IJBPAS, March, 2021, 10(3): 842-851

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IDENTIFICATION AND CHARACTERIZATION OF RESERPINE IN *RAUWOLFIA SERPENTINA* AND GALLIC ACID IN *PHYLLANTHUS EMBLICA BY HPTLC*

NIKHITHA M[1*], INDU BHAVANI M[1*], MADHURI N[1*], PRACHET P[2] AND RAMARAO N[3]

1: UG Scholar, Chalapathi Institute of Pharmaceutical Sciences

2: Assistant professor, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences

3: Principal, Chalapathi Institute of Pharmaceutical Sciences

***Corresponding Author: Madhuri N: E Mail: nathanimadhuri28@gmail.com; Tel: +919490321993**

Received 11th May 2020; Revised 6th June 2020; Accepted 12th July 2020; Available online 1st March 2021

https://doi.org/10.31032/IJBPAS/2021/10.3.5394

ABSTRACT

The aim of present study was to identify and Characterize Reserpine in *Rauwolfia serpentina* (Indian snakeroot) and gallic acid in *Phyllanthus emblica* (Amla) in polyherbal formulation. Dried roots of rauwolfia serpentine and amla fruits were taken and were extracted for their constituents by Soxhelt apparatus **[1]**. The extract was dried using rotary film evaporator. Extract powder was taken and phytochemical tests were performed and results showed that alkaloids and vitamins were present along with many other constituents. These powders of plants along with polyherbal formulation were taken and solubility studies were carried out. Identification and characterization of constituents was done using sophisticated analytical technique HPTLC. Stationary phase used was Pre-coated aluminium TLC plates with silica gel 60F254 of 10 X 10 cm size and mobile phase was Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) with 8 µL/sec spraying rate of sample and detection was done at 254nm and 365nm respectively. R_f values 0.963 and 0.326 respectively. Hence we conclude that Reserpine and gallic acid are the major active constituents present in these plants.

Keywords: HPTLC, *Rauwolfia serpentine, Phyllanthus emblica*

INTRODUCTION

Rauwolfia serpentine is one of the plants belonging to Apocyanaceae family which have importance from ancient time as an antidote to snakebite **[2]**. It constitutes of alkaloid which has an antihypertensive activity **[3]**. In the modern era it is been used for depression, hypochondria and psychosis **[4]**. The main constituents present in the plant were alkaloids it is about 0.7 to 2.4%. It has nearly 80 active alkaloid constituents. Some of them are serpentine, reserpine, raubasine, yohambinine, Ajmalin, Ajmalicin. Reserpine is the active constituent for antihypertensive activity **[5]**.

Phyllanthus emblica is also known as Amla. It has many names based on the region but it belongs to the family Euphorbiaceae. It has many active constituents like quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin and vitamin C. It is used in treatment of anti oxidant activity, diarrhoea, skin disorders, respiratory infections, and premature aging, diabetes. Vitamin C is the active constituent for anti oxidant activity **[6]**.

HPTLC is an advanced technique than TLC to overcome the problems like time consuming, detection errors, accuracy of sampling, quantity of sample used and elution problems.

The present study was aimed to identify and characterize these active constituents in plants and formulation using HPTLC technique **[7]**. By adopting suitable and perfect method and technique these active constituents were extracted and were identified.

MATERIALS AND METHOD

Pre-coated aluminium TLC plates with silica gel 60F254 were procured from Merck manufactures. Various solvents like Toluene, Ethyl acetate, Formic acid, Methanol, Chloroform, Diethyl amine and Acetic acid of AR grade were procured from local suppliers National scientific products. HPTLC equipment manufactured by Aetron was used. Sample Applicator was used with software Spraylin for different sampling rates by hamilton syringe with different sizes of plates can be used. Documentation system was used for documenting the developed plates in 254nm and 365nm and visible light. Just TLC is the software used for analyzing the eluted compounds **[8]**.

METHOD DEVELOPMENT:

Preparation of *rauwolfia* extract sample solution:

Weigh accurately about 100mg of powdered rauwolfia drug and was added to 1ml of ammonia solution and kept aside for 10min.

To the above solution 10 ml of methanol was added and refluxed on the water bath for 10 min and filtered. The methanol extract was concentrated, dissolved, filtered and stored in 1 ml of methanol and used for further analysis **[9]**.

Preparation of amla extract sample solution:

Weigh accurately about 100mg of powdered amla drug and was added to 10ml of methanol solution and kept aside for 10min. The above mixture was refluxed on the water bath for 10 min and filtered. The methanol extract was concentrated, dissolved, filtered and stored in 1 ml of methanol and used for further analysis **[10]**.

Preparation of mixture sample solution:

About 210mg of formulated rauwolfia and amla powdered tablet mixture was weighed accurately. To the mixture sample add 10 ml of methanol and sonicate for 15min and filter it. The methanol extract was concentrated, filtered and stored in 1ml of methanol.

Preparation of mobile phase:

Mobile phase has been selected based on the phyto-constituent chromatogram detected for each extract by TLC. Several trails were conducted to get optimized mobile phase for the separation of active constituents present in the prepared sample solutions **[11]**. The optimized mobile phase used for the separation of the active constituents is toluene: formic acid: methanol: ethyl acetate (3:0.8:0.2:3 v\v\v\v).

A. SAMPLE APPLICATION:

Samples were spotted in the form of bands of band length 6mm and band width of 8mm with a100 µl Hamilton syringe on pre-coated aluminum TLC plates with Silica gel 60 F_{254} of 0.2 mm of thickness and 10 x 10 dimensions with the help of TLC applicator attached to HPTLC system, which was programmed through Spraylin software. 10µl and 30µl extracts of rauwolfia, amla and mixture were applied in six tracks as 8mm bands at a spraying rate of 8 seconds/µl with a injector dead space 20µl. Track 1&2 was 30µl, 10 µl of rauwolfia extract, track 3, 4 was 30µl and 10µl of mixture and track 5, 6 was 30µl, 10µl of amla extracts are applied on the TLC plate **[12]**.

B. CHROMATOGRAM DEVELOPMENT:

Development of the plate up to a distance of ¾ of plate was performed at 27± 2°C with mobile phase for each extracts in a HPTLC twin trough glass chamber previously saturated for 30 minutes with mobile phase **[13]**. Sample elution was carried out in ascending manner according to the

absorption capacity of the active components to be analysed. After elution the, TLC plates were taken out of the development chamber and dried.

C. SCANNING:

After drying, the plate was scanned and visualized under wavelength of 254nm for ultra violet and 366nm for fluorescence detection using the HPTLC documentation system using Aetron software. Images taken were cropped and were saved in the system with separate folder. The saved images are further analysed by using the Just TLC software.

D. QUANTIFICATION:

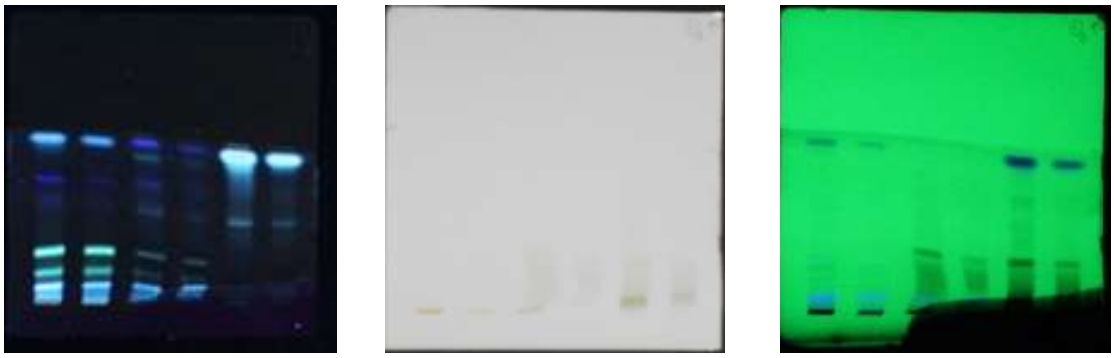
By using the Just TLC software the phyto-constituents present in the each extract of developed chromatographic finger print was detected based on comparing the R_f values of standard with that of sample and were tabulated. The saved images in the system are quantified by using the Just TLC software **[14]**. The 365nm fluorescence detection saved image is open with Just TLC software, choose the region of interest and crop the image. Select the number of tracks for quantification, first select track 1, 2 compare each other and detect the point which shows same fluorescence in both the tracks. 1& 2 tracks are compared with the mixture tracks 3& 4 for detection of active constituent present in mixture is same as that of rauwolfia for determining the Rf value of the rauwolfia. Tracks 5&6 are compared with each other for identifying the active constituent present in it, at a particulars spot they show the same fluorescence which is compared with the tracks 3& 4 for detection of R_f value for the amla extract. Thus, the active constituent present in amla is gallic acid and rauwolfia is reserpine are isolated, and observed in mixture. Mixture also contains both the phyto-constituents reserpine and gallic acid. All the tracks were compared with each other and represented in the graphical format in the form of peaks and R_f values were tabulated.

TRAIL 1:

Chromatographic conditions:

Table 1: Chromatographic Conditions for trail-1

Stationary phase	**Pre-coated aluminum TLC plates with silica gel 60 F_{254}**
Mobile phase	**Toluene: Ethyl acetate: Acetic acid: Formic acid (4; 9:4:1)**
Band length	**8mm**
Spraying rate	**10µl/sec**
Plate dimensions	**10cm x10cm**
Detection wavelength	**254nm and 365nm**
Room temperature	**$25^{\circ}C \pm 2^{\circ}C$**



365nm *Visible* *254nm*

Figure 1: Chromatogram developed using mobile phase with Toluene: Ethyl acetate: Acetic acid: Formic acid (4:9:4:1)

TRAIL 2:

Chromatographic condition:

Table 2: Chromatographic Conditions for trail-2

Stationary phase	**Pre-coated aluminum TLC plates with silica gel 60 F_{254}**
Mobile phase	**Chloroform: Toluene: Ethyl acetate: Diethyl amine (7:7:4:1)**
Band length	**8mm**
Spraying rate	**10µL/sec**
Plate dimensions	**10cm x 10cm**
Detection wavelength	**254nm and 365nm**
Room temperature	**$25^{\circ}C \pm 2^{0}C$**

365nm **Visible** **254nm**

Figure 2: Chromatogram developed using mobile phase with Chloroform: Toluene: Ethyl acetate: Diethyl amine (7:7:4:1)

TRAIL 3:

Chromatographic conditions:

Table 3: Chromatographic Conditions for trail-3

Stationary phase	**Pre-coated aluminum TLC plates with silica gel 60 F_{254}**
Mobile phase	**Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)**
Band length	**6mm**
Spraying rate	**10µL/sec**
Plate dimensions	**10cm x10cm**
Detection wavelength	**254 nm and 365 nm**
Room temperature	**25°C ± 2°C**

365nm ***Visible*** ***254nm***

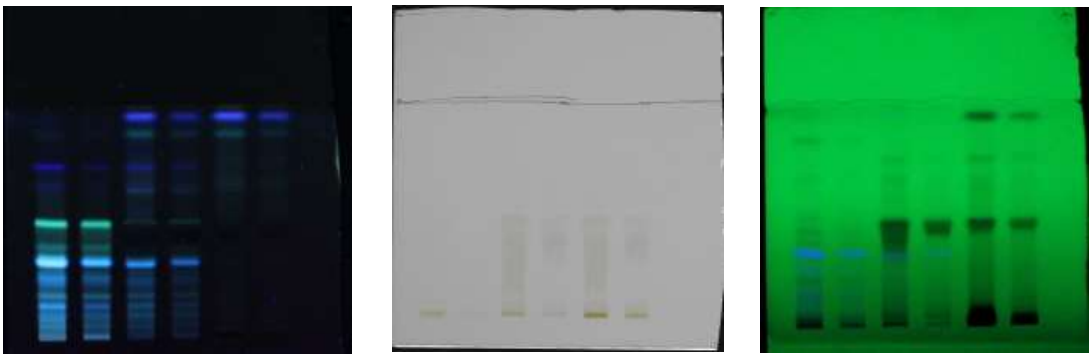
Figure 3: Chromatogram developed using mobile phase with Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)

OPTIMIZED METHOD

Chromatographic conditions:

Table 4: Chromatographic Conditions for optimized method

Stationary phase	**Pre-coated aluminum TLC plates with silica gel 60 F_{254}**
Mobile phase	**Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)**
Band length	**6mm**
Spraying rate	**8µL/sec**
Plate dimensions	**10cm x 10cm**
Detection wavelength	**254 nm and 365 nm**
Room temperature	**25°C ± 2°C**



365nm ***Visible*** ***254nm***

Figure 4: Chromatogram developed using mobile phase with Toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2)

RESULTS AND DISCUSSION

Phytochemical analysis

The chemical tests for various phytoconstituents in the raw materials were carried out and the results were recorded and detailed in **Table 5**.

Characterization using HPTLC:

The characterization was done by considering the sample taken from tablet triturate and analyzed against standard solutions of Amla and Rauwolfia. In all the trails six bands were applied on TLC plate according to the following **Table 6**.

The eluted bands were clearly visible under UV light of 365nm. Hence the further analysis of the compounds was done using the image of plate under 364nm using ***Just TLC software***.

Along with reserpine another compound was eluted in the standard solution of Rauwolfia which exhibits characteristic green fluorescence. The compound was found to be ajmalicine which was not present in the tablet triturate **(Figure 5)**.

R_f values of the eluted bands were listed in the **Table 7** below

The bands with id 1_2 and 2_2 belong to reserpine in standard rauwolfia solution with R_f value **0.333**. The bands with id 3_2 and 4_2 belong to est solution have almost similar R_f value i.e **0.319** and **0.326**.

The bands with id 5_1 and 6_1 belong to gallic acid in standard amla solution with R_f value **0.963**. The bands with id 3_1 and 4_1 belong to test solution have almost similar R_f value i.e **0.963** and **0.956**.

This evidence concludes the presence of reserpine and gallic acid in the prepared polyherbal tablets.

The concentration of the sample used for the study was 10 mg/ml. These are taken as standard parameters in all the trails. Various trails are carried out by changing mobile phase.

Table 5: Phytochemical analysis of Amla and Rauwolfia herbal powders

S. No	Phytochemicals	Amla	Rauwolfia
1.	Alkaloids	+	+
2.	Glycosides	+	_
3.	Flavanoids	+	_
4.	Steroids	_	+
5.	Phenolic compounds	+	+
6.	Tannins	+	+
7.	Terpenoids	_	_
8.	Sterol	_	_
9.	Carbohydrates	_	_
10.	Proteins	+	+
11.	Aminoacids	+	+

(+ *indicates present*, – *indicates absent*)

Table 6: Description of Bands on TLC plate

Sample Bands	Compound	Standard/Test	Volume of sample applied
Band-1	Rauwolfia	Standard	30µL
Band-2	Rauwolfia	Standard	10µL
Band-3	Tablet triturate solution	Test	30µL
Band-4	Tablet triturate solution	Test	10µL
Band-5	Amla	Standard	30µL
Band-6	Amla	Standard	10µL

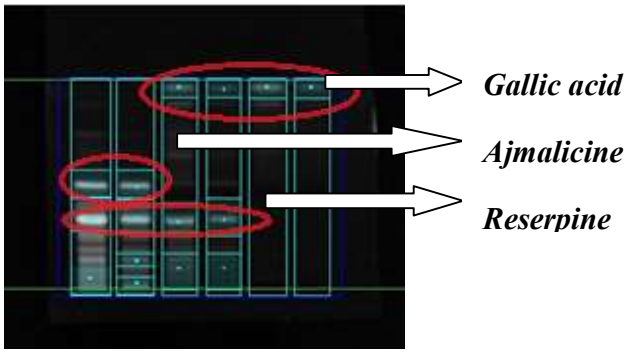


Figure 5: Identification of eluted spots

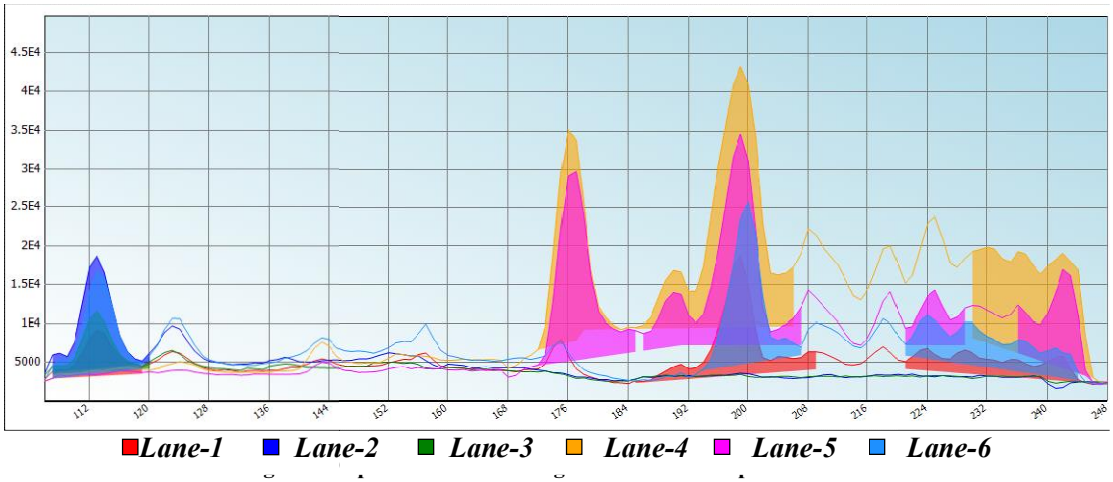


Table 7: R_f values of eluted bands

Band ID	R_f values	Note
1_1	0.489	
1_2	0.333	Reserpine
1_3	0.044	
2_1	0.481	
2_2	0.333	Reserpine
2_3	0.133	
2_4	0.022	
3_1	0.963	Gallic acid
3_2	0.319	Reserpine
3_3	0.096	
4_1	0.956	Gallic acid
4_2	0.326	Reserpine
4_3	0.096	
5_1	0.963	Gallic acid
6_1	0.963	Gallic acid

CONCLUSION

Finally we conclude that the prepared polyherbal formulation of amla and rauwolfia given the best results with acceptable limits. Wet granulation method best suits for the preparation of polyherbal tablets. Later it was characterized by using HPTLC technique for their presence of active chemical constituents in the prepared formulation. So that there by their synergistic activity can be considered and enhanced.

This study helps in reduction of cost, simple process, patience compliance can be enhanced and can be used for routine analysis.

ACKNOWLEDGEMENT

We finally like to acknowledge the our principal of Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur Prof. Rama Rao Nadendla and beloved chairman Sri Y. V. Anjaneyulu sir for providing necessary facilities and giving free hand to do these type research studies.

REFERENCES

[1] Parwez Alam, Dr. Jyoti Guptha and Seema Firdouse, Development of polyherbal solid dosage formulation with Amla, Withania and Tulsi extract in different ratio, World Journal of Pharmaceutical Research (2016), Vol.5, Issue.9, 1632 – 1640.

[2] Fairuz Fatema Priya, Mohammad Sayful Islam, *Phyllanthus emblica* Linn (Amla) – A natural gift to humans: An overview, Journal of Diseases and Medicinal plants (2019), Vol.5, No.(1), 1 – 9.

[3] http://www.ayurveda.hu/api/API-Vol-1.pdf, The Ayurvedic Pharmacopoiea of India, Vol.1, Part – 1.

[4] https://en.wikipedia.org/wiki/Phyllanthus_emblica, Wikipedia.

[5] William. R Livesay et al, Treatment of hypertension with *Rauwolfia serpentina* alone and with other drugs, JAMA (1954), 155(12), 1027-1035.

[6] https://en.wikipedia.org/wiki/Rauvolfia_serpentina, Wikipedia.

[7] Sonia.K *et al*, HPTLC Method Development and Validation: An overview, J. Pharm. Sci. & Res. Vol. 9(5), 2017, 652-657.

[8] Dr. Harish Chandra Andola, Dr. Vijay Kant Purohit, High Performance Thin Layer Chromatography (HPTLC): A Modern Analytical tool for Biological Analysis, Nature and Science 2010, 8(10), 58-61.

[9] Singh M, Kumar D, Naman S, Madhavi N, Singh PA, Bajwa N, Bajwa N, Baldi A, Validation of HPTLC Method for the Simultaneous Estimation of Ascorbic Acid and Gallic Acid in Amla Juice Preparation. JDDT [Internet]. 15Jul.2019 [cited 19Apr.2020]; 9(4):227-31.

[10] Devendra Kumar Pandey *et al*, A validated and densitometric HPTLC method for simultaneous quantification of reserpine and ajmalicine in *Rauwolfia serpentina* and *Rauwolfia tetraphylla*, *Revista Brasileira* de Farmacognosia, Vol.26, Issue.5, Sep – Oct 2016, 553 – 557.

[11] G.S. Panwar and S.K. Guru, Alkaloid profiling and estimation of reserpine in *Rauwolfia serpentina* plant by TLC, HPTLC and HPLC, Asian Journal of Plant Sciences (2011), Vol.10, 393 – 400.

[12] Rungsung W *et al*, Pharmacognostical profiling on root *Rauwolfia serpentina*, IJPPR 2014, Vol.6 (3), 612 – 616.

[13] Sadhana Singh, Vinay Verma, Rashmi Yadav and Brijesh Singh, Pharmacognistical study of Amlaki, Journal of Pharmacognosy and Phytochemisty, 2018, Vol.7(3), 3476 – 3480.

[14] Bhavana Srivastava, Vikas Chandra Sharma, R. Singh, P. Pant and A.D. Jadav, Substitution of roots with small branches of *Rauwolfia serpentina* for therapeutic uses – A phytochemical approach, AYUSHDHARA (An International Journal of Research in AYUSH and Allied Systems, 2015, Vol.2 (6), 373 – 378.