A preliminary Comparative Study of Punarnava roots collected in Grishma and Sharad rutu W.S.R. to Phytochemical Properties & High-performance thin layer Chromatography

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***Abstract*—This classical texts of Ayurveda places great emphasis on prevention and encourages the maintainance of health through close attention to balance in one's life. Ayurveda medicines mainly comprises of herbal formulations, minerals and metals. Medicinal plants are of great importance in Ayurveda therapies. *Dravys* are capable of producing maximum therapeutic effects when their veerya is augumented by collecting the plant in appropriate season. But in today's era, vast expansion of urban area and deforestation is leading to loss of natural resources of plants at very fast rate. Acharya charaka has given importance to place and method of collection and even more importance to the season in which we are collecting. Season for collection of drug plays an important role in field of drug research. Acharya charaka and vaghbhata both have mentioned the root should be collected during *shishira* or *grishma rutu*. The market analysis shows that some formulations of *Punarnava* like *punaranavamandur*, *Punarnavaashtakkwath* etc are being prescribed at large quantity .This plant has proved itself to be beneficial in the past, is a drug of choice nowdays and seems to effective in future. So the plant has been selected for this study.**

As per above reference, the rutu selected for collection of punarnava root is grishma and other rutu is selected randomly i.e. sharad rutu. Alkaloids, Carbohydrates, Carbohydrates, Steriods, Tannins, Flavonoids all of these constituents are present in Punarnava root sample collected in Grishma and Sharad rutu. we can say that roots collected in grishma rutu are qualitatively better and it is preferable to collect them in grishma rutu as compared to sharad rutu.

***Index Terms*—Dravyasangrahakaala, Punarnava, roots.**

I. INTRODUCTION

India is having a very rich diversity of plants.Use of medicinal plants is as old as human civilization. But in today's era, vast expansion of urban area and deforestation is leading to loss of natural resources of plants at very fast rate. According to the analysis done by Natinal Medicinal Plant Bourd, roots/rhizomes and the whole plant based raw material makes more than half of the trade market. Wild variety of medicinal plants occupy 90% of the trade market out of which 2/3rd are harvested by destructive means. This leads to rapid decline in original plant source. Medicinal plants are of great importance in ayurveda therapies.Acharya charaka as well as vaghbhata mentioned four pada of chikitsa in which dravya is included.[1]

Acharya charaka has given importance to place and method of collection and even more importance to the season in which we are collecting. Season for collection of drug plays an important role in field of drug research. Acharya charaka has told that, dravyas are capable of producing maximum therapeutic effects when their *veerya* is augumented by collecting the plants in appropriate season(*kala sampath*) [2]

Punarnava is well known and long established plant in the scientific system of ancient indian medicine. It is an abundant creeping weed found all over india. The whole plant is used especially the root. It is used as *mutravirechaniya*(diuretic), *shothahara* (anti-

inflammatory), *kaashara* (antitussive), *jvarahara* (anti pyretic), *rasayan* (rejuvenator) etc[3].

Nowadays different pharmaceutical companies are emerging due to increasing demand of natural medications. They are collecting the roots or whole plant in any season to make the availability which in turn causes more destruction. This collected plant shows decreased potency of that drug beacause they are not collected appropriately. So above study is chosen to direct them for collecting plant in proper season as acharya charaka mentioned in *dravya sangraha kala.*

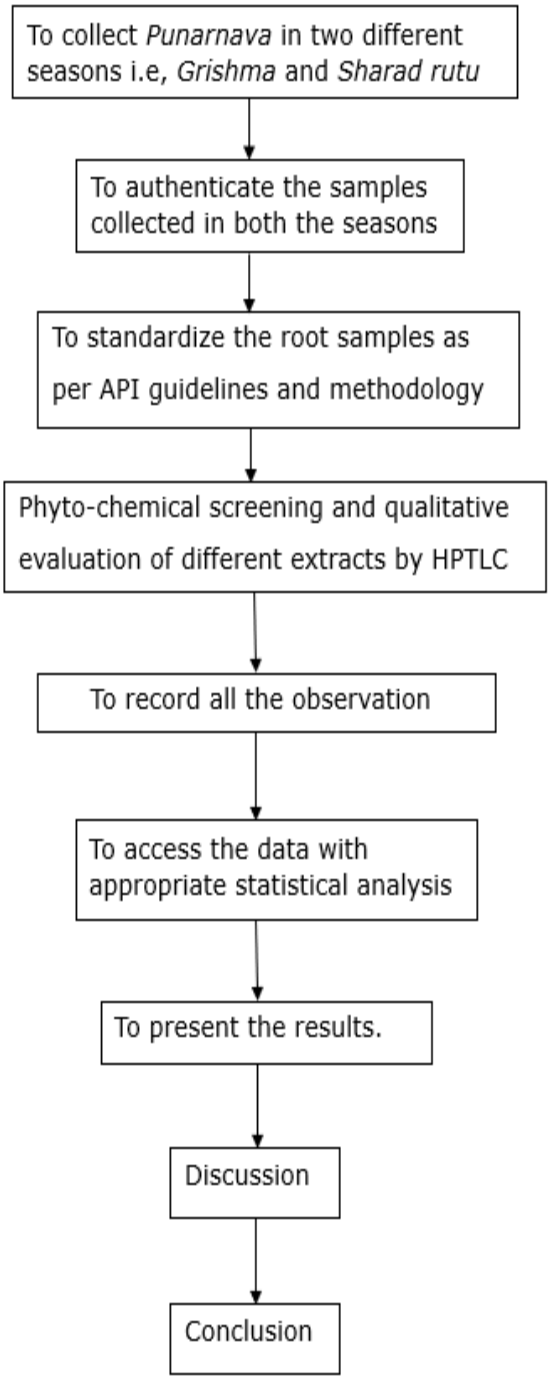
Acharya charaka and vaghbhata both have mentioned the root should be collected during *shishira* or *grishma rutu*. Where as raj nighantu has mentioned root should be collected only during *shishir rutu* [4,5,6]

As per above reference, the rutu selected for collection of punarnava root is grishma and other rutu is selected randomly i.e. sharad rutu.

Methods:-
Primary Objective
To compare phytochemical properties of *Punarnava root* (*Boerhavia diffusa Linn*.) collected in *grishma and sharad rutu*.

Secondary Objevtive
To authenticate and standardize both the samples of *Punarnava* root collected in *grishma and sharad rutu* as per API guidelines.
To do Phyto-chemical screening and evaluation of different extracts by HPTLC of both sample.

Study design:



II. MATERIALS AND METHODS

The present study deals with collection, authentication and standardization of *Punarnava* (Boerhavia diffusa Linn.) root sample collected in *Grishma* and *Sharad* Rutu. Utility of any given drug sample depends upon

its identity, purity, safety, Quality and its storage conditions.

III. MATERIALS

In present study the materials which were studied includes two samples of *Punarnava* root collected in *Grishma* and *Sharad Rutu* respectively. Before actual study the parts of the plants were identified, collected, observed, purified and preserved to facilitate the proper study.

1. Plant identification:

Punarnava root *(Boerhavia diffusa linn.)* was identified on the basis of its Morphology and Family characters of the plant.

2. Collection:

- FIELD COLLECTED SAMPLE-

Sample collection is one of the important part of this study. *Punarnava* root Sample was collected from Wild Place (Forest area which is 30km away from College area) in respective *Rutu* according to dravya *sangraha kala* described in Ayurveda texts, also according to Guidelines on Good field Collection Practices for Indian medicinal plants. So, the first Root sample was collected in *Grishma rutu* Dated (18/6/2020) and Second sample was colleted in *Sharad Rutu* dated (19/10/2020) after collecting the samples were cleaned with water and dried properly avioiding direct sunlight to obtain shushka churna form of the sample required.

AUTHENTICATION

Punarnava (Boerhavia diffusa Linn.) was authenticated from taxonomist and subject experts from well known Government approved Botany institute. The authenticity of these samples was confirmed by comparing their characters with standard herbarium sample and various floras available in taxonomical pattern and specimen sample was vouchered as number 10353.

METHODS OF PREPARATION OF SAMPLE:

The season wise collected samples of *punarnava* were grinded in grinder to form the fine powder and they were sieved for further analytical study.

STORAGE OF POWDER MATERIAL:

The *shushka* powder of both samples was stored in different polythene bags to protect from moisture or any other contamination.

External Morphology of *Punarnava* (Boerhavia diffusa linn.) [7]:

- Habitat-

This weed is fond all over India; it is one of two kinds, one with white and the other with red flowers. Both of them are used in medicines. It is a diffuse herb perennating by means of roots.

Fig No.1 -Whole plant of *Punarnava* (Boerhavia diffusa Linn.)

- Root-stout, woody, Branches- Diffuse Fusiform
- Leaves- opposite, one of the members of the pairs larger while the other is smaller, exstipulate, with long petiole, Lamina simple, broadly triangular ovate, apex obtuse, rounded; margines wavy, upper surface glaberous, lower with fine scales, lamina with reddish tinge.
- Inflorescence- Corymbose-umbel on very long peduncle, either axillary or terminal.
- Flowers- Incomplete, bisexual, regular, actinomorphic, hypogynous, with a whorl of bracteoles. Bracteoles small. As a whole,the flowers are conspicuous

Fig No. 2- Flower of *Punarnava* (Boerhavia diffusa Linn.)

- Perianth- (undifferentiated calyx and corolla) Tapals 5, gamotepalous throughout; the tube constricted above the ovary, expanding thereafter into funnel. The constricted part glandular- viscid, limbs of the funnel plicate – dark pink with five narrow bsnds on the outer face; basal viscid glandular part persistent.
- Androecium- Stamens 2-3, unequal in height; slightly exserted, filament inflexed in bud state.
- Gynaecium- Carpel one,overy, superior, uni-locular with a single ovule, style filiform, stigma small.
- Fruits- Achene with the base of the perianth tube called anthocarp with five glandular ribs over the surface. It is 1.3 cm long. Flowering and fruiting occur in winter.

GEOGRAPHICAL DISTRIBUTION:

It is a perennial herb found Throughout India, Baluchistan and Ceylon.In tropical and subtropical Asia, Africa and America.

PART USED- Whole plant, root and leaf.

RASAPANCHAKA: [8]

Rasa- Madhur, Tikta, Kashay

Vipaka- Madhur

Virya- Ushna

Gunas- Laghu, Ruksha

Doshaghnata- Tridoshhar

Rogaghnata- Shothrog, Agnimandya, Udarrog, Vibandha, Hridrog, Pandu, Kasa, Shwasa, Raktapradara, Mutrakruccha, Kushta, Jwar, Dourbalya.

Karma- Lekhana, Shothhar, Deepana, Anulomana, Rechana, Hridya, Raktavardhaka, Kasahar, Mutrajanana, Swedajanana, Rasayan and Vishaghna.

PHARMACOLOGICAL ACTIVITIES-

DOSAGE-

- Fresh juice 5-10 ml
- Powder 1-3g.

CHEMICAL CONSTITUENTS:

Hentriacontane, β-sitosterol, Oxalic acid, D-glucose, Punarnavoside, Punarnavine-1, Punarnavine-2, Boeravinones A,B,C etc.

IMPORTANT PREPARATIONS-

- Punarnavasava
- Punarnavarishta
- Punrnavadi Mandura
- Punarnavasataka
- Punarnavambu
- Punarnava Guggula
- Punarnavastak Kwath/Churna
- Sukumar Ghrit
- Shthaghna Lepa
- Punarnavadi taila
- Punarnava rasayana
- Punarnava leha

USES: [9]

1. The aqueous extract of roots collected in summer exhibited marked hepatoprotective activity.
2. Decoction of Punarnava is useful in various diseases.
3. The root has Diuretic, Anti-Inflammatory, Fibrnolytic and Cardiotonic Properties.

IV. METHODOLOGY

PHYTOCHEMICAL STUDY[10][11]

1) Glycosides
2) Carbohydrates
3) Steroids
4) Flavonoids
5) Tannins
6) Alkaloids

CHROMATOGRAPHIC STUDY[12]

1) HPTLC

The plant biosynthetizes compounds like Glycosides, Alkaloids, Volatile oils, Tannins etc. that exert a physiological and therapeutic effect in the human body. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. The crude powder and crude drug extractin different drug solvents are tested for detection of various phytoconstituents present n it were carried out using following methods in different test tubes.

Detection of Carbohydrates-

The Carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones or compounds that on hydrolysis produce ether.

Molisch's test-

- Small quantities (200 mg) of alcoholic and aqueous extracts was dissolved separately in 5ml of distilled water and filtered.
- The filtrate is reacted with 0.5 ml of α-naphthol solution.

- The mixture was vortexed and two drops of concentrated sulphuric acid was added from the side of test tubes.
- Formation of purple ring at the junction of two liquid showed the presence of carbohydrate.

Fehling's test-

- In this test, 1ml Fehling's A and 1 ml of Fehling's B solution were added and boiled for 1 minute.
- Equal volume of test solution was added and heated in boiling water bath for 5-10min.
- Formation of brick red ppt confirmed the presence of carbohydrates.

Detection of Glycosides-

Glycosides are the condensation products of sugar and aglycon. Glycosides are secondary metabolites of pharmaceutical significance. These are soluble in water, as well as alcohol.

- In this test, few g of samples was taken in test tube. Then test tube was covered with filter paper soaked in dilute NaOH. After few minutes, expose filter paper to UV light – green fluorescence is produced showed the presence of glycosides.

Detection of Steroids/ Phytosterols-

Libermann-Burchard's test-

- Small quantities of both extracts reacted with 3ml of acetic anhydride and 2 drops of concentrated Sulphuric acid.
- Bluish green colour indicated presence of steroids.

Salkowaski test-

- In a small portion of extract, 2 ml of chloroform and 2 ml of concentrated Sulphuric acid were added from side of the test tubes.
- The test tubes were shaken for few minutes. The formation of red colour in chloroform layer indicates presence of steroids.

Detection of Flavonoids-

Flavonides are group of plant metabolites thought to provide health benefits through cell signalling pathways and antioxidant effects.

Shinoda test

- In this test 3-4g of powder is taken and 5 ml of methanol is added then few drops of concentrated HCL and required amount of magnesium turnings is added in sample.
- Formation of pink, orange red to purple color indicates the presence of flavonoids.

Detection of Tannins-

Tannins are present in cell sap, soluble in water and alcohol. They give blue –black or green colours with iron compounds. They have several medicinal properties and hence used as drugs.

$FeCI_3$ test

- To the 2 ml of aqueous extract were taken in test tube and added few drops of $FeCI_3$ solution.
- Apperance of dark green colour precipitate indicates the presence of tannins.

Detection of Alkaloids

Alkaloides are the basics nitrogenous secondary metabolites having marked physiological action, if taken internally. They occur in plants as salt of nicotinic, quinic, citric or oxalic acid. They are found in seeds, barks, leaves, roots etc.Alkaloides are used as highl potent medicaments and posseses curative properties. They are detoxicating agents andreservoirs of protein synthesis.

i) Mayer's test

- The filtrate was tested with Mayer's reagent. Cream coloured precipitate confirms presence of Alkaloids.

ii) Dragendorff's test

2ml of the filtrate were taken in different test tubes, when treated with few drops of dragendorff's reagent leads to formation of orange to reddish colourewd precipitate confirming the presence of alkaloids.

HPTLC

Instrument: CAMAG Linomat 5

Linomat 5 application parameters:

Spray gas	Inert gas
Sample solvent type	Methonol
Dosage speed	150nl/s
Predosage Volume	0.2ul

Sequence
Syringe size: 100µl
Number of tracks: 2

No.	Appl. Position	Appl.Volume	Vial#	Sample ID	Active
>1	15.0mm	10.0 µl	1	Punarnava Grishma rutu sample 210201029	Yes
>2	85.0mm	10.0 µl	2	Punarnava Sharada rutu sample 210201030	Yes

Application position Y: 8.0mm
Band length: 8.00mm

No.	Appl. Position	Appl.Volume	Vial#	Sample ID	Active
>1	15.0mm	10.0 µl	1	Blank	Yes
>2	85.0mm	10.0 µl	2		Yes

Development- Glass tank
Chamber type- Twin Trout Chamber 20x10cm

Pre- Conditioning

Mobile phase	Chloroform:Methanol(8:2)
Solvent front position	70.0mm
Volume	10.0ml
Dryig device	Oven
Temperature	60ºC
Time	5 Minute

Detection- CAMAG TLC Scanner
Information
Application position 8.0mm
Solvent front position 70.0mm

Number of Tracks	2
Position of first track	15.0mm
Distance between tracks	70.0mm
Scan start pos. Y	5.0mm
Scan start pos. Y	75.0mm
Slit dimention	4.00x0.30mm, Micro
Optimize optical system	Light
Scanning speed	20 mm/s
Data resolution	100 µm/step

Integration Properties-

Data Filtering	Savitsky-Golay 7
Baseline correctin	Lowest Slope
Peak threshold min. Slope	5
Peak threshold min. Height	10 AU
Peak threshold min. Area	990 AU

Track start position	5.0mm
Track end position	75.0mm
Display scaling	Automatic

Mesurement Table-

Wavelength	254
Lamp	D2 & W
Measurement Type	Remission
Measurement mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	185 V

V. RESULT

Priliminary Phytochemical results of Grishma and Sharad Rutu Sample of Punarnava Root (Boerhavia diffusa Linn.)

Sr. No.	Qualitative tests	Grishma Rutu Sample		Sharad Rutu Sample	
		WATER EXTRACT	ALCOHOL EXTRACT	WATER EXTRACT	ALCOHOL EXTRACT
1.	Test for Alkaloid a)Hager's Test	+	+	+	+
2.	Test for Carbohydrates a)Molisch Test b)Fehling's Test	-	-	-	-
3.	Test for Steriod a)Salkowaski Test	+	-	+	-
4.	Test for Glycosides a)Kellar kiliani Test	+	-	+	-
5.	Test for Tannins a) $FeCl_3$	-	-	-	-
6.	Test for Flavonoids a)Shinoda Test	+	+	+	+

HPTLC OBSERVATIONS:

HPTLC Profile of Grishma & Sharad Rutu Sample of Punarnava root at 254 nm and 366 nm wave length (With Solvent Methanol)-

Visualization	Track 1		Track 2	
	No. Of Spots	Max Rf value	No. Of Spots	Max Rf value
254nm	7	-0.04 0.03 0.05 0.14 0.54 0.56 0.85	4	0.02 0.29 0.74 0.87

366nm	6	0.03 0.05 0.14 0.20 0.32 1.01	3	-0.03 0.64 0.97

HPTLC Profile of Grishma and Sharad Rutu Sample of Punarnava root at 254 nm and 366 nm wave length (Exposure with iodine)-

Visualization	Track 1		Track 2	
	No. Of Spots	Max Rf value	No. Of Spots	Max Rf value
254 nm	8	-0.04 -0.03 0.05 0.14 0.29 0.39 0.54 0.85	4	0.02 0.27 0.74 0.87
366 nm	11	0.00 003 0.14 0.20 0.32 0.44 0.48 0.50 0.65 0.76 0.88	9	0.02 0.11 0.18 0.21 0.43 0.67 0.79 0.82 0.89

VI. DISCUSSION

Alkaloids, Carbohydrates, Carbohydrates, Steriods, Tannins, Flavonoids all of these constituents are present in Punarnava root sample collected in Grishma and Sharad rutu.

7) HPTLC study:

High performance thin layer chromatography study was done under short UV 254 nm and long UV 366 nm.

Grishma rutu sample of punarnava under short UV 254nm track shows 7 peaks with Rf values- -0.04, 0.03, 0.05, 0.14, 0.54, 0.56, 0.85. Under Iodine exposure it shows 8 peaks with Rf values- -0.04, -0.03, 0.05, 0.14, 0.29, 0.39, 0.54, 0.85. And under long UV 366nm track shows 6 peaks with Rf values- 0.03, 0.05, 0.14, 0.20, 0.32, 1.01. This sample under iodine exposure shows 11 peaks under Rf values- 0.00, 0.03, 0.14, 0.20, 0.32, 0.44, 0.48, 0.50, 0.65, 0.76, 0.88.

Sharad rutu sample of punarnava under short UV 254nm track shows 4 peaks with Rf values 0.02, 0.29, 0.74, 0.87. Under Iodine exposure it shows 4 peaks with Rf values- 0.02, 0.27, 0.74, 0.87. And peaks under long UV 366nm track shows 3 peaks with Rf values- -0.03, 0.64, 0.97. This sample under iodine exposure shows peaks under Rf values-0.02, 0.11, 0.18, 0.21, 0.43, 0.67, 0.79, 0.82, 0.89.

Maximum 7 spots were found of Grishma rutu collected sample of punarnava roots at wavelength 254 and 6 spots at 366nm wavelength. On the other hand only 4 and 3 spots were found of Sharad rutu collected sample. This indicates that, It will be more beneficial

to use Grishma rutu collected sample of punarnava in Ayurvedic drug formulations to attain maximum benefits.

VII. CONCLUSION

C) PHYTOCHEMICAL ANALYSIS [7][8]

Different preliminary phytochemical tests showed the presence of alkaloid, steroid, glycosides, flavonoids in extract of water and alcohol extract showed the presence of only alkaloids and flavonoids in both grishma and sharad rutu collected sample of punarnava roots.

D) CHROMATOGRAPHIC SYUDY

HPTLC study showed that there is difference in chemical nature of both samples. There are different Rf values found in both the samples collected of punarnava root.

From this study conducted, observation and results obtained from above parameters, it can be concluded that there is a difference in properties of Punarnava Root sample collected in Grishma and Sharad rutu.

Accordingly Alternate hypothesis is accepted for both the entities.

Hence we can say that roots collected in grishma rutu are qualitatively better and it is preferable to collect them in grishma rutu as compared to sharad rutu.

VIII. FUTURE SCOPE OF THE STUDY

1. Advance study could be done using Standard Marker Compounds.
2. Advanced phytochemical evaluations could be incorporated into thestudy.
3. Comparative study of efficacy of punarnava root grown in different flora should be done.
4. By knowing the above results, Preclinical and clinical studies would have been done.

Conflicts of Interest: None

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Diagrams: -

Roots of Boerhavia diffusa Linn. Collected In Grishma Rutu.

Roots of Boerhavia diffusa Linn. Collected In Sharad Rutu.

Grishma rutu collected sample of punarnava in churna form

Sharad rutu collected sample of punarnava in churna form

Densiographs:

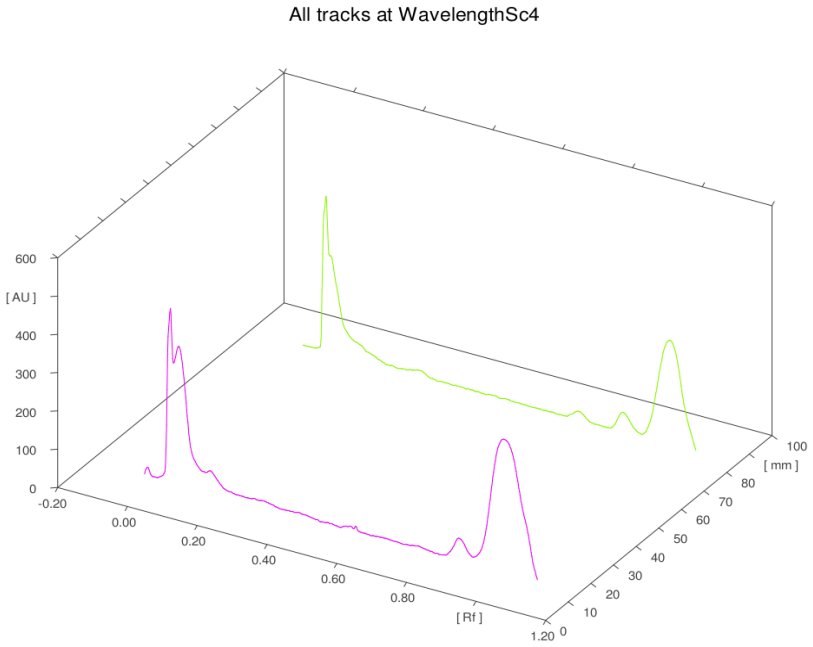

Fig no. 7- All tracks at wavelength 254nm

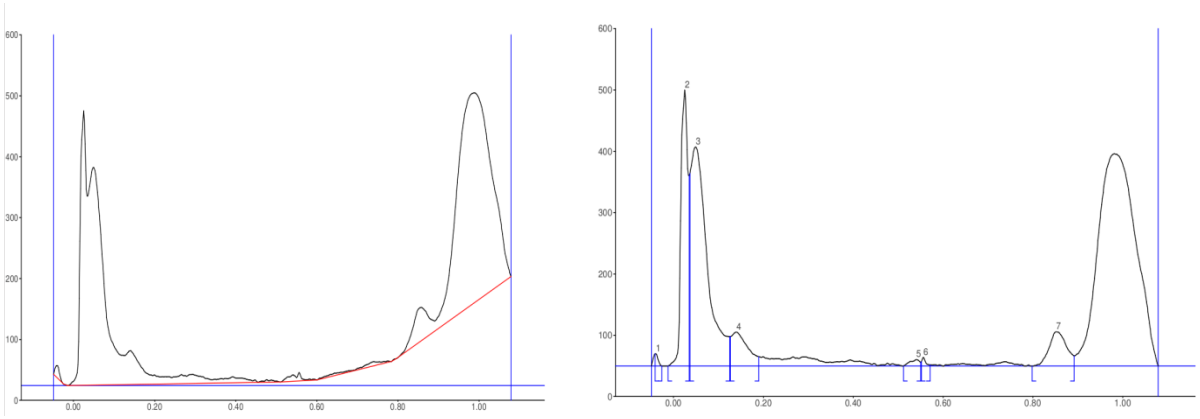

Fig no. 8-Track 1, ID: Punarnava Grishma rutu sample 210201029

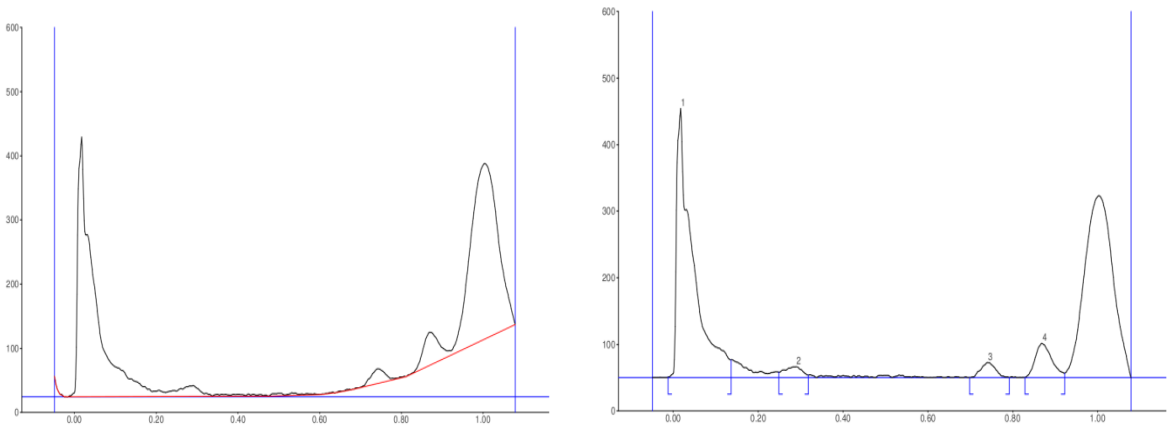

Fig No. 9-Track 2 ID: Punarnava Sharad rutu sample 210201030

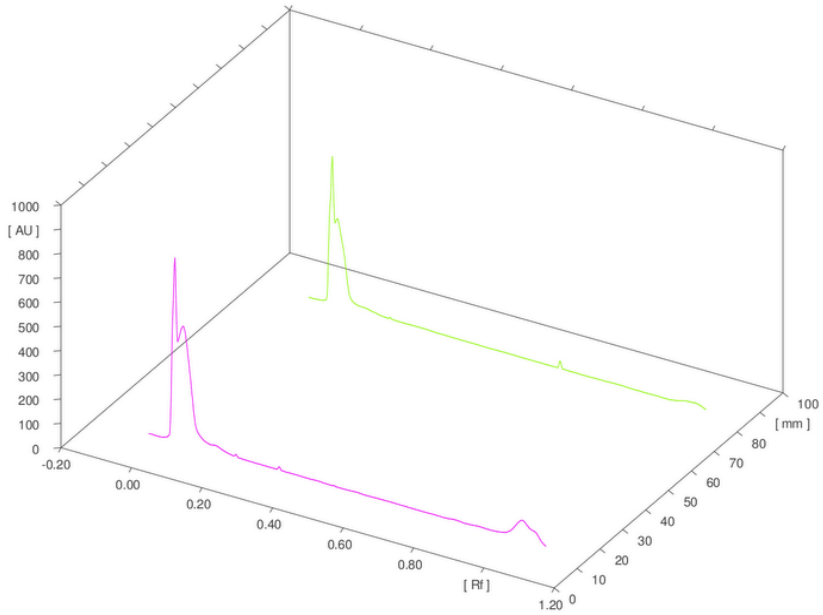

Fig No.10-All Tracks at wavelength 366 nm

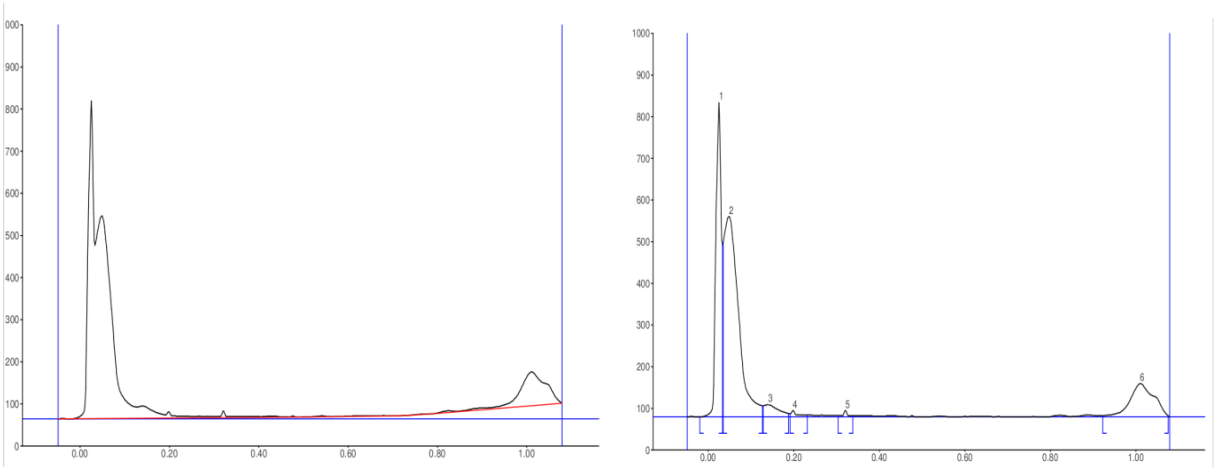

Fig No.11-Track 1, ID: Punarnava Grishma rutu sample 210201029

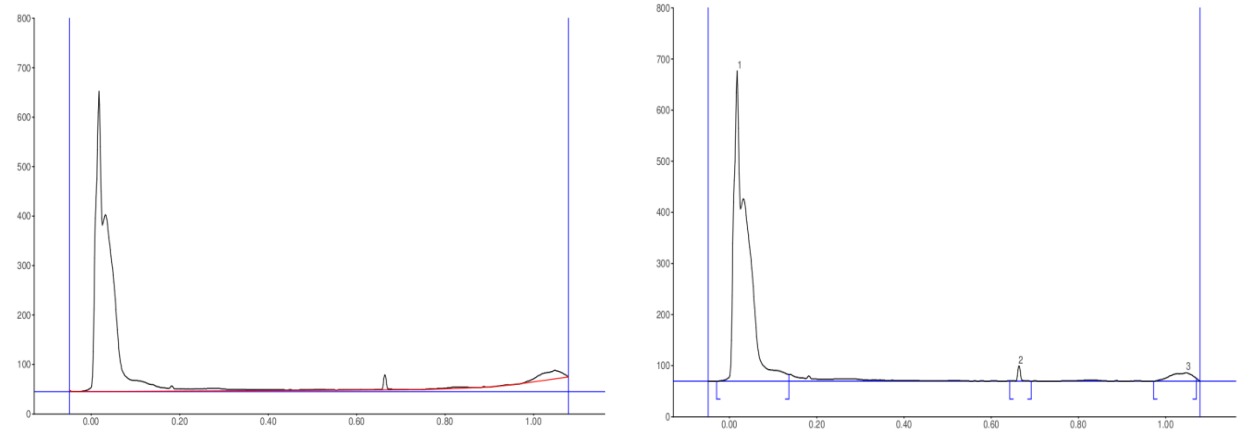

Fig.No.12-Track 2 ID: Punarnava Sharad rutu sample 210201030

On exposure to iodine-

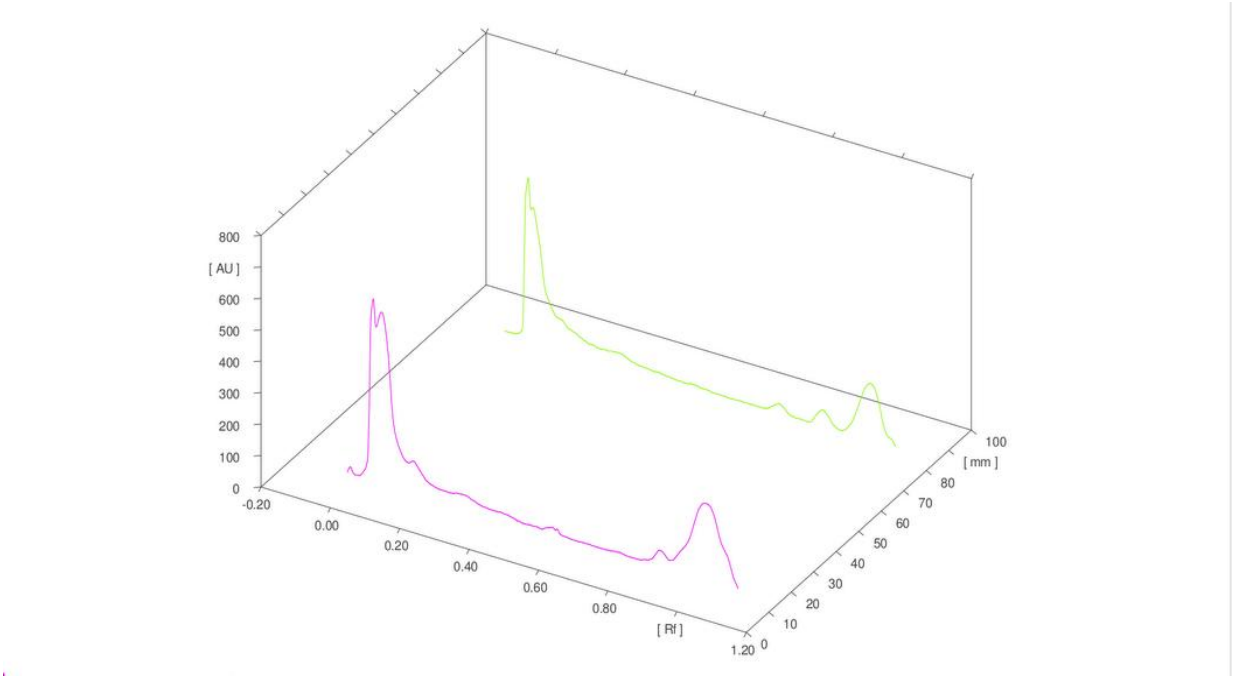

Fig No.13-All tracks at wavelength 254nm

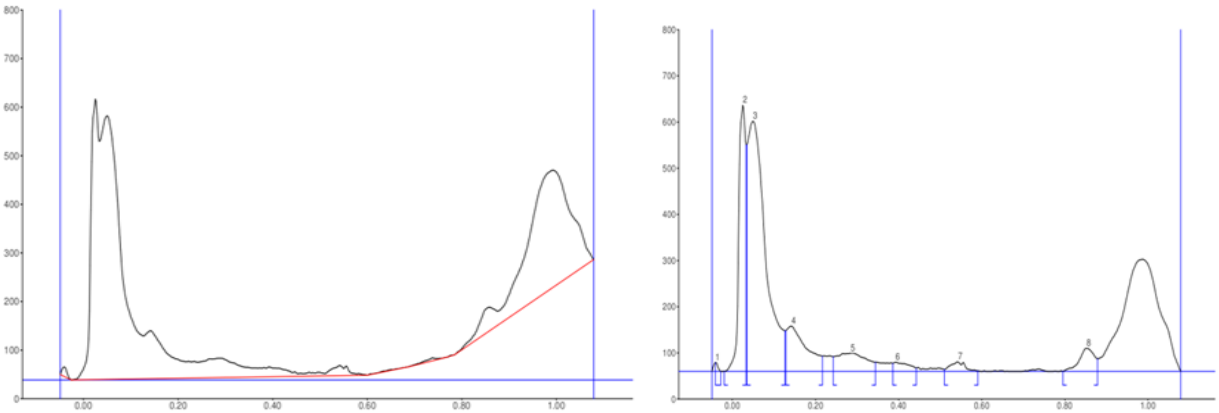

Fig no. 14-Track 1, ID: Punarnava Grishma rutu sample 210201029

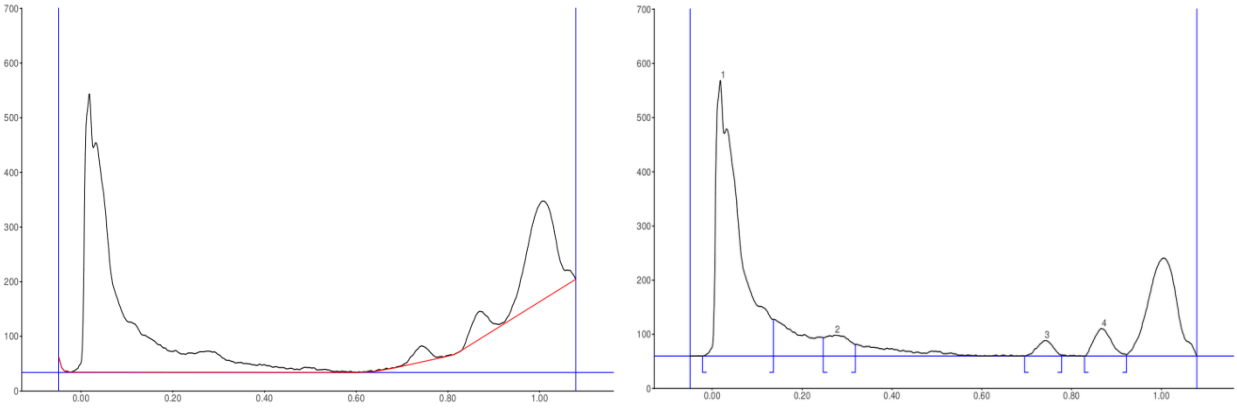

Fig no. 15-Track 2 ID: Punarnava Sharad rutu sample 210201030

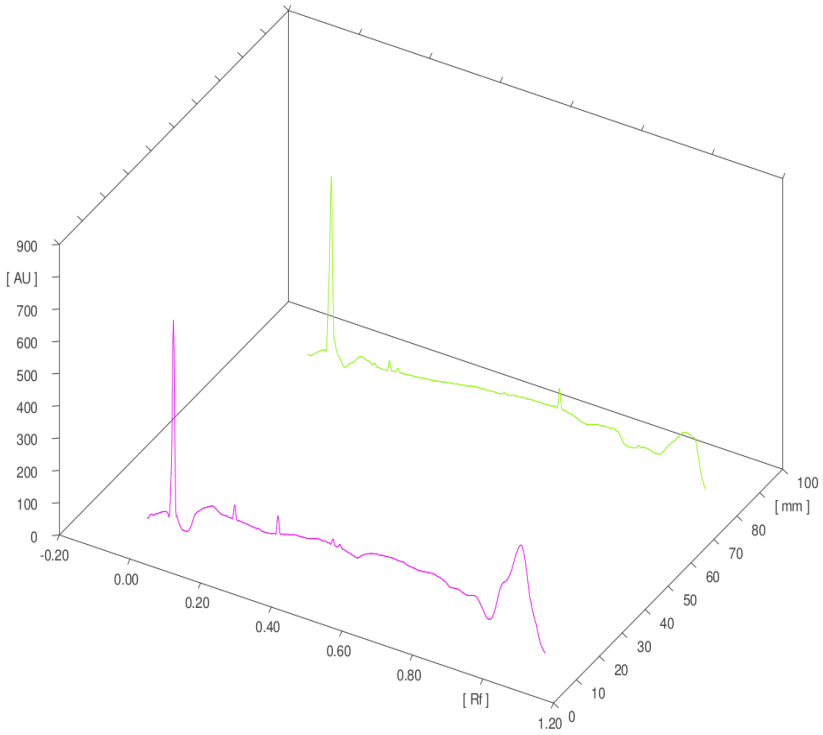

Fig no. 16-All Tracks at wavelength 366 nm

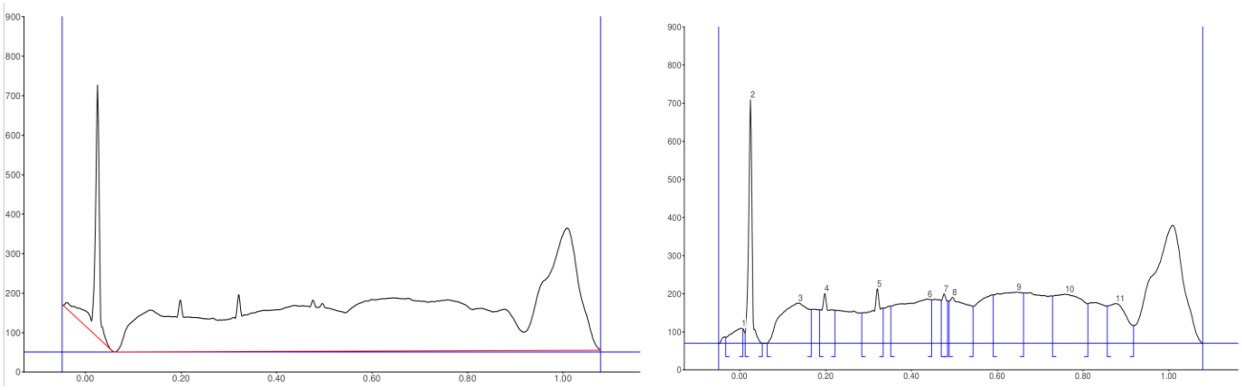

Fig no. 17-Track 1, ID: Punarnava Grishma rutu sample 210201029

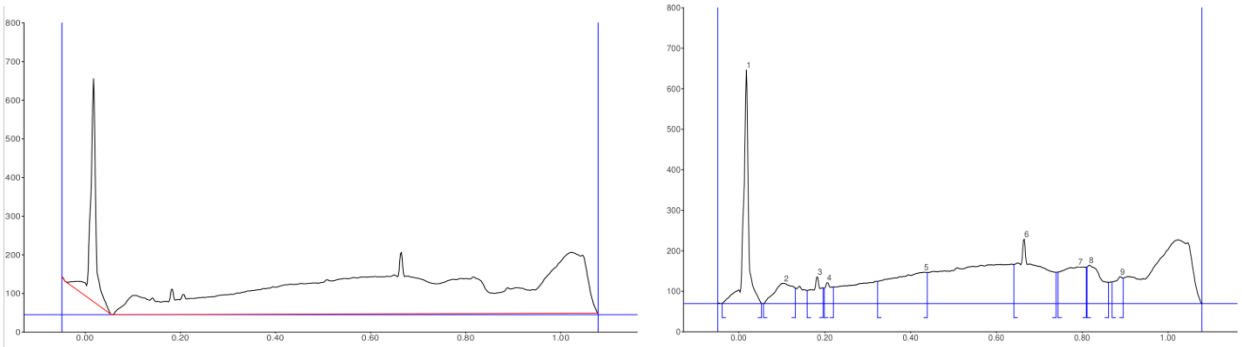

Fig no. 18-Track 2 ID: Punarnava Sharad rutu sample 210201030

HPTLC Images:

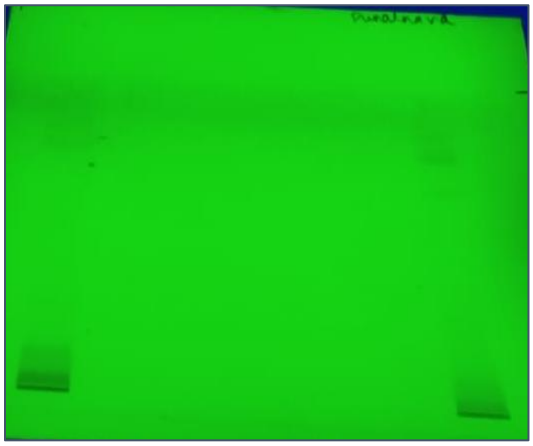

Fig No.19: All the sample tracks under short UV 254nm after development

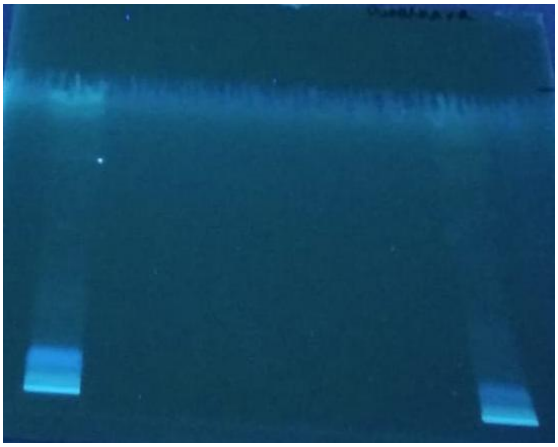

Fig No.20: All the sample tracks under short UV 366nm after development