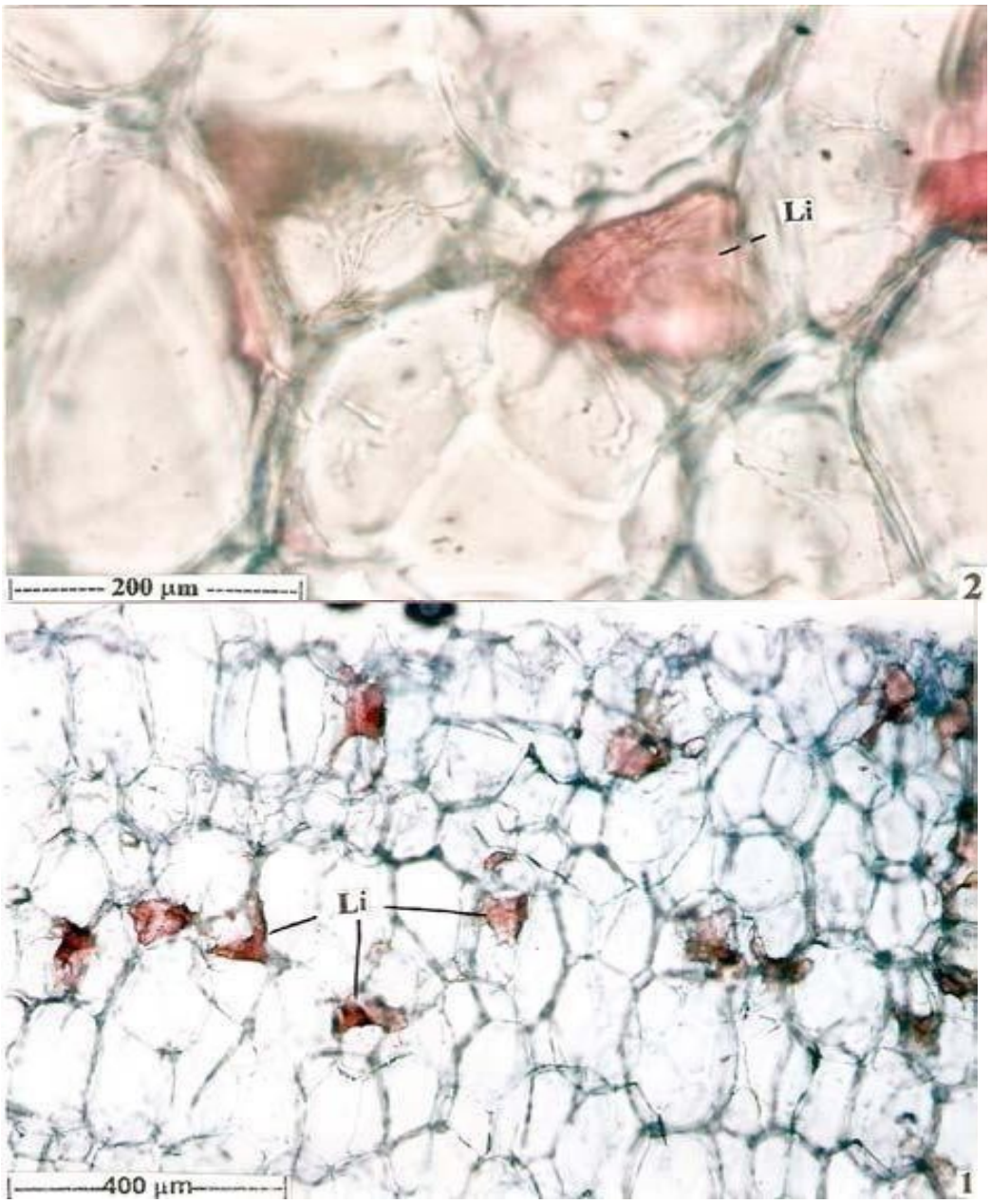

Fig. 4: Powder Microscopic Structure of the Pericarp of *Gymnemasylvestre* Ground parenchyma cells stained with Sudan-III to show the lipid bodies (4.1) and lipid bodies (4.2) Li: Lipid

Table 1: Physico-chemical evaluation of *Gymnema sylvestre*

Parameters	Results
Foreign Matter	2±0.76
Total Ash	02±0.58
Water soluble Ash	1.1±2 .0
Acidin soluble Ash	0.55±0.22
Sulfated Ash	0.5±012
Moisture Content	2.42±1.42
Swelling Index	1.5ml/gm
Foaming Index	15

Table 2*:* Yield of extracts and fractions of *Gymnema sylvestre*

Plant	Drug taken in gms	Extract/Fractions	Total yield in gm	% Yield in gm w/w
Gymnema sylvestre	625	Pet. Ether	1.17	0.18
		Chloroform	0.51	0.081
		Ethyl acetate	41.24	6.76
		Ethanol	40.27	6.6
		Water fractions	15.0	2.4
	100	Aq. Alcoholic extract	8.65	17.3

Table 3: Qualitative Phytochemical screening of *Gymnema sylvestre*

S. No.	Chemical Tests	Aq. Alc.	Pet. Ether	Ethyl acetate	Ethanol	Water fraction	Chloroform
1.	**Tests for Sterols**						
	A) Salkowskitest	+	+	-	-	-	-
	B) Liebermann-Burc	+	+	-	-	-	-
2.	**Tests for Triterpenes**						
	A) Salkowski test						
	B) Liebermann- Burchard test	-	-	-	-	-	-
		-	-	-	-	-	-
3.	**Tests for Saponins**						
	A) Foam test	+	-	-	+	-	-
	B) Haemolysis test	-	-	-	-	-	-
4.	**Tests for Alkaloids**						
	A) Wagner's test	-	-	-	-	-	-
	B) Mayer's test	-	-	-	-	-	-
	C) Dragendorff's test	-	-	-	-	-	-
	D) Hager's test	-	-	-	-	-	-

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5.	**Tests for Carbohydrates** A) Fehling's test B) Molisch's test	 + +	 - -	 + +	 + +	 + +	 - +
6.	**Tests for Lactones /Cardiac Glycosides** A. Legal's test B. Baljet's test	 + +	 + +	 + +	 + +	 + +	 + +

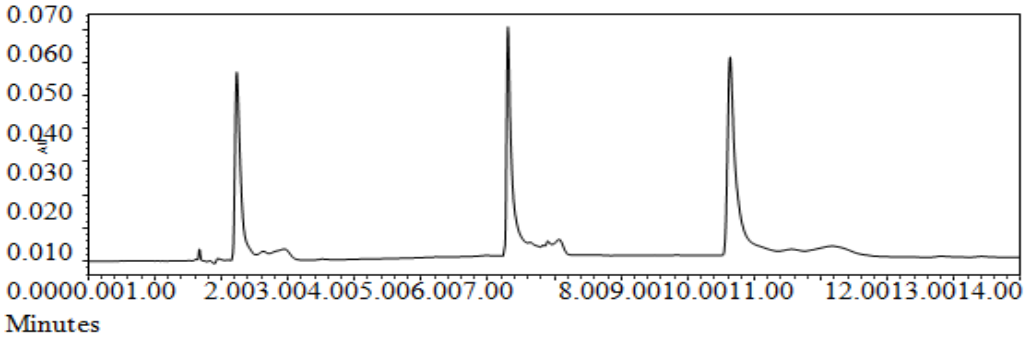

Fig 5: Overlay chromatogram of standard (Gymnemic acid)

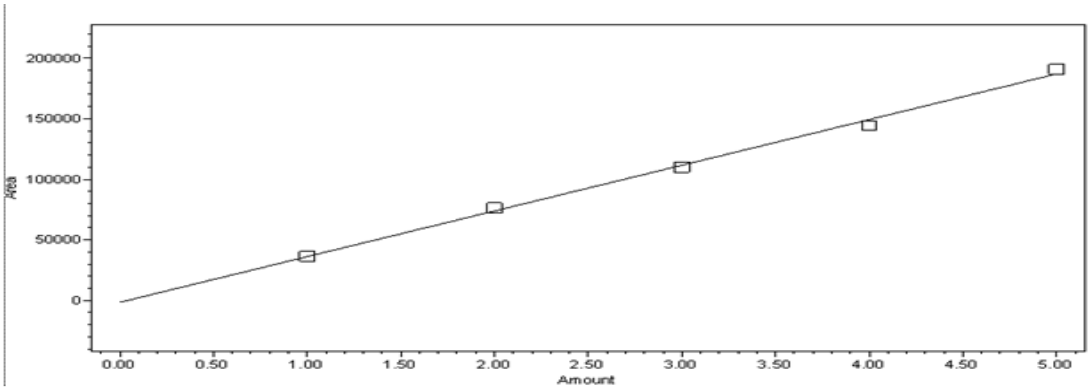

Fig 6: Calibration curve of Gymnemic acid by RP-HPLC

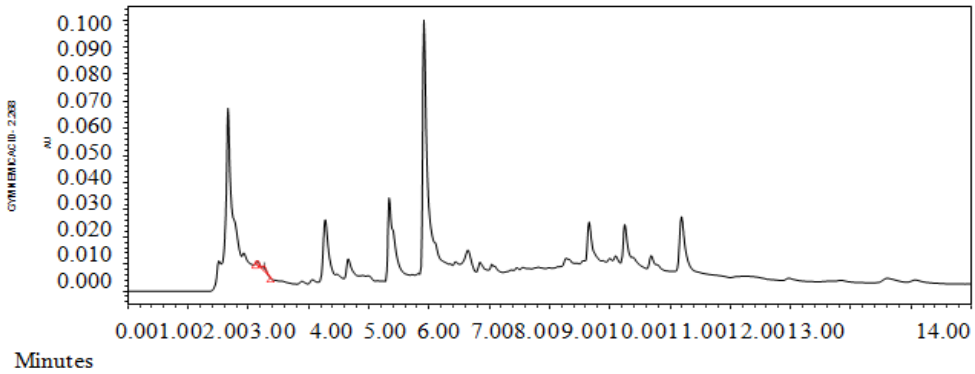

Fig. 7: Chromatogram of Gymnemic acid in the ethanol extract of *Gymnema sylvestre*

Table 4: HPLC analysis of Gymnemic acid in *Gymnema sylvestre*

Plant extract	Constituents	Amount found (% w/w)
Ethanol Fraction	Gymnemic acid	0.0424

Conclusion

The plant material was investigated anatomically microscopic and other physical parameters i.e. total ash, acid insoluble ash, water-soluble ash and sulphated ash, loss on drying, swelling and foaming index etc. Plant material was extracted using water, petroleum ether, butanol, ethyle acetate and aqueous alcoholic and chloroform solvents and the extracts were evaluated for qualitative estimation of various photochemical present in the plant, to determine the presence of alkaloids, carbohydrates, glycosides, saponins, proteins, steroids saponins, etc. Estimation of Gymnemic acid in *Gymnema sylvestre* methanol extract was carried out by RP-HPLC method using the optimized chromatographic conditions.

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