Development of HPTLC Fingerprint of *Eclipta alba* L. for Quality Evaluation

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Abstract

The present study was designed to determine the HPTLC profile of the medicinally important plant *Eclipta alba* L. The chloroform : methanol (7:3) was employed as mobile phase for phyto-constituents. Linear ascending development was carried out in 20cm x 10cm twin trough glass chamber (Camag, Mutenz, Switzerland) saturated with the mobile phase and the chromatoplate development for two times with the same mobile phase to get good resolution of phytochemical contents. The developed plate was seen under UV light 254 nm and 366 nm. The methanolic extract of whole parts of *Eclipta alba* L showed the presence of 10 different types of phyto-constituents with different Rf. values. The developed HPTLC fingerprints will help the herbal drug industry to distinguish the adulterant and standardization of herbal formulations. Such chemo finger printing will act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies

Keywords: *Eclipta alba* L., Weadelolactone, HPTLC fingerprint, Phytochemistry

Introduction

Eclipta alba L., 'Bhringaraj' in Hindi and 'King of the Hair' and 'False Daisy' in English, belongs to family Asteraceae. Plant is native to the tropical and sub tropical regions and grows as common weeds throughout India, ascending upto 1800m in the Himalayas, common in areas of upper Gangetic plains, in pasture lands, roadsides in Utter Pradesh, all districts of Bihar, Madhya Pradesh, Uttar Pradesh, Orissa and Punjab. It is an annual herbaceous plant, erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate (Sharma *et al.,* 2001; Roy *et al.,* 2008). The genus name comes from the Greek word meaning "Deficient," with reference to the absence of the bristles and awns on the fruits. The specific *Eclipta alba* L. means white which refers to the color of the flowers (Mehra and Handa, 1968; and Kapoor, 2001).

The major chemical constitutes of plants are wedelolactone, desmethylwedelolactone, furanocoumarins, eclalbatin oleanane and taraxastane glycosides (Sikroria *et al*., 1968; Sarg and Khafagi, 1981; Jain & Singh 1988; Singh, 1988; Singh & Bhargava, 1992). The plant is commonly used in hair oil for healthy black and nourishment (Khare, 2004). The fresh juice of leaves is used for increasing appetite, improving digestion and as a

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mild bowel regulator and in viral hepatitis to promote bile flow and protect the parenchyma and enhance memory (Singh *et al.,* 2001). It is also used as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder. Charaka advises taking the juice of plant with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. This plant is well documented and several *in vitro* and *in vivo* studies describe its antiageing agent, hepatoprotective, anti inflammatory diuretic, hypertensive, immune stimulant, anti hyperglycaemic acid, and analgesic, antivenom, anticancer, antioxdent, antiviral, antibacterial, antifungal, spasmogenic property ,inflammations, gastric disorders, anorexia, worm infection, skin diseases, ulcers, ophthalmic disorders, headache, hypertension leprosy, fever, and jaundice (Wanger *et al*., 1986; Wagner and Fessler, 1986; Jayaram *et al*., 1987; Chandra *et al.,* 1987; Sharma *et al*., 1989; Saxena, 1993; Singh, 1993; Zhang and Chen, 1996; Pandey *et al*., 1997; Kirtikar & Basu, 1999; Leal *et al.,* 2000; Upadhyay, 2001; Syed, 2003; Jayatirtha and Mishra, 2004; Joshi, 2004 and Sawant *et al.,* 2004). It is also used in catarrhal jaundice and for skin diseases (Dixit and Achar, 1981; Sankaran, 1984; Ananthi *et al*., 2003; Thakur and Mengi 2005).

Present study deals with the HPTLC finger print profiling of *Eclipta alba* L. which will help the herbal drug industry to distinguish the adulterant and standardization of herbal formulations.

Material and Methods

a) Plant material and extraction

Fresh plant materials were collected from Ghaziabad and Haridwar, India in the month of August 2011. The collected plant materials were authenticated with the help of standard floras and pharmacopoeial reference (Anonymous, 2010). The whole plant was shade dried and powdered then extracted with 500 ml methanol for 8 to 12 hours by using Soxhlet apparatus. Extracts was filtered through Whatman paper no. 42 and were concentrated under reduced pressure and finally vacuum dried. The yield of the methanolic extract was 11.2 % w/w. The protocol for preparing sample solutions was optimized for high quality fingerprinting and also to extract the marker compounds efficiently. Since the marker compounds were soluble in methanol, therefore methanol was used for extraction. For the experimental work pre-coated silica gel 60 F_{254} HPTLC plates, reference standard 'wedelolactone' (Purity: 90 % w/w) and analytical reagent (AR) grade chemicals were used.

b) Physico-chemical studies

Physico-chemical characters were determined as per standard methods described in Indian Pharmacopoeia (IP), (1996), Lala (1993) and Kokate *et al*. (2005).

c) HPTLC analysis

A densitometric HPTLC analysis was carried out to develop characteristic fingerprint profile of methanol extract of both the sample with reference 'weadelolactone' for standardization purpose; 5 mml sample solution of each of methanol extract with reference were applied on silica gel 60 F254 precoated (E-Merck, India) plate of uniform thickness of 0.2 mm using CAMAG Linomet-5 automated TLC applicator with the nitrogen pressure 4kg/cm² from applicator syringe. Condition of work kept constant throughout the analysis. Following sample application bands were developed in a twin trough glass chamber that had been pre saturated with the mobile phase of chloroform : methanol (7:3 v/v) till proper separation of bands up to 8cm height. After development bands were scanned using CAMAG TLC scanner 3.

Observations

Diverse compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The separation was achieved using Chloroform : Methanol (7:3) as the mobile phase. The methanol extract of whole aerial parts of Eclipta alba L. showed the presence of 10 different types of phyto-constituents (at 254 nm) and 8 (at 366 nm) with different Rf. values (Figure 1 & 2 and Table 4 & 6). Reference standard 'weadelolactone' showed peak at Rf. 0.41 at 254 nm and Rf. 0.41 at 366 nm.

Table 1: Preliminary phytochemical tests

Nature Product	Test Performed	Result
Steriod	Liber mann's Reagent	+ ve
Flavonoid	Shinoda Test	+ ve
Tannin	Neutral FeCl3	+ ve
Carbohydrate	Molesch Test	+ ve
Starch	Iodine Solution	+ ve
Protein	Million's Solution	– ve
Saponin	NaOH Solution	+ve
Mucilage	Swelling in Water	– ve

Alkaloid	Mayer's Test, Dregandroff's Test, Wanger Test, Hager's Test	+ ve
Amino Acid	Ninhydine	– ve
Coumaine Glycoside	Alkaline Solution	+ ve
Fat and Oil	Pt. ether ext.	Trace
Diterpenoids	Picric acid (alkaline)	- ve

Table 2: Phytochemical screening of different extractions

Constituents	Dichloromethane extract	Methanolic extract	Aqueous extract
Alkaloids	+	+	+
Amino Acids	–	+	+
Flavonoids	+	+	+
Glycosides	+	+	+
Saponins	+	+	+
Steroids	+	+	–
Tannins	–	+	+
Terpenoids	+	+	+

Table 3: Peak display of weadelolactone at 254 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.41	2.0	0.89	77.7	100.0	0.91	0.00	1279.0	100.0

Table 4: Peak display at different Rf values of *Eclipta alba* methanol extract at 254 nm

Peak	Start rf	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1	0.00	6.2	0.02	696.1	68.68	0.07	50.4	13799.6	60.93
2	0.08	50.7	0.10	69.7	6.87	0.13	18.4	2011.7	8.88
3	0.14	18.2	0.17	30.0	2.96	0.19	21.1	1024.1	4.52
4	0.19	21.2	0.22	38.8	3.83	0.23	35.7	936.1	4.13
5	0.24	37.2	0.27	56.3	5.55	0.29	6.8	1487.1	6.57
6	0.30	6.9	0.31	21.6	2.13	0.39	0.2	455.3	2.01
7	0.37	0.8	0.40	55.0	5.43	0.45	1.1	1399.8	6.18
8	0.41	4.4	0.49	11.2	1.10	0.52	3.5	283.0	1.25
9	0.55	2.0	0.61	18.6	1.84	0.64	4.7	809.1	3.57
10	0.72	0.1	0.75	16.2	1.60	0.78	1.2	444.2	1.96

Table 5: Peak display of weadelolactone at 366 nm

Peak	Start Rf.	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1.	0.41	3.6	0.93	15.4	100.0	0.95	0.7	305.8	100.0

Table 6: Peak display at different Rf values of *Eclipta alba* at 366 nm

Peak	Start rf	Start height	Max Rf.	Max height	Height %	End Rf.	End height	Area	Area %
1	0.0	15.1	0.02	719.6	46.23	0.07	83.0	16411.9	39.84
2	0.07	83.1	0.1	127.0	8.24	0.14	66.1	4905.3	11.91
3	0.15	67.8	0.18	79.3	5.01	0.19	76.8	2271.6	5.51
4	0.22	89.7	0.27	150.2	9.75	0.29	15.9	5308.6	12.87
5	0.30	16.0	0.31	62.7	4.07	0.36	0.1	1347.9	3.27
6	0.41	1.5	0.4	191.0	12.4	0.45	1.5	3921.6	9.52
7	0.62	4.5	0.61	73.8	4.79	0.66	8.0	2788.1	6.77
8	0.74	0.0	0.75	137.8	8.95	0.79	0.2	4246.4	10.31

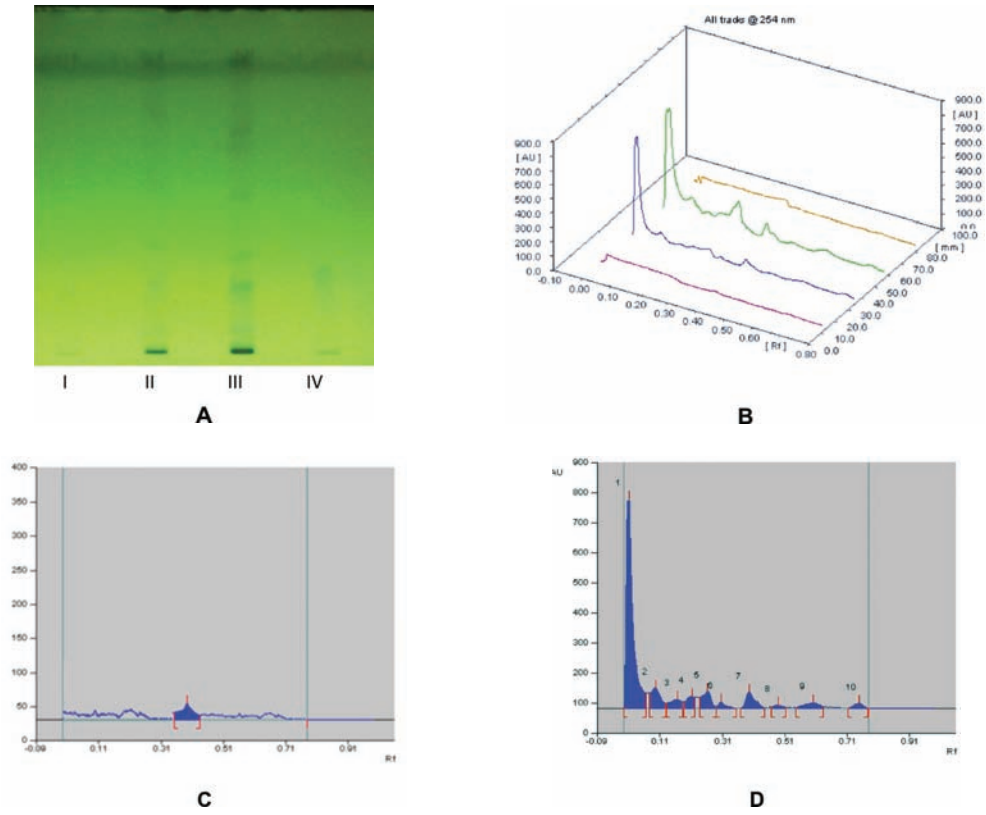

Fig. 1. HPTLC fingerprinting profile under UV 254 nm

A. HPTLC fingerprints profile of methanol extract and reference standard 'weadelolactone' (**I.** 3µg/ml of wedelolactone; **II.** 5µg/ml of methanol extract of sample; **III.** 10µg/ml of methanol extract of sample; **IV.** 6µg/ml of wedelolactone)

B. Overlay Chromatogram

C. Chromatogram of weadelolactone

D. Chromatogram of methanol extract

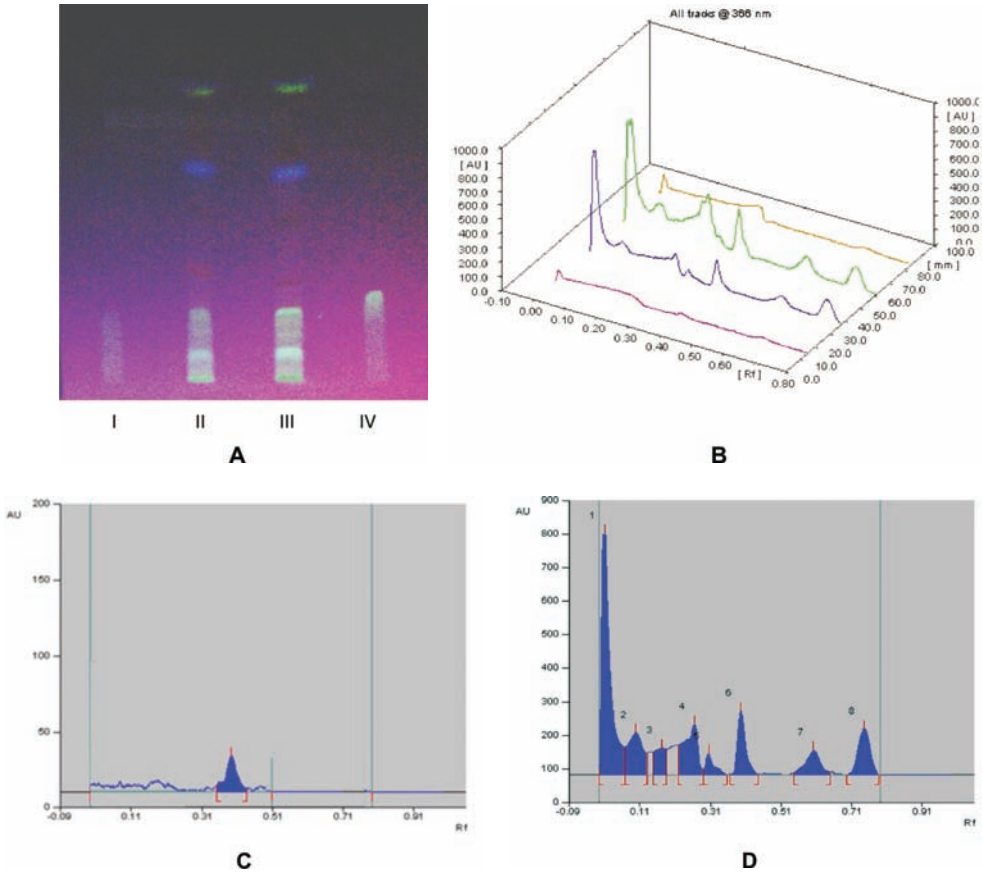

Fig. 2. HPTLC fingerprinting profile under UV 366 nm

A. HPTLC fingerprints profile of methanol extract and reference standard 'weadelolactone' (**I.** 3µg/ml of wedelolactone; **II.** 5µg/ml of methanol extract of sample; **III.** 10µg/ml of methanol extract of sample; **IV.** 6µg/ml of wedelolactone)

B. Overlay Chromatogram

C. Chromatogram of weadelolactone

D. Chromatogram of methanol extract

Results and Discussions

The results of the preliminary phytochemical studies confirm the presence of flavoniods, steroids, alkaloids, tannin, glycosides, carbohydrates, and saponins (Table-1 & 2). The HPTLC finger print analysis of methanol extracts of *Eclipta alba* L. showed the presence of various phytoconstitutents. The isolation and identification of these bioactive compounds can be used to formulate new drugs to treat various diseases and disorders. In recent times during this molecule era in addition to morphological characters in plant taxonomy anatomical, cytological, biochemical and molecular markers are also being used to classify the plants. HPTLC finger printing profile is useful

as phytochemical marker and also a good estimation of genetic variability in plant populations. The data generated from the present study would help in the authentication and quality control for *Eclipta alba* L. Such chemo finger printing will also act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

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