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## PRECISE AND SENSITIVE HPTLC METHOD FOR QUANTITATIVE ESTIMATION OF WEDELOLACTONE IN *ECLIPTA ALBA* HASSK

Mitesh D. Phale\*, Purnima D. Hamrapurkar, Manasi A. Chachad,

Priti S. Patil, Sandeep B. Pawar

Department of Pharmaceutical Analysis,

Prin. K. M. Kundnani College of Pharmacy,

Jote Joy Building, Rambhau Salgaonkar Marg,

Cuffe Parade, Colaba, Mumbai- 400 005, India

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### ABSTRACT

Wedelolactone, a characterizing compound present in the plant *Eclipta alba* is used in *viral hepatitis*, liver diseases, skin and hair care. Wedelolactone was extracted from the plant using Soxhlet and supercritical fluid extraction technique and the compound was isolated using column chromatography. The compound was then characterized by spectroscopic techniques. The method employed HPTLC aluminum plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of toluene: ethyl acetate: formic acid (5:5:0.1v/v). Densitometric analysis of wedelolactone was carried out in the absorption mode at 351 nm. Quantitative evaluation was achieved by measurement of peak intensity. The method was validated for precision, accuracy, robustness and recovery. The HPTLC method can be applied for identification and quantitation of wedelolactone in herbal extracts of *Eclipta alba*.

**Keywords:** *Eclipta alba*, Wedelolactone, Sensitive, Quantitative estimation.

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### INTRODUCTION

*Eclipta alba* Hassk, belonging to family Asteraceae, commonly known as Bhringaraj, Bhangra, Mochkand, and Maka is an annual herb grown throughout India and Southwestern U.S. in moist and dump land.<sup>1</sup> *E. alba* has been mentioned in ancient texts to

be a nervine tonic,<sup>2,3</sup> in addition to possessing hepatoprotective, hair growth promoting and antiaging properties. The plant has been extensively studied for its hepatoprotective activity and a number of herbal preparations comprising *E. alba* are available for treatment

of jaundice and viral hepatitis.<sup>4-7</sup> Additionally, studies on antianaphylactic activity of the plant are also carried out.<sup>8</sup> The plant is reported to contain the phytoconstituents eclalbatin, alpha-amyrin, ursolic acid, oleanolic acid<sup>9</sup> ecliptasaponin, daucosterol, stigmasterol-3-O-glucoside,<sup>10</sup> and coumestans like wedelolactone and demethyl wedelolactone as main active principles.<sup>11</sup> Wedelolactone (Figure 1.) forms the major constituent of the plant for its use in the treatment of viral hepatitis. Thus, considering the major hepatoprotective activity of wedelolactone, an appropriate analytical procedure for the quantitative determination of wedelolactone in *E. alba* is of considerable importance.

For the quantitative estimation of plant products, HPTLC is a preferred chromatographic method over other chromatographic techniques like HPLC because of its simplicity, accuracy, cost-

## MATERIALS AND METHODS

### Plant material

The aerial plant of *Eclipta alba* Hassk was procured from Mumbai, and authenticated at Agharkar Research Institute, Pune, India. (Certificate dated 12/02/07)

### Chemicals and reagents

The wedelolactone standard was obtained from Natural Remedies Pvt. Ltd., Bangalore, India and characterized by Ultra-violet (UV), Infrared, Proton nuclear magnetic resonance and by mass spectroscopy to confirm their identity and purity. HPLC grade Toluene, and Ethyl acetate was purchased from Merck (Darmstadt, Germany). And Methanol, Formic acid of AR (Analytical Reagent) grade was procured from Qualigens Fine Chemicals, Mumbai, India. Deionised and ultra pure water used in all experiment was obtained from Milli-Q system (Millipore, USA).

effectiveness and rapidity. Literature survey reveals that various chromatographic methods such as HPLC<sup>12-14</sup> and HPTLC<sup>15-16</sup> have been reported for quantification of wedelolactone. However these methods suffer from drawback such as the poor resolution, reproducibility and sensitivity. Literature survey further reveals that extraction of wedelolactone has been carried out using the classical method of extraction such as soxhlet method. In this proposed work an attempt has been made to extract wedelolactone using 'state-of-the-art' technique i.e., Supercritical Fluid Extraction (SFE) technique. An attempt has also been made to develop and validate HPTLC method for the analysis of wedelolactone which would be highly sensitive, having good resolution, shorter retention time and reproducible. Various validation aspects of the analysis, namely peak purity, recovery and the limits of detection and quantification etc., have been measured.

### Sample Extraction

#### *Soxhlet method for extraction*

The powdered aerial parts of *E. alba* were extracted with methanol using Soxhlet apparatus and the extract obtained was then diluted appropriately with methanol.

#### *Supercritical fluid extraction (SFE)*

The plant material was powdered and passed through sieve of # 40 mesh size. 1.0 gm of powdered plant material were weighed and transferred to the SFE vessel. The material was extracted using CO<sub>2</sub> gas (supercritical fluid) and methanol as co-solvent. The conditions of SFE were optimized. The optimized conditions thus followed were; Gas-CO<sub>2</sub>, Pressure-150 bar, Temperature 45°C, Modifier- methanol, Flow rate- 0.2 ml/min, CO<sub>2</sub> flow rate – 2.5 ml/min, and extraction time- 30 min. The extract was then concentrated on removal of solvent under

vacuum. The concentrated extract was then diluted appropriately with methanol.

### **Phytochemical Evaluation of *Eclipta alba***

To assure the presence of phytoconstituents in the plant and also to check adulteration if any, preliminary studies like phytochemical screening of the plant are of considerable importance before starting the analysis. In addition to screening of the plant, the estimation of extractive values also helps in optimizing the solvent for extraction processes.

#### ***General qualitative chemical tests for the presence of phytoconstituents***

Sample of plant material were subjected to phytochemical tests for alkaloids, glycosides, phenols and tannins to confirm literature reports of phytoconstituents of the plant.

#### ***Physicochemical properties of *E. alba****

Physicochemical properties namely water soluble extractive, alcohol-soluble extractives, ash values, foreign organic matter and moisture content were determined.

### **HPTLC**

#### ***Prewashing of Plates***

HPTLC was performed on 10 cm x 20 cm precoated HPTLC plates from E.Merck (Darmstadt, Germany). The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried and activated for 30 min at 110°C.

#### ***Procedure***

A methanolic solution of wedelolactone (1 mg/ml) was prepared. This solution was

further diluted with methanol to yield a solution containing 100 µg/ml. Different concentrations of wedelolactone were applied on plates as 6 mm bands, 6 mm apart and 1 cm from edge of the plate, by means of Camag Linomat IV automatic sample applicator fitted with 100 µl Hamilton syringe. A methanol blank was applied to parallel track. After drying of bands the TLC plates were placed in the Camag twin trough glass chamber saturated with mobile phase containing toluene: ethyl acetate: formic acid (5:5:0.1 v/v). Each TLC plate was developed to a height of about 80 mm under (25– 30°C and 40–50% relative humidity) laboratory conditions. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 206 nm, using a deuterium lamp, by means of Camag scanner III densitometer. The densitogram obtained by HPTLC of wedelolactone standard and extract are shown in Figure 2 and 3 respectively. This method was followed for all quantitative analysis. CATS software (Version 3.17) was used for data acquisition and processing of the plate. The scanning speed was 20 mm s<sup>-1</sup>, the offset was 10 %, and the sensitivity (SPAN) was optimized to 20 min. Peak height and peak area were integrated for the entire track. The calibration plot was constructed by plotting peak area against concentration of wedelolactone. The reproducibility of this method was ascertained by repeating the experiment three times.

#### **Method Validation**

The method was validated for specificity, accuracy, precision by use of calibration standards of wedelolactone and it is applied to extracts of *E. alba*. Limit of detection and limit of quantitation was determined by spotting different concentrations of wedelolactone with visualization under UV and scanning at 351 nm, using a deuterium lamp, by means of Camag scanner III

densitometer. The lowest concentration that could be detected for three replicate spots was regarded as the limit of detection. The lowest concentration for which the RSD [%] of six replicate spots was less than 5 % was regarded as the limit of quantitation. Accuracy was determined by measurement of the recovery of wedelolactone standard added at three different levels to plant extract, each being analyzed as described for the assay. Intraday and interday precision was determined by applying 100, 600, 1000 ng standard wedelolactone. After development and densitometric scanning of the plates the peak-area response was measured and

## RESULTS AND DISCUSSION

Results from general qualitative chemical testing of *E. alba* for the presence of phytoconstituents reveals the presence of glycoside, tannins, sterols, carbohydrates and amino acids. Quantitative estimation of the physicochemical properties expressed as (%w/w) of the dry *E. alba* plant were : moisture content 7.40, water soluble extractives 28.43, alcohol soluble extractives 14.00, total ash 8.41, acid insoluble ash 0.27, water insoluble ash 0.17, foreign organic matter 1.02. From all the obtained results it was observed that water soluble extractive value was higher indicating that the amount of polar phytoconstituents are more. Thus taking into account the polarity of phytoconstituents present, the composition of mobile phase was selected and optimized during method development.<sup>19</sup>

The development of the HPTLC method for the quantitative estimation of herbals has received considerable attention in recent years because of its importance in routine quality control analysis. The extraction solvent selected plays an important role and should take into account the chemical nature and polarity of the sample constituents. Considering the objective which was

precision was calculated as RSD [%]. The developed method was validated as per ICH guidelines. Further extraction, method development and experimental calculations were performed with help of a listed reference papers.<sup>17, 18</sup>

### Application of the validated method

Extracts of plant material *E. alba*, obtained as described in sample extraction, were diluted with methanol and later with mobile phase. All samples were analyzed in duplicate and the amounts of wedelolactone present in the sample were determined by use of calibration plots obtained.

quantitation of the compound wedelolactone, which was more soluble in methanol as compared to water, the solvent chosen for extraction was methanol using classical method and supercritical fluid extraction technique. Various mobile phases reported were tried and modified for chromatography of wedelolactone in this study to overcome the drawbacks of the reported methods. Considering the nature of wedelolactone, solvent system for polar compound i.e. Chloroform: Methanol was tried in different ratios. But it failed to achieve the separation and resolution due to the high polarity index of the methanol. The system also failed to give the required separation because of the high evaporation rate of chloroform due to which the composition was not remaining constant during development stage. Hence Methanol was replaced with Ethyl acetate and chloroform was replaced with Toluene. The system used was toluene: ethyl acetate (1:1v/v). The system gave considerable separation but wedelolactone being polar did not give good resolution with smudging on the plate. Formic acid was added to the solvent system to alter the polarity of the mobile phase and overcome this problem. Hydrogen bonding between wedelolactone and formic acid, aided wedelolactone to give

good resolution and a good peak shape with shorter retention time. Thus after considerable efforts the optimized developing system for quantitative analysis of wedelolactone by HPTLC method consisted of toluene: ethyl acetate: formic acid (5:5:0.1v/v) which gave a good separation and resolution of the constituents of *E. alba*. The plate material employed was normal phase silica gel F<sub>254</sub>. Scanning of wedelolactone on HPTLC plate showed UV max at 351 nm, hence quantitation was carried out at 351 nm using Camag TLC scanner II.

The method developed was validated for limit of detection (LOD) which was found to be 40 ng per spot, limit of quantitation (LOQ) was found to be 100 ng per spot. The method was found to be linear over the range 100 – 1000 ng/spot with coefficient of regression 0.999. Intra-day and inter-day precision studies showed a % CV was less than 2.00%, indicating the method was precise. The accuracy values obtained, in the range 99.77 – 100.27 % for wedelolactone in *E. alba* are

## CONCLUSION

A rapid, simple, accurate, sensitive and specific HPTLC method for the detection and quantitative estimation of wedelolactone from *E. alba* extract had been successfully developed, with optimum retention time. Apart from the greater precision and sensitivity attained using this HPTLC method, the specificity offered is undoubtedly another advantage compared to the other

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## REFERENCES

1. Kirtikar, KR and Basu, BD (1998), “*Indian medicinal plant*”, 2<sup>nd</sup> Ed.,

indicative of excellent accuracy and recovery. There was no evidence of peaks or any other interfering co eluting peaks at the RF of wedelolactone (0.43). This indicates the method is specific. Stability studies were carried out for standard. It was found to be stable in sample solution, prior to development and after development. The summary of validation parameters and accuracy, precision is shown in Table. 1 and 2 respectively.

The developed method was then validated and successfully applied for quantitation of wedelolactone from various extracts obtained from soxhlet extracts and SFE of *E. alba*. The % w/w yield of wedelolactone in Supercritical fluid was 0.88, whereas in soxhlet extract % w/w yield was 0.79 %. Time required for extraction of wedelolactone by soxhlet method was 6.0 hr whereas for supercritical fluid extraction only 0.5 hr. This shows supercritical fluid extraction has more extraction efficiency and reduced extraction time than Soxhlet method.

methods/techniques of analysis. The outcome justifies the use of HPTLC and the validation data convincingly demonstrates the adequate performance of the proposed method of analysis that may help in fingerprinting analysis as well as quantitative estimation of wedelolactone from different geographical variety of *E. alba*. Also the study concludes that SFE technique used has more extraction efficiency over traditional methods of extraction.

Mumbai, India for supercritical fluid extraction facilities, provided.

International Book Distributors, Dehradun, 1360-1361.

2. Uniyal, RC; Sandhu, S and Chandok, JK (1998), “*Herbology: The Ayurvedic*



- Encyclopedia*”, Sri Satguru Publications, India, 77-88.
3. Satyavati, GV; Rana, MK and Sharma, M (1976), “*Medicinal Plants of India*”, Vol. 1, Cambridge Printing Works, Delhi, 370-372.
  4. Saraf, AP; Joglekar, SN; Nabar, SD and Mulay, MS (1991), “Evaluation of protective effect of 'Hepatogard' a compound herbal formulation in carbontetrachloride induced liver damage”, *Antiseptic*, Vol. 88, 511-516.
  5. Singh, B; Saxena, AK; Chandan, BK and Agarwal, SG et al. (1993), “Hepato protective effect of ethanolic extract of *Eclipta alba* on experimental liver damage in rats and mice”, *Phytother Res*, Vol. 7, 154-158.
  6. Saxena, AK; Singh, B and Anand, KK (1993), “Hepatoprotective effects of *Eclipta alba* on subcellular levels in rats”, *J Ethnopharmacol*, Vol. 40, 155-161.
  7. Singh, B; Saxena, AK; Chandan, BK and Agarwal, SG et al. (2001), “*In vivo* hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves”, *Indian J Physiol Pharmacol*, Vol. 45, 435-441.
  8. Patel, MB; Panchal, SJ and Patel, JA (2009), “Antianaphylactic activity of alcoholic extract of *Eclipta alba*”, *J Young Pharm*, Vol. 1, 244-250.
  9. Upadhyay, RK; Pandey, MB; Jha, RN and Pandey, VB (2001), “Eclalbatin, a triterpine saponins from *Eclipta alba*”, *J Asian Nat Prod Res*, Vol. 3, 213-217.
  10. Zhang, M and Chen, Y (1996), “Chemical constituents of *Eclipta alba* (L.) Hassak”, *Zhongguo Zhong Yao Za Zhi*, Vol. 21, 480-481.
  11. Wagner, H; Geyer, B; Kiso, Y and Hikino, H et al. (1986), “Coumestans as main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulaceae*”, *Planta Med*, Vol. 5, 370-373.
  12. Murali, B; Amit, A; Anand, MS and Samiulla, DS (2002), “Estimation of wedelolactone and demethylwedelolactone in *Eclipta alba* Hassk by improved chromatographic analysis”, *J Nat Rem*, Vol. 2, 99-101.
  13. Wagner, H; Geyer, B; Kiso, Y and Hikino, H et al (1986), “Coumestans as the Main Active Principles of the Liver Drugs *Eclipta alba* and *Wedelia calendulaceae*”, *Planta Med*, Vol. 52, 370-374.
  14. Hamrapurkar, P; Chachad, M and Phale, M (2009), “Quantitative Estimation of Wedelolactone in *Eclipta alba* Hassk using High Performance Liquid Chromatography”, *Natural Products: An Indian Journal*, Vol. 5, 57-60.
  15. Thorat, RM; Jadhav, VM; Kadam, VJ and Kamble, SS et al (2009), “Development of HPTLC method for estimation of Wedelolactone, Quercetin and Jatamansone in Polyherbal Formulation”, *International Journal of ChemTech Research*, Vol. 1, 1079-1086.
  16. Patel, MB; Kadakia, VM and Mishra, SH (2008), “Simultaneous estimation of andrographolide and wedelolactone in herbal formulations”, *Ind J Pharm Sci*, Vol. 70, 689-693.
  17. Hamrapurkar, P; Pawar, S and Phale, M (2010), “Quantitative Estimation of Phyllanthin in *Phyllanthus amarus* using High Performance Thin Layer Chromatography”, *J Planar Chromatogr – Modern TLC*, Vol. 23, 112-115.
  18. Hamrapurkar, P; Phale, M and Shah, N (2009), “Quantitative estimation of efavirenz by high performance thin layer chromatography”, *J Young Pharm*, Vol. 1, 359-363.
  19. Hamrapurkar, PD; Thakurdesai, LV and Phale, MD (2010), “Quantitative

estimation of silybin in *Silybum marianum* mother tincture using high

performance layer chromatography”, *Int J Green Pharm*, (in press).

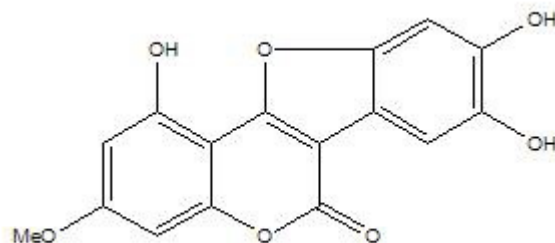
**Table 1:** Validation parameters

Parameters	Values
Detection limit (ng/ml)	40 ng/ml
Quantitation limit (ng/ml)	100 ng/ml
Calibration range (ng/ml)	100 – 1000 ng/ml
Repeatability (RSD <sup>a</sup> , n=3)	0.11-1.33
Correlation coefficient (r <sup>2</sup> )	0.999

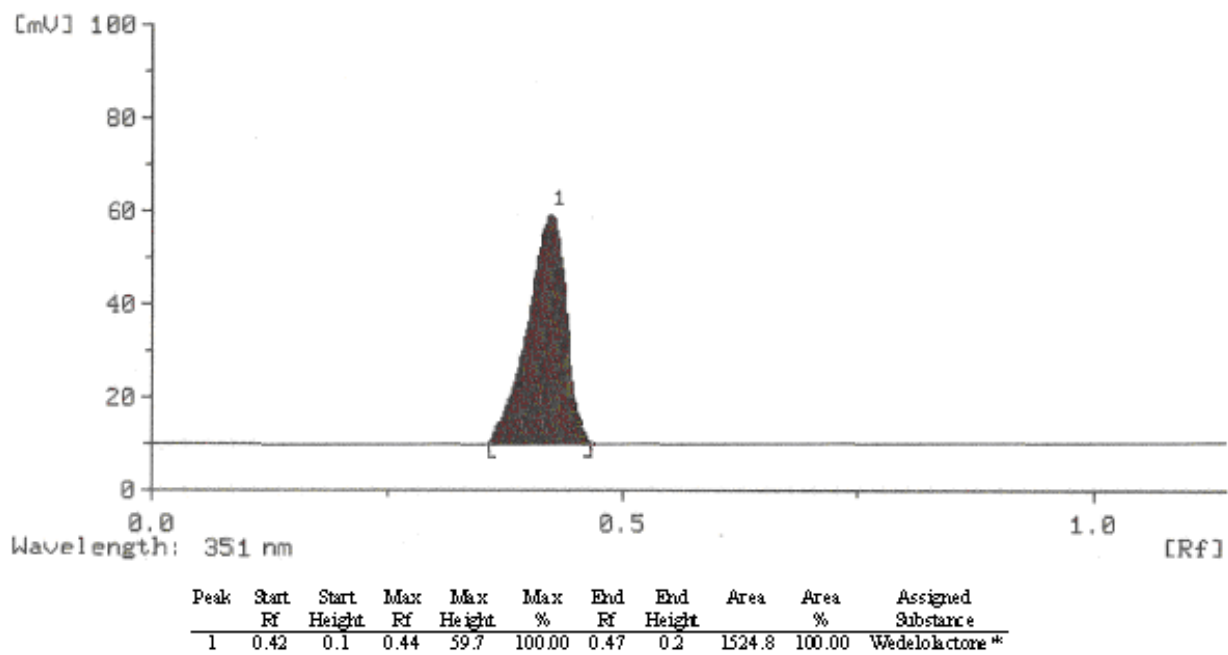
<sup>a</sup>=Relative Standard Deviation

**Table 2:** Summary of precision and % recovery

Precision		% Recovery		
Actual Concentration (ng/ml)	Measured Concentration (ng/ml) ± S.D.; % R.S.D.			
	Intra-day	Inter-day	Intra-day	Inter-day
100	100.3 ± 1.332; 1.33	99.7 ± 0.869; 0.87	100.27	99.77
600	600.3 ± 0.681; 0.11	599.9 ± 1.191; 0.20	100.05	99.99
1000	999.8 ± 1.394; 0.14	1000.1 ± 2.278; 0.23	99.98	100.02

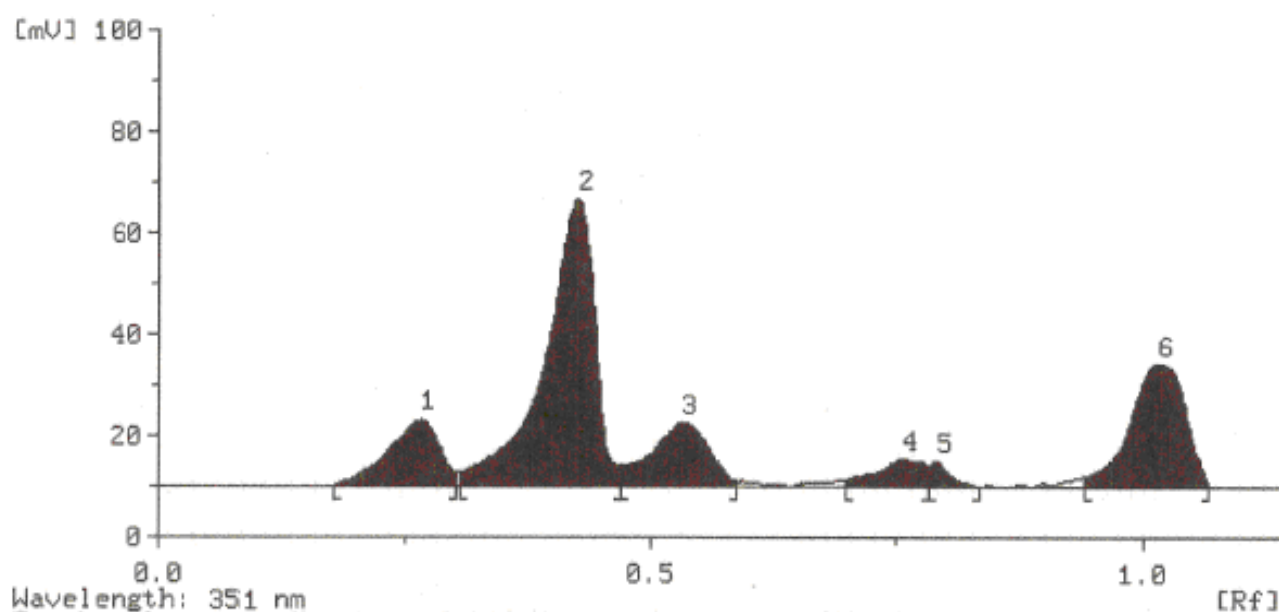


**Figure 1:** Chemical structure of wedelolactone



**Figure 2:** Representative Chromatogram of standard wedelolactone at 351 nm





Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned Substance
1	0.18	0.1	0.27	13.1	11.20	0.30	2.8	557.3	11.35	Unknown*
2	0.31	2.8	0.43	56.7	48.37	0.47	4.1	1614.3	47.65	Wedakblactone*
3	0.47	4.1	0.53	12.6	10.72	0.59	1.0	601.5	12.25	unknown*
4	0.70	1.3	0.76	5.5	4.69	0.79	4.0	217.3	4.42	unknown*
5	0.79	4.0	0.79	5.1	4.33	0.84	0.0	79.5	1.62	unknown*
6	0.94	1.9	1.02	24.3	20.69	1.07	0.0	1015.5	22.71	unknown*

**Figure 3:** Representative HPTLC Chromatogram of extract at 351 nm