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A COMPARATIVE STUDY OF PHARMACOGNOSTIC AND PHYTOCHEMICAL PROPERTIES OF FIELD COLLECTED AND MARKET SAMPLES OF *PATALA* (STEREOSPERMUM SUAVEOLENS, ROXB. DC)

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ABSTRACT

Introduction: Patala is one of the most important drug of Dashmoola and widely use in different diseases. It is important content of many ayurvedic preparations. Ayurvedic formulations are considered safe under the impression that they are derived from herbal origin. To study the adulteration of Patala in market, difference in pharmacognostic and phytochechical properties of field collected and market samples of Patala (Stereospermum suaveolens, Roxb. DC) with special reference to Adulteration; which alters the effectiveness of drug, this topic taken for study. **Aim:** To compare pharmacognostic and phytochemical properties of Field collected and Market samples of Patala (Stereospermum Suaveolens, Roxb. DC). **Objectives:** 1) To do Physico- chemical screening of all the samples. 2) To do qualitative evaluation by HPTLC (High Performance Thin Layer Chromatography) of all the samples. **Materials and Methods:** In present study four samples of *Patala* were taken for analysis; FS i.e. field collected sample and 3 market samples from 3 different zones of Maharashtra (M1, M2 & M3). These four samples were analised with Pharmacognostic, Phytochemical, Physicochemical properties and HPTLC. **Conclusion:** The above study revealed that in Ayurvedic formulations we should use Field collected sample.

KEYWORDS: Patala, Pharmacognostic study, Phytochemical study, Physicochemical study, HPTLC.

INTRODUCTION

Ayurveda is the repository of safe and therapeutically officious remedies and plant based preparation play a major role in Ayurvedic healing process. Drug is considered as potent as *amrut* and compared to *visha*, *shashra* and *agni* acting like a weapon to fight the disease. It is included in *Dhashvidha Pariksha* as a '*Karan'* by Acharya Charaka, which acts according to its properties.[1]

Treatment is effective only when *dravya* is used with proper knowledge about its properties and in pure form.[2] Ayurvedic recipes are formulated by following proper norms of the *dravya* like collection, part to be used, storage, preservation and medicinal preparation of plants explained by *Charaka* and after centuries of trial and experience, thus these are well known to be free from toxicity.

Dashmoola is a famous Ayurvedic combination of roots of ten medicinal herbs, which used together. It contains *Brihat Panchmool* i.e. roots of five big trees and *Laghu Panchmool* i.e. roots of five small herbs.[3] This is the most common polyherbal combination used in the production of many Ayurvedic formulations that are indicated in the treatment variety of ailments especially in *Vata Roga*. *Patala* is one of the most important drug of *Dashmoola* and widely use in Ayurvedic preparations. Any kind of negligence regarding its preparation knowingly or unknowingly affects its efficacy.

Frequent and abundant use of raw material needed for these pharmaceutical purposes, give rise to the scarcity of drug, which contributes the possibility of Adulteration. Urbanization, pollution and immense cutting down of forest causes insufficiency of the raw materials needed for Ayurvedic formulations. To fulfill this paucity, substitutes have been added to market without following proper formulating methods and with adulterations, thereby lowering their efficacy against the indicated disease.

It is necessary to examine the plants as a potential source in development of many new formulae. Sometimes adulterated drugs cause harmful effect on the body. Many times some of the drugs which are labelled in the formulation, qualitatively and quantitatively may not go with the product at all, on the whole the patient is at losing end. Nowadays the proper

identification of these herbs is one of the major challenges, Many times due to improper knowledge a wrong drug or a drug of low potency is mistakenly chosen for production which leads to mismanagement of the disease, so there is need of evolution of certain methodology for the identification and standardization of plant products.

In ancient times physicians were directly involved in drug collection so that there was only minimal chance of Adulteration. In present days *Ayurvedic* physicians are mainly dependent on market, both for raw drugs as well as for formulations. Indiscriminate use of adulterants in place of genuine drug in herbal markets is becoming commercial and most of the physicians are not aware of genuinity of the raw drug which has direct impact on the quality of medicinal preparations and efficacy also. So standardization of every raw drug is a must to get the desired safety and efficacy of the formulations.

In order to study the adulteration of *Patala* in market, difference in pharmacognostic and phytochechical properties of field collected and market samples of *Patala* (*Stereospermum suaveolens*, Roxb. DC) with special reference to Adulteration; which alters the effectiveness of drug, this study is carried out.

MATERIAL AND METHODS

- **MATERIALS**

1. Plant identification

Patala root *(Stereospermum suaveolens, Roxb.DC.)* was identified on the basis of its Morphology and family characters of the plant.

2. Collection

- **Fieid collected sample**

Patala field collected sample (FS) was collected from forest area 50 km from our city in *Grishma rutu* according to *Dravya sanghraha kala* described by Charaka[4] and according to the Guidelines on Good Field Collection Practices for Indian Medicinal Plants. Complete intact roots were collected without doing any harm to the roots. Then this sample was cleaned with water and dried under shade to avoid direct sunlight.

- **Market samples**

Three Market samples (M_1, M_2 and M_3) were collected from three well known registered Ayurvedic raw drug venders of the three different zones in Maharashtra.

Date of collection

M_1-26/04/2019

M_2-20/05/2019

M_3- 01/06/2019

Figure 1: FS, **Figure 2:M_1,** **Figure 3:M_2,** **Figure 4: M_3.**

3. Authentication

Field collected sample of *Patala* (*Stereospermum suaveolens*, Roxb. DC) will be authenticated from taxonomist, Department of Botany of well known research institute. The authenticity of the samples was confirmed by comparing their characters with standard herbarium sample available at the Botany department with the help of Subject experts and the specimen sample was vouchered as 10105.

4. Preparation of Powder

Dried roots of FS and Market samples were powdered in grinder and sieved with the help of 20 mm sieve for further studies.

Figure 5: Powder of FS, Figure 6: Powder of M_1, Figure 7: Powder of M_2, Figure 8: Powder of M_3.

5. Storage

Polythene bags were used to store powdered drug to protect from moisture and any other contamination.

- **METHODOLOGY**

Ayurvedic system of medicine uses drug as a whole where as the modern system of medicine has given much importance to the fractions of the drugs.

1. Pharmacognostic Study[5]

- It may be defined as a branch of bioscience, which treats in detail medicinal or related products of crude or primary type obtained from plants, animals or mineral origins.
- It includes both macroscopic and microscopic study of the samples.

a) Macroscopic study/ Organoleptic Study (*Panchabhautik Parikshana*)

Morphological or organoleptic evaluation means conclusion drawn from studies resulted due to impression on organ of senses. It helps to evaluation of drugs by colour, taste, size, shape and special features like touch, texture etc.

b) Microscopic study & Powder microscopy

This method allows more detailed examination of a drug and it can be used to identify the organized drugs by their known histological characters. It is mostly used for qualitative evaluation of organized crude drugs in entire and powdered forms. Microscopic study with powder microscopy was carried out in the well-known Botany Institute.

2. Physico-chemical Analysis[6]

- It is an important component of qualitative evaluation, useful in establishing quality profile of a crude drug and constitute.

a) Foreign matter

b) Loss on drying

c) Ash value

d) Acid insoluble ash

e) Water soluble extraction value

f) Alcohol soluble extraction value

g) Determination of pH.

3. Prelimnary Phyto-chemical Analysis[7]

The extracts obtained in physic chemical analysis are subjected to qualitative tests for the identification of various plant constituents like glycosides, alkaloids, tannins, etc.

4. High Performance Thin Layer Chromatography[8]

- High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials.
- With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses.

- **OBSERVATIONS AND RESULTS**
- **Comparative organoleptic characters of all the samples (FS, M_1, M_2 & M_3) of *Patala* root (*Stereospermum suaveolens*, Roxb.DC.)**

Sr. No.	Characteristics	FS	M_1	M_2	M_3
1.	**Shabda** (Fracture)	Hard with sound, Fibrous	Hard with sound, Fibrous	Hard with sound, Fibrous	Hard with sound, Fibrous
2.	**Sparsha** (Touch)	Ruksha- Kathin (Rough due to scaling, inner side is soft)	Ruksha-Kathin	Ruksha-Kathin	Ruksha-Kathin
3.	**Roopa** Appearance Colour Shape Size	Root Outer light brown, inner pale yellow Cylindrical branched cut pieces 20-50 cm long, 2-4 cm thick	Root Pale yellow Cylindrical pieces 1-3 cm long, 0.5cm thick	Root Light Brown Half Cylindrical 2-5cm long, 1-2 cm thick	Root Brown Half Cuboid pieces 3-5 cm long, 2-3 cm thick
4.	**Rasa** (Taste)	Tikta, Kashaya	Tikta, Kashaya	Tikta, Kashaya	Tikta Kashaya
5.	**Gandha** (Odor)	Typical strong odor	Characteristic	Characteristic	Characteristic

Table No. 1: Comperative Organoleptic Characters.

- **Comparative organoleptic characters of powder of all samples (FS, M_1, M_2 & M_3) of *Patala* root (*Stereospermum suaveolens*, Roxb.DC.).**

Sr. No.	Features	FS	M_1	M_2	M_3
1.	Colour	Yellowish brown	Light brown	Light brown	Brown
2.	Texture	Granular	Granular	Granular	Granular
3.	Odor	Typical	Characteristic	Characteristic	Characteristic

		strong			
4.	Taste	Bitter	Bitter	Bitter	Bitter

- **Comparative Microscopic features of all the four samples (FS, M_1, M_2 & M_3) of *Patala* root (*Stereospermum suaveolens,* Roxb.DC).**

Table No. 2: Comperative Microscopic characters.

Table No. 21: Comperative chart of Organoleptic Characters of powder of all samples.

Sr. No.	Characteristics	FS	M_1	M_2	M_3
1	Bark thick and lencelate	+	-	+	-
2	Cortex	25-30 layers	-	15-20 layers	-
3	Secondary cortex	5-6 layers of parenchyma cells Polyhedral shaped, Stone cells +	2-3 layers of parenchyma cells	4-5 layers of parenchyama cells	1-2 layers of parenchyma cells
4	Phloem	Wide secondary phloem	Much reduced	Fairly broad	Broad
5	Xylem	Wide, Starch & crystals +	Starch grain +	Calcium oxalate crystals +	Porous, Starch grains +
6	Vessels	Simple	Simple	Simple	Simple
7	Medullary rays	+ Multiseriate	+	+ Multiseriate	+
8	Pith	+	-	+	-

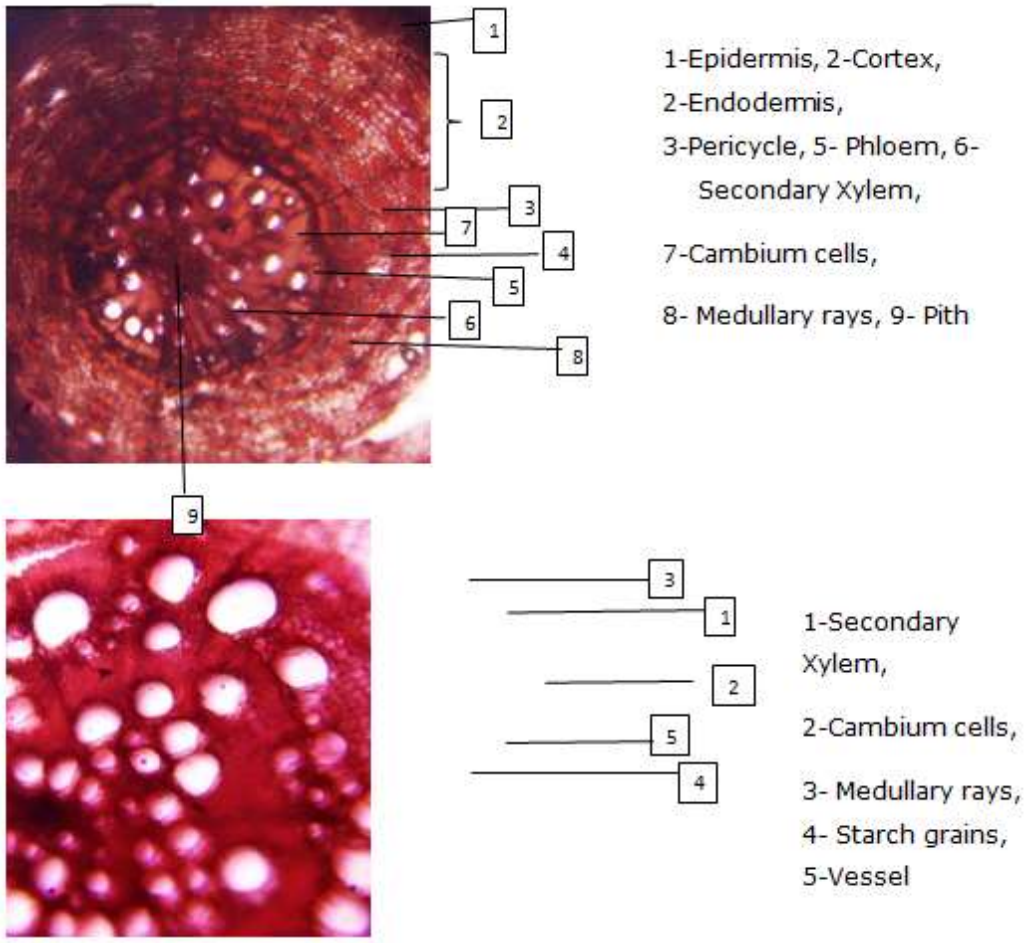


Figure 9: T. S. of root of FS.

Microscopic characteristics of M_1

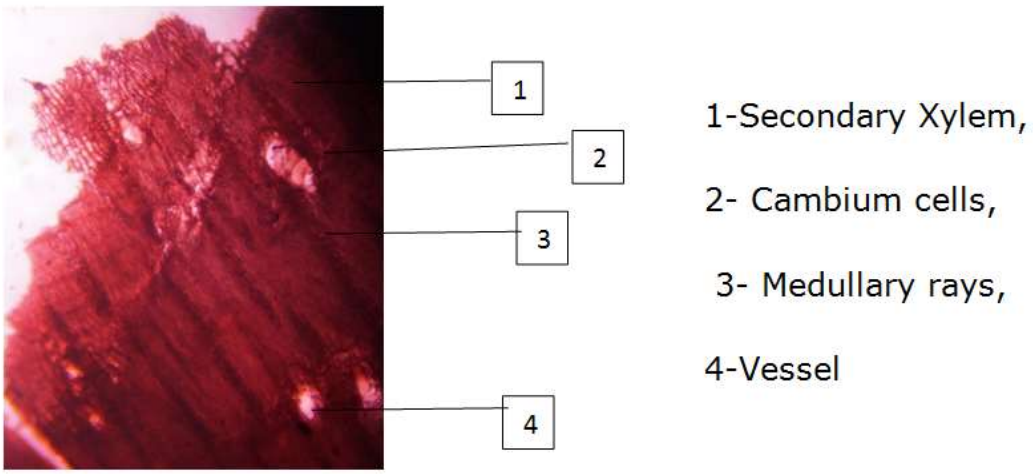


Figure 10: T. S. of root of M_1.

Microscopic characteristics of M_2

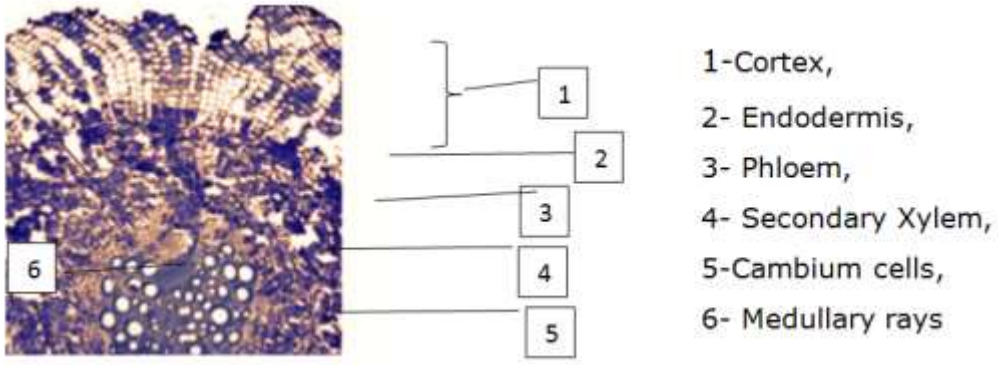


Figure 11: T.S. of root of M_2.

Microscopic characteristics of M_3

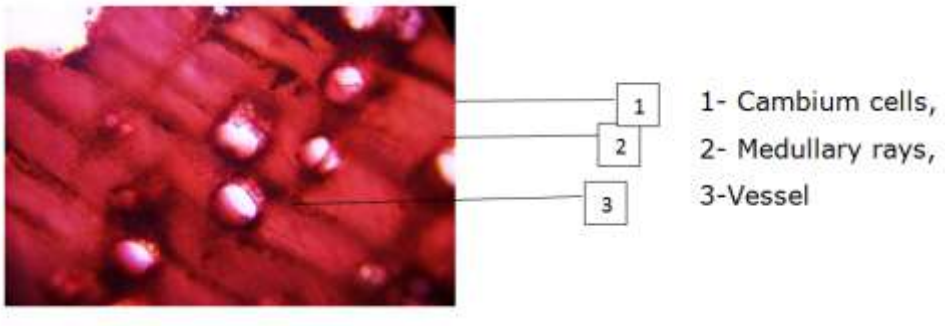


Figure 12: T. S. of root of M_3.

- **Comperative Powder Microscopic Characters of all four samples (FS, M_1, M_2 & M_3) of *Patala* root (*Stereospermum suaveolens,* Roxb.DC).**

Table No. 3: Comperative Powder Microscopic characters of all samples.

Characters	FS	M_1	M_2	M_3
Fibres	+	+	+	+
Calcium oxalate crystals	+	-	+	-
Crowded pits	+	-	+	-
Vessel elements	+	-	+	+
Phloem	+	-	+	+
Starch grains	+	+	+	+

Fibres & vessel elements Phloem Starch grains.

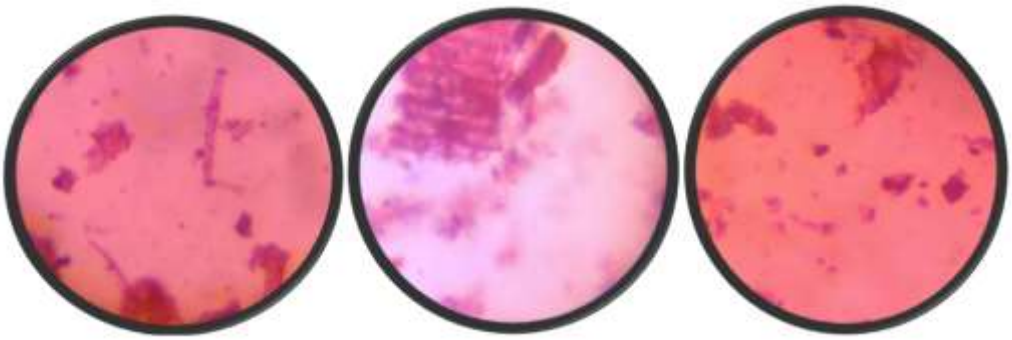


Figure 13: Power microscopic images of FS.

Figure 14: Power microscopic images of M_1.

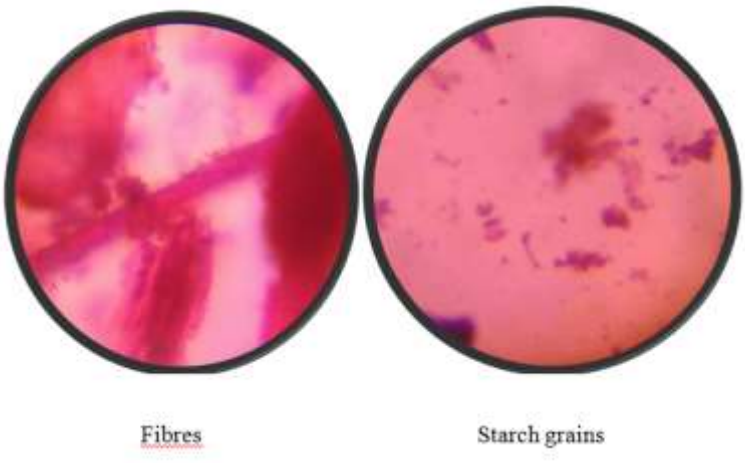
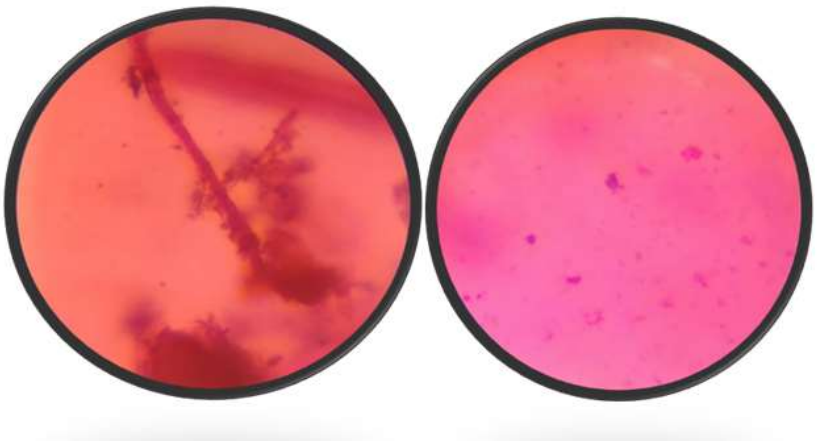


Figure 15: Power microscopic images of M_2.



Fibres **Starch grains**

Figure 16: Power microscopic images of M_3.

Table No. 4: Comperative Physico-chemical Values of all samples.

Parameter	As per API guideline	FS	M_1	M_2	M_3
Foreign Matter	Not more than 2%	0.90%	2.10%	1.90%	1.8%
Loss on Drying		6.00%	8.50%	7.50%	9.50%
Total ash	Not more than 8%	4%	5%	5%	5%
Acid insoluble ash	Not more than 6%	0.40%	1.65%	0.78%	1.05%
Water soluble Extractive	Not less than 20%	22.8%	20.4%	21.4%	19.4%
Alcohol soluble Extractive	Not less than 10%	11.8%	12.8%	12.4%	13%
pH Value		5.12	4.98	5.01	4.97

Table No. 5: Comperative Phyto-chemical values of all samples.

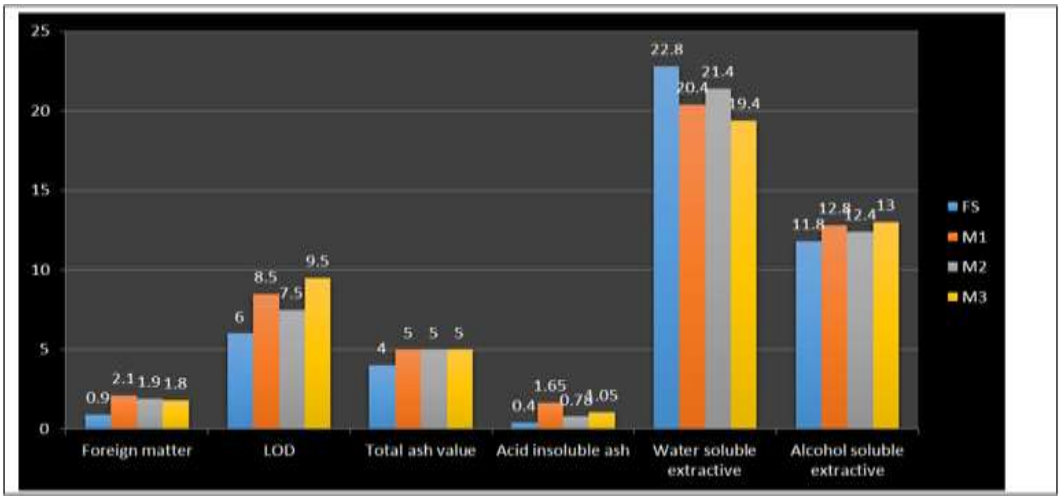
Sr. No.	Test		Result							
			Water soluble extractive				Alcohol soluble extractive			
			FS	M_1	M_2	M_3	FS	M_1	M_2	M_3
1.	Carbohydrates	Molish's test	-	-	-	-	-	-	-	-
2.	Glycosides		+	-	+	-	+	-	+	+
3.	Steroids	Liberman Burchard's test	+	+	+	+	+	+	+	+
		Salkowaski test	+	+	+	+	+	+	+	+
4.	Flavonoids		-	-	-	-	-	-	-	-
5.	Tannins		-	-	-	-	-	-	-	-
6.	Alkaloids	Mayer's test	+	+	+	+	+	+	+	+
		Dragendroff's test	+	+	+	+	+	+	+	+
7.	Proteins	Biuret test	-	-	-	-	-	-	-	-
		Xanthoproteic test	-	-	-	-	-	-	-	-
8.	Amino acids		-	-	-	-	-	-	-	-
9.	Saponins		-	-	-	-	-	-	-	-

Peak table of HPTLC

Table No. 6: Peak table of HPTLC.

Wave length	Track 2- FS		Track 3- M_1		Track 4- M_2		Track 5- M_3	
	No. of spots	Max.Rf Value	No.of spots	Max.Rf value	No.of spots	Max.Rf Value	No.of spots	Max. Rf Value
254 Nm	13	-0.03 0.02 0.09 0.16 0.23 0.30 0.38 0.50 0.62 0.73 0.81 0.86 0.97	12	-0.03 0.02 0.08 0.16 0.23 0.39 0.46 0.51 0.63 0.73 0.88 0.96	12	-0.03 0.02 0.08 0.16 0.24 0.40 0.46 0.51 0.59 0.62 0.73 0.95	13	-0.03 0.02 0.08 0.16 0.24 0.35 0.40 0.46 0.51 0.59 0.63 0.73 0.95
366 Nm	13	-0.03 0.02 0.11 0.28 0.38 0.45 0.49 0.57 0.64 0.73 0.81 0.92 1.04	12	-0.03 0.02 0.12 0.39 0.46 0.50 0.58 0.64 0.68 0.73 0.81 0.93	14	-0.03 0.02 0.11 0.39 0.46 0.50 0.58 0.65 0.67 0.72 0.81 0.89 0.91 0.99	12	-0.03 0.02 0.11 0.39 0.43 0.46 0.50 0.58 0.68 0.72 0.80 0.92

GRAPHS



Graph No. 1: Comparative Physico-chemical values of four samples of Patala root.

HPTLC IMAGES

- **Under 254nm After Development**

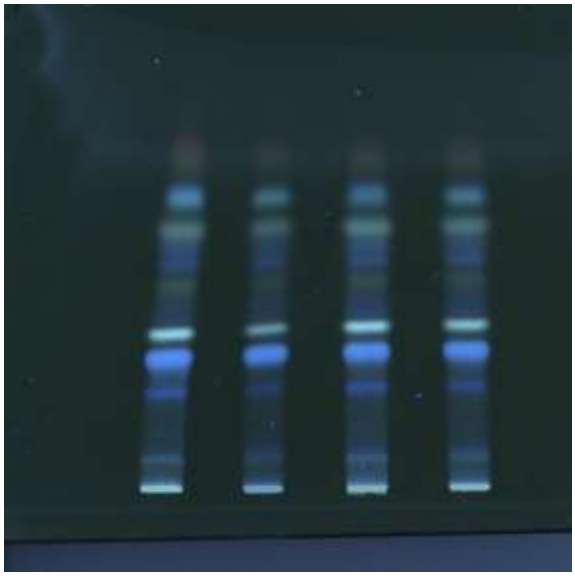


Figure 17: HPTLC image under 254nm after Development.

- **Under 366 After Development**

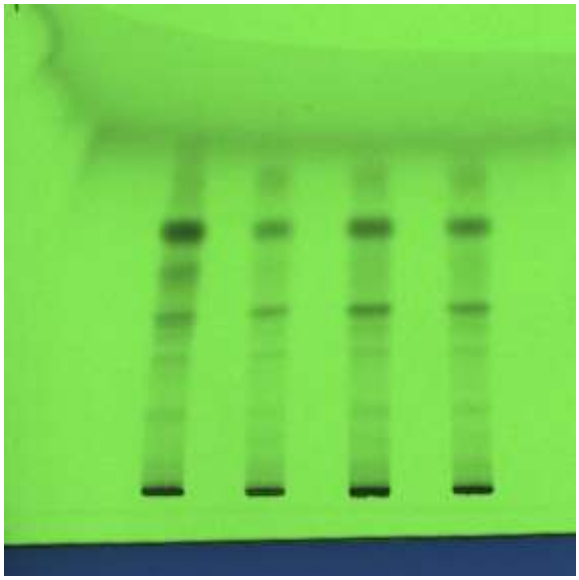
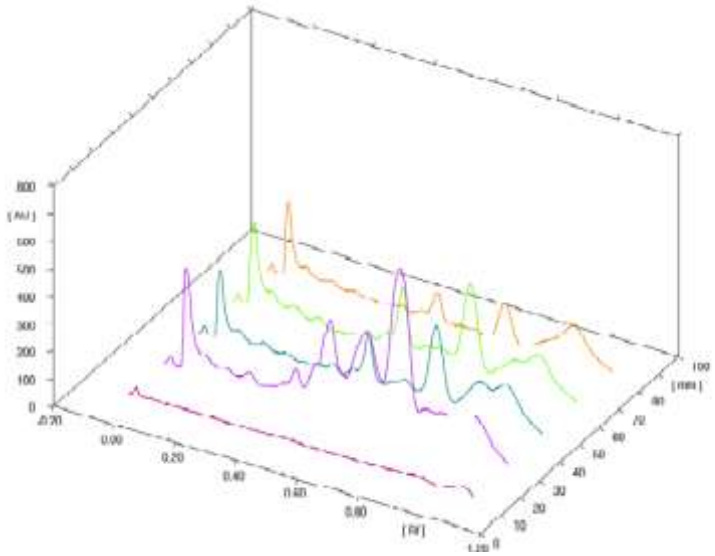


Figure 18: HPTLC image under 254nm after Development.

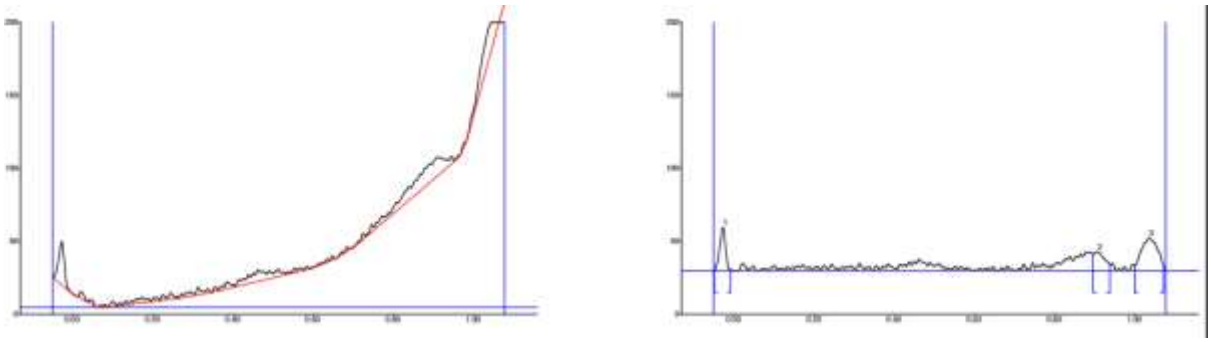
Densiographs

- **All tracks at wavelength 254**



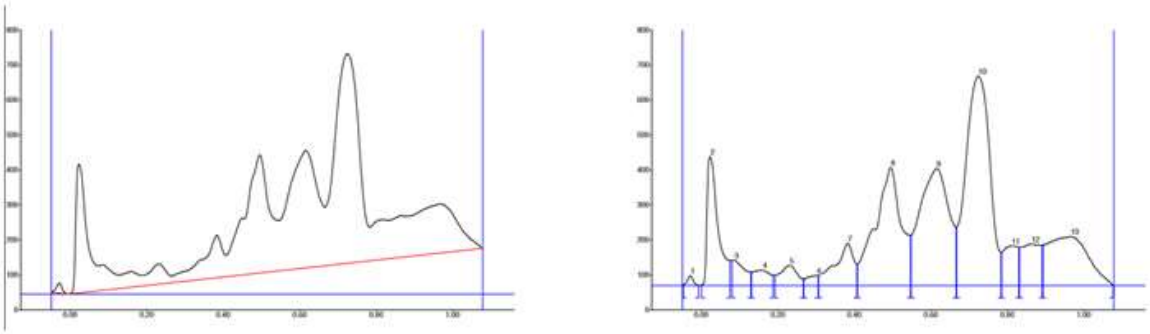
Graph 2: All tracks at wavelength 254 nm.

- **Track 1- Blank**



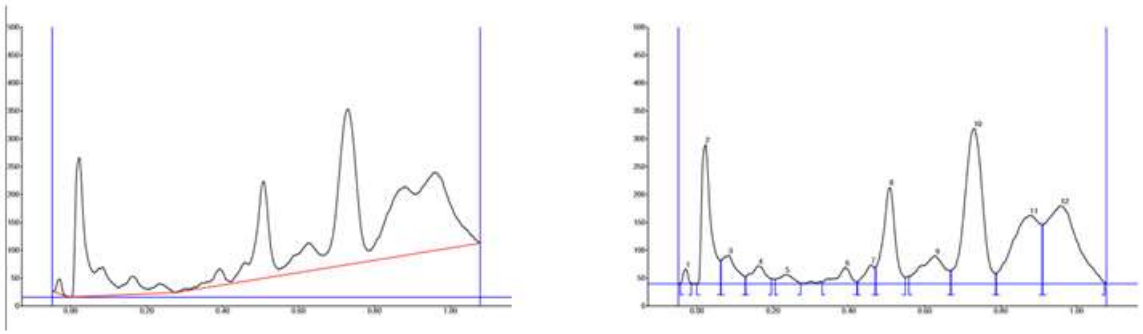
Graph 3: Track 1- Blank at wavelength 254 nm.

- **Track 2- FS**



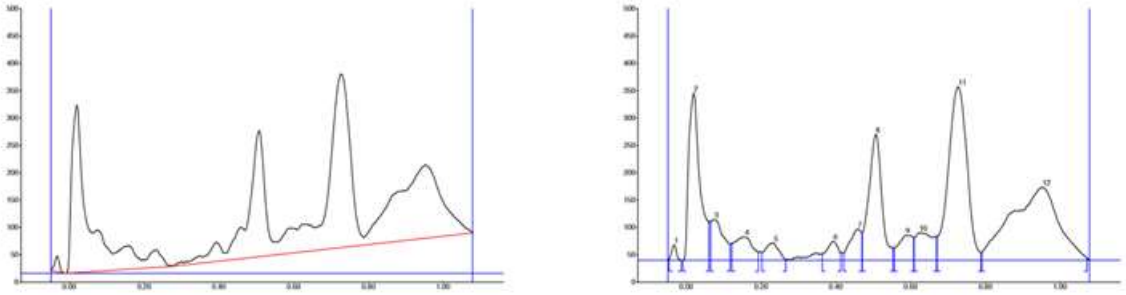
Graph 4: Track 2-FS at wavelength 254 nm.

- **Track 3- M_1**



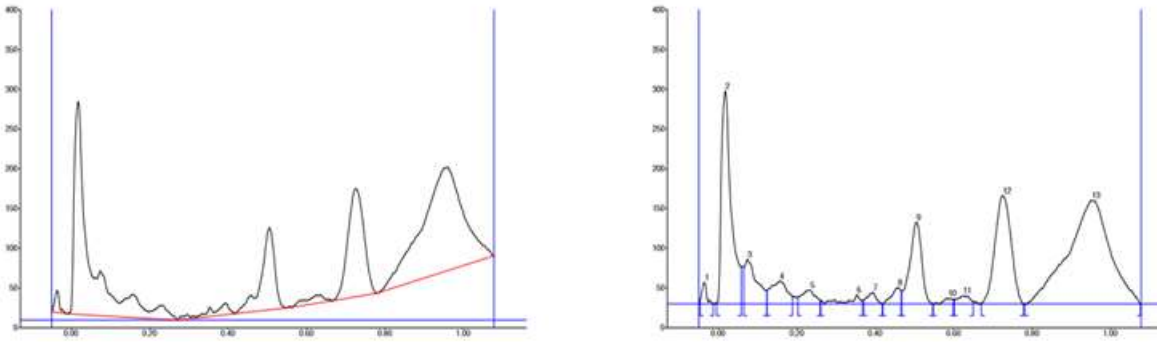
Graph 5: Track 2-M_1 at wavelength 254 nm.

- **Track 4- M_2**



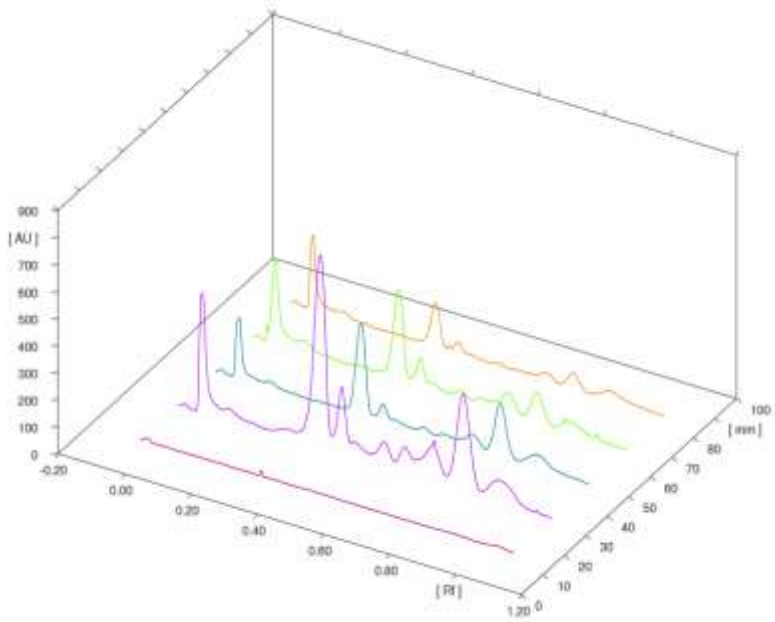
Graph 6: Track 2-M_2 at wavelength 254 nm.

- **Track 5- M_3**



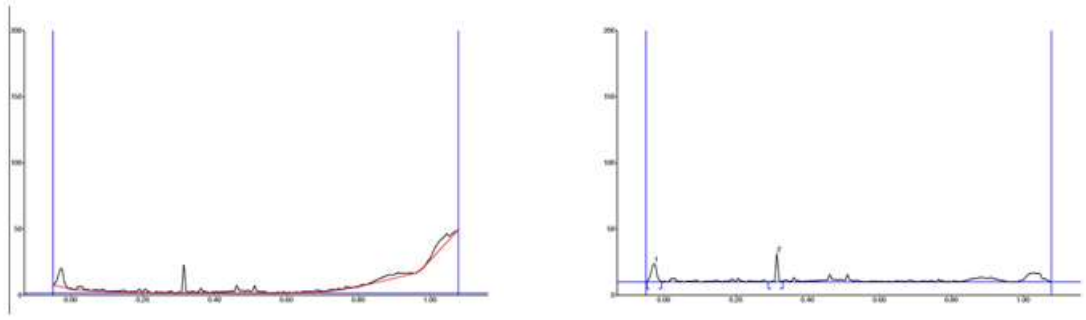
Graph 7: Track 2-M_3 at wavelength 254 nm.

- **All tracks at wavelength 366 nm**



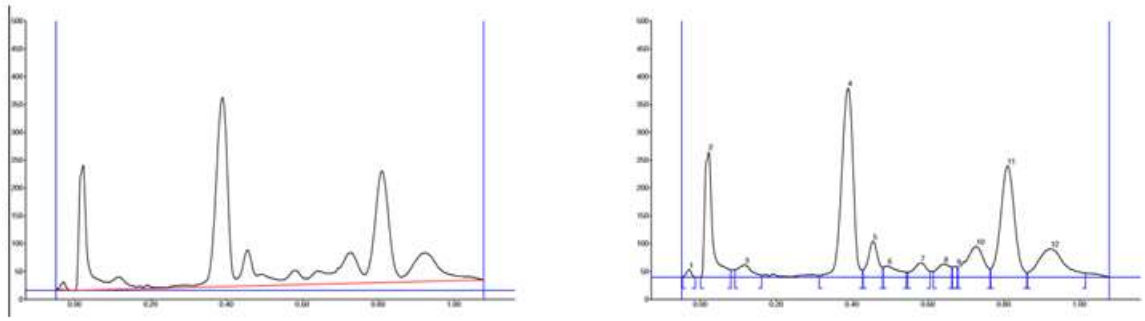
Graph 8: All tracks at wavelength 366 nm.

- **Track 1- Blank**



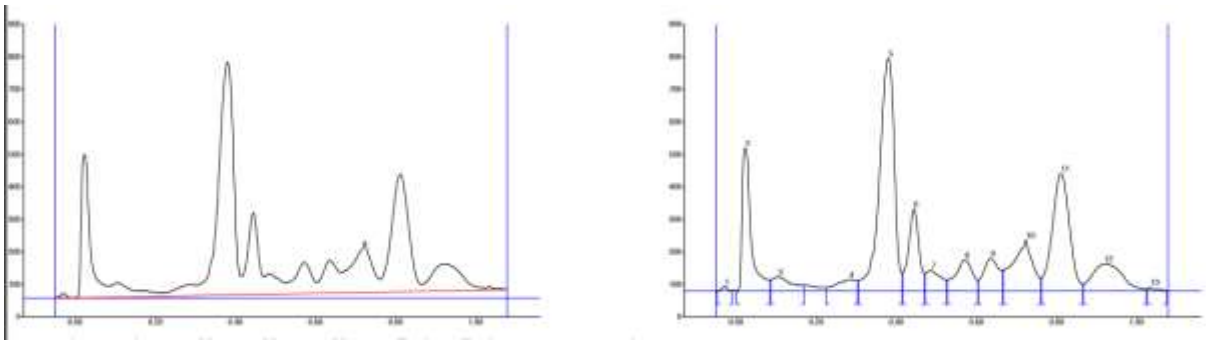
Graph 9: Track 1-FS at wavelength 366 nm.

- **Track 2- M_1**



Graph 10: Track 2-M_1 at wavelength 366 nm.

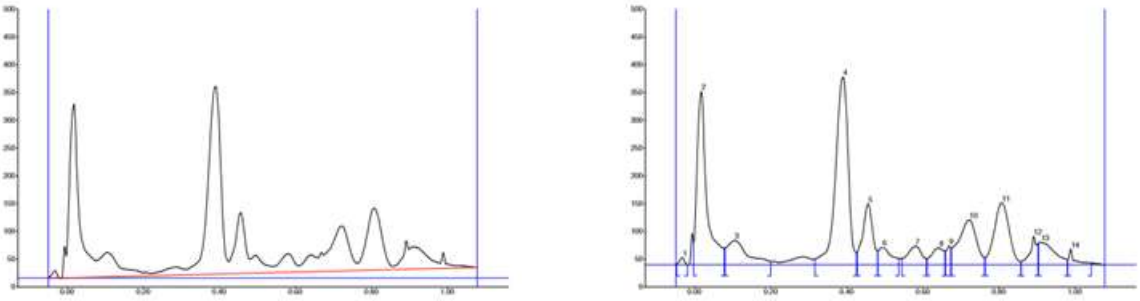
- **Track 3- M_2**



Graph 18: Track 3-M_2 at wavelength 366 nm.

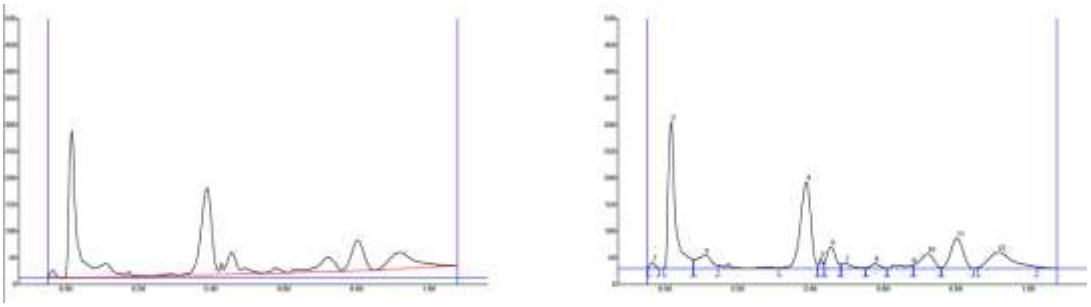
Graph 11: Track 2-M_2 at wavelength 366 nm.

- **Track 4- M_3**



Graph 12: Track 3-M_2 at wavelength 366 nm.

- **Track 5- M_3**



Graph 13: Track 3-M_2 at wavelength 366 nm.

DISCUSSION

- Root of FS was long and thick, but roots of Market samples were thicker and were cut down in short piece available in dried condition.
- In physical appearance, colour of FS appeared light brown in colour. M_1 looked pale yellow, M_2 light brown and M_3 was brown colour.

- FS had typical strong odour, while all Market samples had characteristic odour.
- Slight structural differences were seen in Microscopy of all four samples of *Patala*.
- FS had 25-30 layer of cortex and M_2 had 15-20 layers of cortical cells were present. There was absence of cortex in M_1 and M_3.
- Secondary cortex was differing by no. of layers of parenchyma cells. Secondary cortex of FS was broad and had more no. of layers than others.
- Starch grains were present in all samples except M_2. Calcium oxalate crystals aggregations were seen in FS and M_2.
- Medullary rays were present in all samples.
- Pith cells were present in FS and M_2 only.

In powder microscopy

- Fibres and starch grains are seen in all samples.
- Calcium oxalate crystals and pith are found only in FS and M_2.
- Vessel elements and phloem are seen in all samples except M_1.

Physicochemical analysis

- % of foreign matter found Minimum in FS, higher in M_2 and M_3, but less than 2, M_1 exceeds the API limits.
- LOD value of M_2 was close to that of FS which is lower as compare to other samples.
- Total ash values for all the samples are different, but within the value of API standard, it was higher and same for all Market samples i.e. 5.00% while that of FS it was 4.00%.
- FS had least value of Acid insoluble ash, it is more than FS, but within the standard limit for all Market samples.
- For *Patala* root water soluble extractive value was more than alcohol soluble extractive value.

  Water soluble extractive: Slightly lower than API standard for M_3 sample, it was close to standard value for other samples.

  Alcohol extractive value: highest in M_3 sample and least in FS.
- pH value: Though pH values of the all four sample were different, all were acidic in nature, it was higher for FS then decreasing in order for M_1, M_2 and M_3.

Prelimnary phytochemical analysis

- Glycosides are present in Water soluble extractives of FS and M_1, while in Alcohol soluble extractives of all samples except M_1.
- Steroids are found in all samples of *Patala* in both extracts.
- In both extracts of all samples Alkaloids are present.

HPTLC

- High performance thin layer chromatography study was done under short UV 254nm and long UV 366nm.
- 13 spots were found in FS and M_3 which is maximum, while 12 peaks in other sample. All Rf values resemble almost similar. Identical values indicates similar component present in all samples.

CONCLUSION

- All the four samples (FS, M_1, M_2 and M_3) show resemblance with characters mentioned in API i.e Ayurvedic pharmacopeia of India.
- The HPTLC study revealed some common Rf values and spots and on there is considerable similarity found in all market samples and differ from that of Field collected sample.
- **The above study revealed that the FS sample of *Patala* is very nearer to the parameters given with the API guideline in nearly all aspects.**
- Market samples show considerable similarities with Field sample among them M_2 is more similar than M_1 and M_3.
- Field collected sample is qualitatively best in all parameters than market samples
- **From above study we conclude that, in Ayurvedic formulations we should use Field collected sample.**

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