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CONSERVATIVE CULTIVATION OF *RAUVOLFIA SERPENTINE* -A COMPARATIVE QUANTITATIVE STUDY WSR TO KUNAPAJALA

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ABSTRACT

Ever since Ayurveda attained world attention medicinal plants Scarcity turned out to be one among the prime lacunas in the research and development of ayurvedic medicines. It's a fact that we are lacking genuine drugs in the dry drug market because of unscientific drug collection and deforestation. So it's the need of the hour to start with scientific cultivation and conservative collection by rediscovering some of our ancient cultivation measures. Vrikshayurveda is a branch of Ayurveda where we can find enough references regarding the ancient cultivation techniques and different preparation techniques of natural manures one among them is kunapajala (organic liquid manure) so here in this research study we are assessing the efficacy of kunapajala in cultivation of *Rauvolfia serpentina* which is one among the red listed plants and is reported to be commonly adultered with *R.tetraphylla*.

Keywords: Kunapajala, Vruksha ayurveda, Organic farming, Sarpagantha, Histology & Chemical analysis of *Rauvolfia*

INTRODUCTION

If the scarcity of original drugs persists then we have to compromise the quality of our ayurvedic medicinal preparations which will definitely reflect in the clinical outcomes also. so we need to ensure maximum yield from the medicinal plant cultivations. For attaining the goal of maximum yield we have to rediscover such techniques which will not only increase the rate of production in medicinal plant

cultivation but also it should increase the potency of medicinal plants. So here in this study we are accessing the efficacy of kunapajala an organic liquid manure explained in Surapala Nighantu on the cultivation of Rauvolfia serpentine [1]The earliest references Of Vrukshayurveda can be traced from the Rigveda and Atharveda. The other books that provide valuable information are Kautilya's Arthasastra, Amarkosha, Krishi-Parashara and Varahmihir's Brhat Samhita etc. But no independent texts seem to have been written on the subject. The oral tradition however regarded Surpala's Vrikshayurveda as a credible compendium.[2] Sadly the actual text of Surpala is not available. Surapala nighantu is a marvellous book in Ayurveda where we can see so many systematic techniques and guidance about vrukshayurveda .in this book it mainly deals with cultivation and conservative collection of plants .one among them is nourishing therapy by [3]Kunapajala. Kunapajala preparation technique is mentioned in this book and it is told that it acts as a good nourishing agent and a catalyst in growth and development of plant parts.

METHODOLOGY

Method of collection of data

We have identified and collected 1kg of *Rauvolfia serpentina* seeds from Raairat gardens ,kerala and germinated according to standard cultivation protocol of *Rauvolfia serpentina* at parul institute of ayurved and research ,Vadodara, Gujarat. After one month 60 healthy saplings were selected and planted in mud filled sacks for conservative cultivation in two different groups, For one group the standard cultivation protocol of *Rauvolfia serpentina* were followed ,in one group normal water were be supplied once in two days but in group the second group along with normal water 60 ml of kunapajal were supplied once in a month[4]

Duration- 2 years in this study two groups of saplings each were made by random selection method and the growth was allowed in same habitat and climatic conditions[5].

Source of data Seeds of *Rauvolfia serpentina* from Raairat gardens, Kerala

Sample size: 60 Group A (Study group): 30 Group B (Control group): 30

Trial drug: *Rauvolfia serpentina* with Kunnapajala

ASSESSMENT CRITERIA:

Pharmacognostic study

Physical comparison

TLC

HPTLC (Quantification of reserpine) of both the groups

Comparative analysis of phytochemicals in the roots of *Rauvolfia serpentina* of both

the groups have been done after the harvest[6]

physical comparison of the roots done by weighing both the total yields separately from both the groups, after harvest have been done[7]

RESULTS

TLC study

TLC Specifications

Technique: One-Way Ascending

Stationary Phase: Silica Gel G

Mobile Phase: Chloroform: Diethyl amine = 90:10

Temperature: Room temperature

Sample preparation: 0.025%w/v solution of test sample

Standard preparation: 0.025%w/v solution of Reserpine

Visualization: Under U.V.light followed by spraying with Dragondorff's reagent

The result under the applied conditions shows that from the four spot, Spot 1 and 2 are unknown constituents and not identified due to lack of standard sample .Spot 3 is test sample which has Rf value resembles to the standard reserpine so reserpine is present in both the samples.

Table 1: TLC of rauwolfia root
(The solvent moved = 7.9cm)

Sr. No.	Substance	Distance Travelled	Rf value	Results
1	Spot 1	6.2	0.78	Unknown
2	Spot 2	6.2	0.78	Unknown
3	Spot 3	6.3	0.79	Test sample
4	Spot 4	6.4	0.81	Standard sample

HPTLC QUANTIFICATION REPORT

Sample	:	**Sharpagandha Powder**
Name of Scholar	:	Dr. Prasanth R. Krishna, Professor, Parul Institute of Ayurved & Research, Vadodara.
Sample ID	:	AD/21/340
Date of Report	:	22.10.2021

Preparation of Standard solution (S1): Accurately weigh 10 mg of standard Reserpine into 10 mL volumetric flask, dissolve in Chloroform and make up the volume up to 10 mL with Chloroform. Use the Standard solution thus obtained for HPTLC fingerprinting.

Preparation of Test solution (T1) and (T2): Accurately weigh 1 g of sample in a Iodine flask. Add few ml of Ammonia till Sample is moistened. Keep the Iodine Flask on rotary shaker for 30 minutes. Then after, add 20 ml Chloroform and reflux for 30 minutes. Filter the sample through Whatmann Paper No. 1 then again add 20 ml Chloroform to remaining Residue and again reflux the sample for 30 minutes. Then after, filter the sample through same Filter paper and pool and evaporate the collected chloroform to concentrate the sample till 7 to 8 ml remains. Ultimately, Take this sample in 10 ml volumetric flask and make up the volume up to mark with Chloroform. Use the Test solution thus obtained for HPTLC fingerprinting.

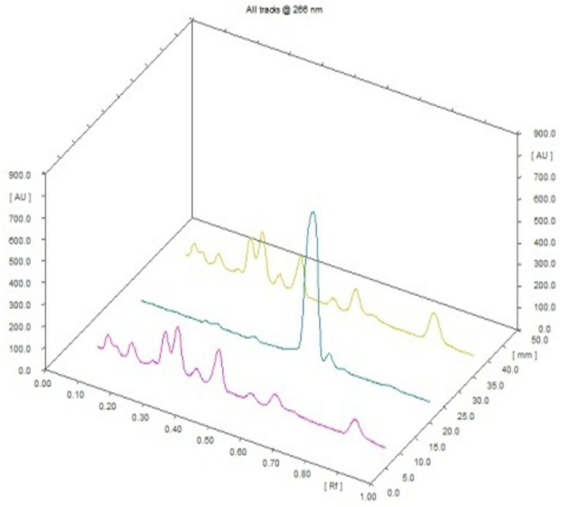
Chromatographic Conditions:

Application Mode	CAMAG Linomat 5 - Applicator
Filtering System	Whatman filter paper No. 1
Stationary Phase	MERCK - TLC / HPTLC Silica gel 60 F_{254} on Aluminum sheets
Application (Y axis) Start Position	10 mm
Development End Position	80 mm from plate base
Space Between Band	10 mm
Distance Between Tracks	15 mm
Sample Application Volume	(T1) 5.0 µL and (T2) 5.0 µL
Standard Application Volume	10.0 µL
Development Mode	CAMAG TLC Twin Trough Chamber
Chamber Saturation Time	30 minutes
Mobile Phase (MP)	Toluene : Ethyl Acetate :Diethylamine (7 : 2 : 1 v/v)
Visualization	@ 254 nm
Quantification Wavelength	266 nm

Sample	Reserpine	Sharpagandha Powder-A (Non-Kunapjala)	Sharpagandha Powder-B (Kunapjala)
Weight	10.3 mg	1.029 gm	1.009 gm
Area	21344.6	1778.9	2537.4
% Reserpine	--	0.153 %	0.222 %

Figure 1: HPTLC quantification of reserpine

HPTLC Chromatogram @ 266 nm



3D chromatogram

R_f value

Spot No.	T1	S1	T2
1	0.10	—	0.10
2	0.13	—	0.12
3	0.17	—	0.17
4	0.23	—	0.23
5	0.27	—	0.27
6	0.31	—	0.31
7	0.36	—	0.36
8	0.43	—	0.43
9	0.53	—	0.53
10 (Reserpine)	**0.60**	**0.60**	**0.60**
11	0.66	—	0.66
12	0.85	—	0.85

T1: Sharpgandha Powder-A

S1 : Standard Reserpine

T2 : Sharpgandha Powder-B

HPTLC Plate (254 nm)

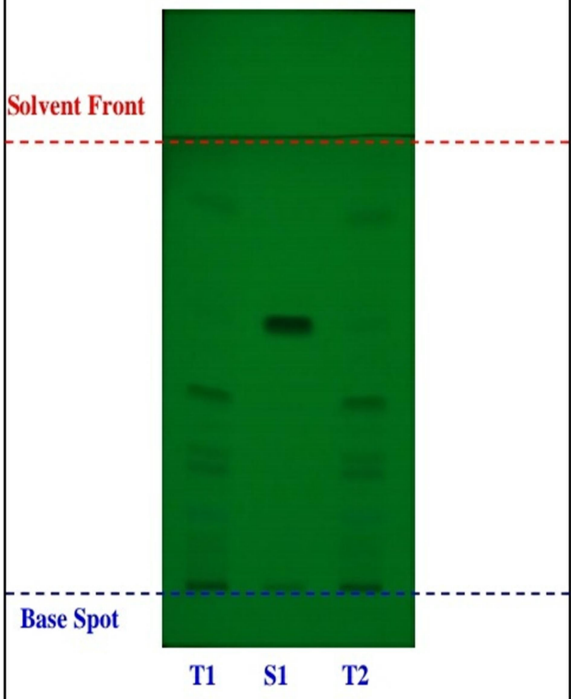


Figure 2: HPTLC quantification of reserpine

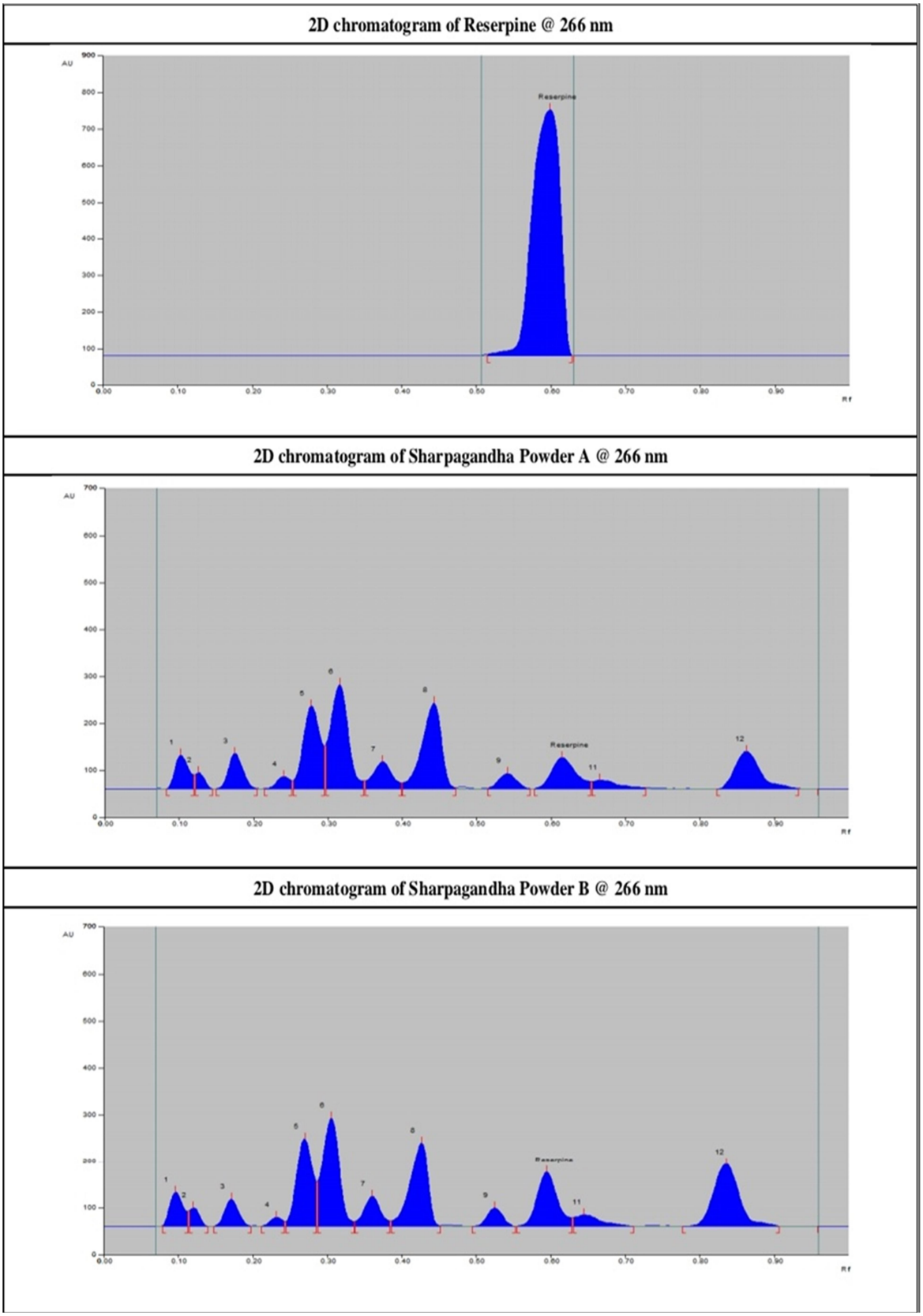


Figure 3: HPTLC quantification of reserpine

DISCUSSION

TLC study

The result under the applied conditions shows that from the four spot ,Spot 1 and 2 are unknown constituents and not identified due to lack of standard sample .Spot 3 is test sample which has Rf value resembles to the standard reserpine so reserpine is present in the both the samples.

The optimized solvent system was used for the estimation of reserpine in Kunapjala Rauwolfia extract and Non- Kunapjala Rauwolfia extract . The resolution was good and components were observed at different Rf value, as shown in Figures was matched with the Rf value of standard sample.

The proposed HPTLC method of provided simple, accurate and reproducible quantitative analysis for simultaneous determination of reserpine in Kunapjala Rauwolfia extract and Non- Kunapjala Rauwolfia extract . This method was validated as per ICH guideline. Statistical tests indicate that the proposed HPTLC method reduce the duration of analysis and appear to be equally suitable for routine determination of reserpine in Kunapjala Rauwolfia extract and Non- Kunapjala Rauwolfia extract.10 grams of sample root powder was taken from each group and quantification of reserpine was done then the the group A that is Non kunapajala group showed the presence of 0.153% of reserpine where as the group B that is the Kunapajala group showed 0.222% of reserpine

CONCLUSION

So this study can be concluded that the kunapajala is improving not only the yield of sarpagandha but also it has improved the quality of the harvested root as compared to the standered group ,by refering the TLC study the prescence of reserpine is detected in both the samples when compared with standered TLC sample.when refering the HPTLC quantification of reserpine it also shows that the kunapajala group have more persentage of reserpine in it. 10 grams of sample root powder was taken from each group and quantification of reserpine was done then the the group A that is Non kunapajala group showed the presence of 0.153% of reserpine where as the group B that is the Kunapajala group showed 0.222% of reserpine . so the study shows that the 0.069% improvement is there for kunapajala group

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Conflit of interest -There was no conflit of interests arised during the study

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