DETERMINATION AND COMPARISON BETWEEN THE HIGH PERFORMANCETHIN LAYER CHROMATOGRAPHY OF PUNARNAVASAVA AND PUNARNAVINE

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ABSTRACT.

Punarnavasavam is a widely used anti-inflammatory and anti-oedema tic Ayurveda medicine. This medicinal property is due to Punarnavine, an alkaloid present in Boerrhavia diffuse (Nyctaginaceae). Quality control for Punarnava is highly essential for obvious reasons. Hence an attempt has been made by isolating Punarnavine from the plant, Boerrhavia diffuse and melting point of the isolated compound has been determined for confirming the purity. HPTLC was performed and the peak found and the area under the curve was determined.

INTRODUCTION

Thin layer chromatography studies are among the key identity tests in most Pharmacopoeial monographs. An extension of TLC is high performance thin layer chromatography (HPTLC) is robust, simplest, rapid and efficient tool in quantitative analysis of the compounds, offers better resolution and lower limit of detection. Punarnavavsava is a self-generated alcoholic formulation containing Punarnava commonly used to treat arthritic inflammation has been selected for the comparative study with the isolated Punarnavine alkaloid from Boerrhavia diffusa.

MATERIALS AND METHODS

MATERIALS

Punarnavasava formulation – 100ml (Bhushan 1389)
Dried and powdered Boerrhavia diffusa -100gm.
Ethanol 95%, Diethyl ether, Ammonia solution, Chloroform.

METHOD

Isolation of Punarnavine from Punarnava – 100g powdered drug was extracted with ethanol 95% by soxhlation at 70ºc and solvent was recovered, dried, the extract was concentrated and Boerhaavic acid was filtered off. Filtrate was concentrated and extracted with hot water and again concentrated to yield Potassium nitrate. It was filtered and made Ammoniacal and extracted repeatedly with Chloroform. The extract was then evaporated and the residue was macerated with diethyl ether, evaporated and obtained amorphous Punarnavine was crystallized from ethanol. Isolation of punarnavine from punarnavasava – 25 ml of the asava was concentrated to complete dryness and the residue was extracted with 3 successive portions of chloroform. The Chloroform layer was separated and evaporated to dryness to leave a pale yellow residue.

CONDITIONS OF CHROMATOGRAPHY

1. Test plate : HPTLC Silica gel 60 F254 Aluminium sheets 20x20 cm
2. Starting Position: 10mm
3. Plate Width: 40mm
4. Band width: 4mm
5. Space Width: 10mm

6. Rate: 4µl/sec
7. Volume: 2µl
8. Separation technique: Ascending
9. Development chamber: Twin-trough glass chamber (10 X 10 cm2)
10. Mobile phase : Chloroform: Ethyl Acetate: Methanol (4:4:2)
11. Spraying reagent: 10% Alcoholic Sulphuric Acid
12. Scanner: CAMAG TLC SCANNER 3

RESULTS AND DISCUSSION

Chemical Tests for the identification of isolated Punarnavine

Reagents	Result
Ferric Chloride	Green colour
Concentrated Sulphuric Acid	Greenish Yellow
Concentrated Nitric Acid	Red
Concentrated Hydrochloric Acid	No colour
Dragendorff reagent	Brown Precipitate

The melting point of the isolated Punarnavine was found to be 234°C

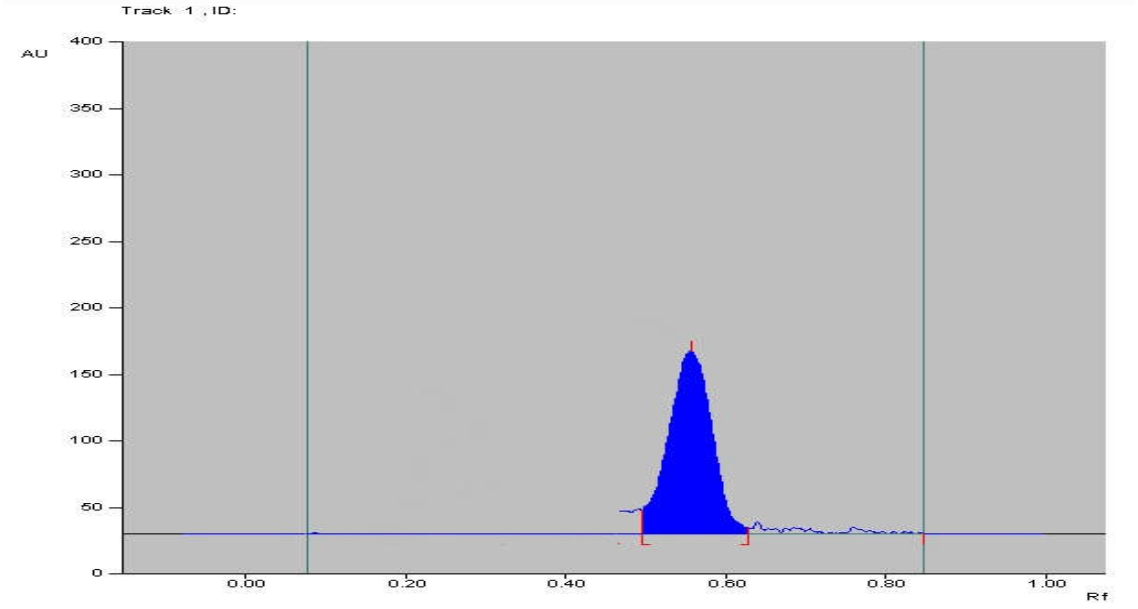
PHYSICO-CHEMICAL EVALUATION OF PUNARNAVASASAVA

PARAMETERS	VALUE
COLOUR	DARK BROWN
ODOUR	CHARACTERISTIC
TASTE	CHARACTERISTIC
TOTAL SOLIDS	12.34%
SPECIFIC GRAVITY	1.0216g/ml
Ph	3.81
ALCOHOL CONTENT	6.62%v/v

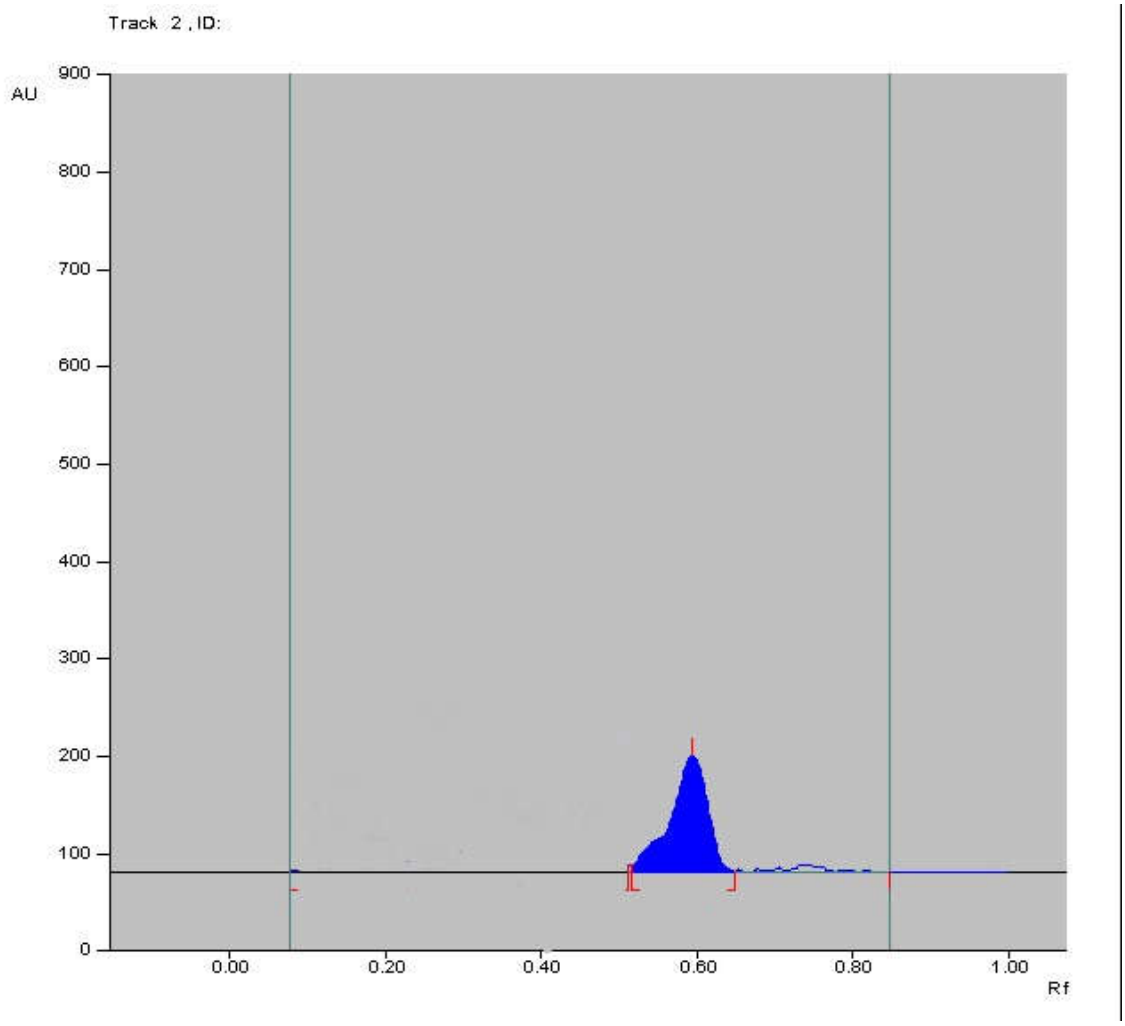
DETERMINATION OF HPTLC OF PUNARNAVASASAVAM AND PUNARNAVINE

Sample	Spot no.	Rf	Area	Area%
Punarnavine (Punarnavasava)	1	0.63	5803.7 AU	49.60%
Punarnavine(Crude drug)	2	0.65	4328.6 AU	20.59%

HPTLC OF THE EXTRACTED PUNARNAVINE



HPTLC OF THE ISOLATED PUNARNAVINE



The amount of Punarnavine present in Punarnvasava = 0.013 mg/ml
The amount of Punarnavine present in the crude drug = 0.020mg/ml

DISCUSSION AND CONCLUSION

Punarnavine was isolated from Boerrhavia diffuse and confirmed by chemical tests and the melting point was determined. The physic-chemical parameters of Punarnavasava were determined and the alkaloid Punarnavine was extracted. Both the isolated and extracted Punarnavine were spotted and HPTLC was performed and compared. From the Area under curve the amount of Punarnavine present in the crude drug and that in Punarnavasava was calculated. The amount of Punarnavine present in the crude drug was found to be greater than that in the Punarnavasava.

REFERENCES

The Ayurvedic Pharmacopoeia of India,Part II,Volume II,Formulations, page no. 55-57
Indian Journal of Experimental Biology Vol 52, August2014, page no.799-807