Comprehensive Phytochemical Profiling and HPTLC Fingerprinting of Eclipta alba (L.) Hassk.

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Abstract:

Eclipta alba (L.) Hassk., 10g dried aerial plant powder was extracted with 100mL of four different solvents; methanol, petroleum ether, toluene and water. All four extracts were evaluated for HPTLC analysis; with the three reference standard compounds gallic acid, quercetin and rutin. The qualitative analysis has been carried out for all four extracts of alkaloids, flavonoids, steroids, cardiac glycosides, carotenoids, saponins, tannins, phenolics, carbohydrates and reducing sugars detection. The quantitative assessments were done with methanolic extract for phenolics, flavonoids and tannins content. HPTLC profiling revealed the separation of seventeen compounds from methanolic extract higher than petroleum ether, toluene and water extracts detected at 366nm. The methanolic extract has shown total tannin 114.02□g/mL, flavonoid 81.10□g/mL and phenolic 20.91□g/mL. The results indicate that the Eclipta alba was the rich source of alkaloids, tannins, flavonoids, phenolics, saponins, glycosides and steroids.

Introduction:

Medicinal plants represent a vital source of bioactive compounds for the development of new therapeutic compounds. Plant derived secondary metabolites such as flavonoids, terpenoids, steroids, glycosides and alkaloids exhibit diverse pharmacological activities. Herbal drugs are widely used due to their broad safety profile and therapeutic potential (Chandran & Indira, 2016; Patel et al., 2016). Eclipta alba is an annual herbaceous plant, commonly known as bhangro, bhringraja or “false daisy” in English (Jahan et al., 2014). It is an erect or prostrate, branched, stem and branches strigose with appressed white hairs, the leaves are opposite, sessile and oblong-lanceolate. It belongs to the family Asteraceae (Kirtikar et al., 1975). Eclipta alba is used for the treatment of liver cirrhosis, liver enlargement, infective hepatitis and other related disease of liver (Yadav et al., 2017). Eclipta alba has also demonstrated anthelmintic and alexipharmic activity and also found useful to cure inflammations, hernias, eye diseases, skin diseases, itching, bronchitis, asthma, syphilis etc. It is also found to be useful for prevention of abortion and miscarriage and for uterine pains after delivery. In Unani system it is used for fever, spleen diseases, toothache, headache, liver pain and vertigo (Kirtikar et al., 1975). Plant has reportedly shown versatile pharmacological actions such as rejuvenating, antimicrobial, antioxidant, antileprotic, antimycotoxic, antiviral, analgesic, hypotensive, immunomodulatory, nootropic and spasmogenic activity (Nelson et al., 2020; Yadav et al., 2017). The plant has been reported to contain phytosterol, β-amyrin, triterpenoids like eclalbasaponins I-VI, echinocystic acid, ursolic acid; saponin such as ecalbatin; flavonoids such as luteolin, apigenin and coumarin such as wedelolactone, demethylwedelolactone (Jahan

et al., 2014; Patel et al., 2016). The aim of the present study was qualitative, quantitative and HPTLC aided phytochemical analysis of Eclipta alba collected from Gujarat.

Materials and Methods

Plant Collection and Authentication

Eclipta alba aerial plant collected from the campus of Veer Narmad South Gujarat University, Surat (Lat 21.154292o, long 72.787169o) Gujarat, India. The identification was confirmed by taxonomist Prof. Minoo H. Parabia consulting the Flora of Gujarat and Flora of India (Shah, 1978; Hajra et al., 1995). The herbarium sample has been deposited at Shri Bapalal Vaidya Botanical Research Centre, Department of Biosciences, VNSGU with the voucher specimen number "VNSGU/BVBRC/2024/05/TC-43". The collected aerial plant part was washed with running tap water, shade-dried and reduced into fine powder using mixer grinder and stored in air tight container for further analysis.

Extraction

Dried aerial plant part powder (10g) was extracted through cold maceration with selected nonpolar to polar solvents (100mL). Solvents used for extraction were petroleum ether (ϵ1.9-2.1), toluene (ϵ 2.3-2.4), methanol (ϵ 32.6-33.0), and water (ϵ 78.3-80). The mixture was kept on a rotary shaker for 6 hours followed by a static period of 18 hours. The extracts were filtered by Whatman filter paper No.1. All four extracts were subjected to qualitative phytochemical analysis while only methanolic extract was utilized for quantitative analysis.

High Performance Thin Layer Chromatography (HPTLC)

HPTLC analysis performed with the respective extracts derived from plant powder dispersing 1g in 10mL solvent. Leave for overnight in static condition at room temperature, next day filtered by 0.22µm syringe filter and further dilute to 1:2 before loading. HPTLC was performed on silica gel 60 F254 plate (12cm X 10cm with 0.2mm thickness, Sigma-Aldrich-1055540007) used as stationary phase. CAMAG system with CAMAG Linomat 5 sample applicator were driven by the software winCATS version 1.00.13. The four extracts were loaded in 6µL volume with standard 1µL (1mg/mL) gallic acid (Researchlab-00765), quercetin hydrate (Loba-05526) and rutin trihydrate (Loba-05621), were applied in 8mm wide bands. Mobile phase Toluene: Ethyl acetate: Formic acid: Methanol (6: 6 :1.5: 0.5) (Shivanandappa et al., 2023) and plate was developed in a solvent system in CAMAG glass twin chamber saturated for 20min. Plate was dry and observed under the CAMAG UV Cabinet. After development, the plate was sprayed with anisaldehyde sulphuric acid reagent and heated at 105ºC for 5-10min (Wagner & Bladt, 1996). Plate was scanned through CAMAG TLC Scanner 3 with UV254 and UV366 illumination.

Qualitative Phytochemical Analysis:

Qualitative phytochemical screening of methanol, petroleum ether, toluene and water extracts were carried out for the detection of alkaloids (Mayer's, Dragendorff's and Wagner's test), flavonoids (Shinoda and Alkaline reagent test), steroids (Salkowski's test), cardiac glycosides (Killer killiani and Sodium hydroxide test), carotenoids, saponins (Froth's test), tannins (Ferric chloride and Gelatine test), phenolics, reducing sugars (Fehling's test), and carbohydrates (Molisch's and Barfoed's test) using standard procedure (Hoque, 2019; Kumar et al., 2020; Nortjie et al., 2022; Shaikh & Patil, 2020; Talmale et al., 2014).

Quantitative Phytochemical Analysis:

Estimation of total phenolic content determined by the Folin-Ciocalteu method. Total flavonoid content was determined by the aluminium chloride method. Estimation of total tannin content by the Folin-Ciocalteu method using standard procedure by (Chandran & Indira, 2016; Sahu et al., 2021; Le et al., 2017; Patel et al., 2016; Tripathi et al., 2021).

Results And Discussion

Qualitative Analysis

The phytochemical screening of the aerial part of E. alba revealed the presence of alkaloids, flavonoids, steroids, cardiac glycosides, saponins, tannins, phenolics, and carbohydrates in different solvent extracts (Table1). Carotenes and reducing sugars yielded negative results in all solvents. However, carbohydrates have shown negative results with Barfoed's test but the same has shown positive results with Molisch's test in all four extracts. This diverse profile highlights the extracts potential for various pharmacological applications. The earlier report indicates that flavonoids were absent in chloroform, methanol and water extract (Kumar et al., 2020) correlates in the line of the present findings.

Table 1. Qualitative analysis of *Eclipta alba*

Sr. No.	Test		Methanol	Petroleum ether	Toluene	Water
1	Alkaloids	Mayer's test	+++	+++	+++	+++
		Dragendorff's test	-	-	-	++
		Wagner's test	+++	+++	+++	+++
2	Flavonoids	Shinoda test	-	-	-	++
		Alkaline test	+++	+++	+++	+++
		Ethyl acetate test	++	+++	+++	+++
3	Steroids	Salkowski's test	+++	+++	+++	+++
		Ethyl acetate test	+++	+++	+++	+++
4		Killar killani test	+++	+++	+++	-

	Cardiac glycosides	Sodium hydroxide test	+++	+++	+++	+++
5	Carotenoids	-	-	-	-	-
6	Saponins	Froth's test	+++	+++	+++	+++
7	Tannins	Ferric chloride test	++	-	-	++
		Gelatin test	++	+++	++	++
8	Phenolics		+++	+++	+++	+++
9	Carbohydrates	Molisch's test	++	+++	+++	++
		Barfoed's test	-	-	-	-
10	Reducing sugars	Fehling's test	-	-	-	+

Quantitative Analysis

Quantitative analysis performed with methanolic extract, derived 100mL from 10g powder of E. alba, showed total phenolic 20.91±2.14µg/mL against standard gallic acid (Fig 1(a)), total flavonoids 81.10±7.05µg/mL against standard quercetin (Fig.1(b)), and total tannin 114.02±14.5µg/mL against standard tannic acid (Fig. 1(c)).

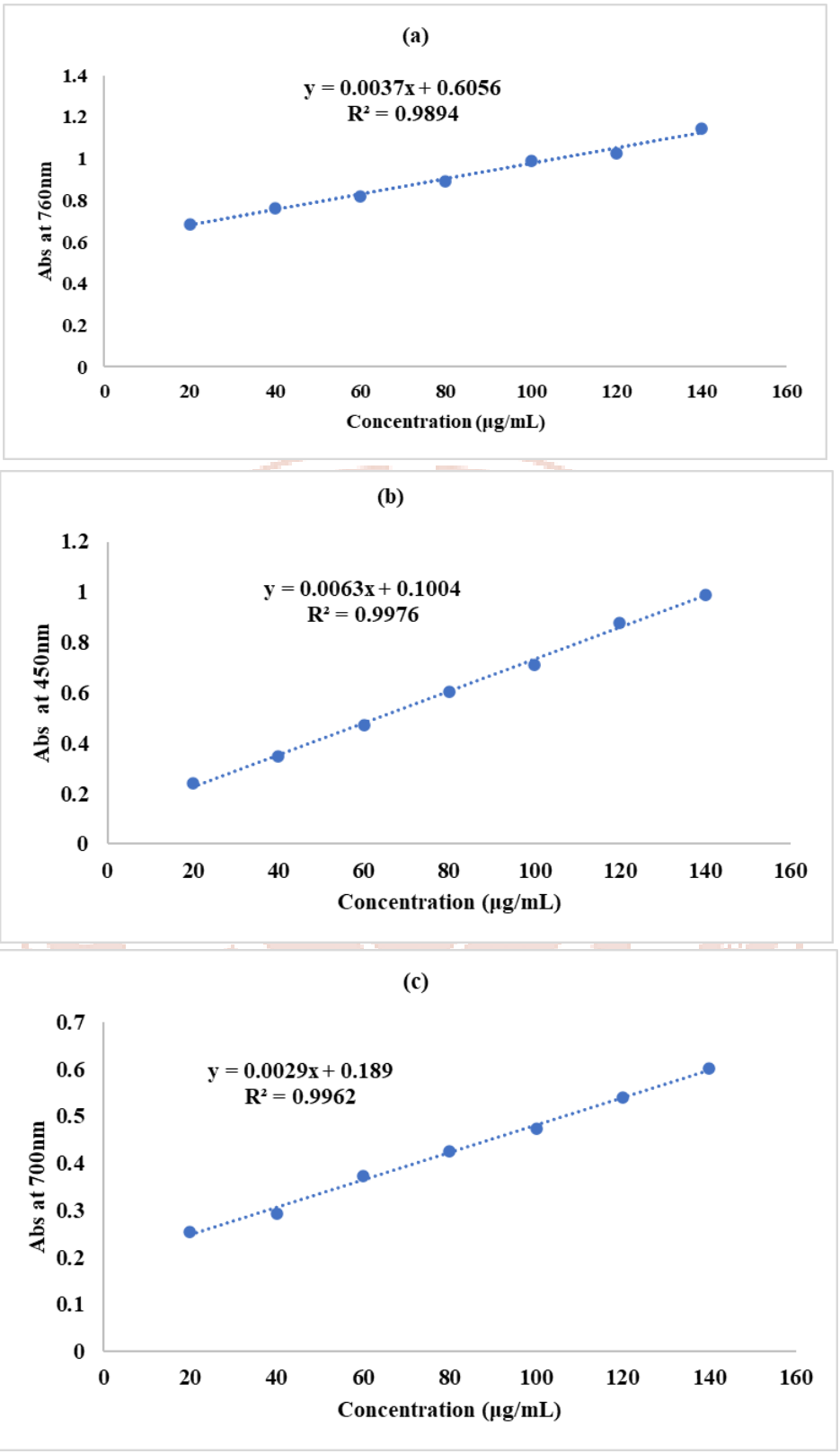

Figure 1: Standard graph of (a) gallic acid for total phenol estimation, (b) quercetin for total flavonoid estimation, and (c) tannin acid for total tannin estimation for quantitative analysis of *E. alba*.

High Performance Thin Layer Chromatography (HPTLC)

Eclipta alba extracts methanol, petroleum ether, toluene and water evaluated for HPTLC fingerprinting with standard reference compounds gallic acid, quercetin and rutin. HPTLC methanolic extract generated significant fingerprinting with sixteen peaks at 254nm (Figure 2 and 3; Table 2) and seventeen peaks at 366nm. Methanolic extract of *E. alba* has shown ten common compounds between 254nm and 366nm (Table 2). Gallic acid detected at Rf 0.47, quercetin at Rf 0.61, and rutin at Rf 0.04 (Figure 2) in 254nm and 366nm. However, anisaldehyde failed to detect gallic acid. Somehow, quercetin and rutin have shown additional bands at Rf 0.67 and Rf 0.61 respectively in 254nm and 366nm, which resolved by anisaldehyde, ensure the presence of correct compound at characteristic Rf.

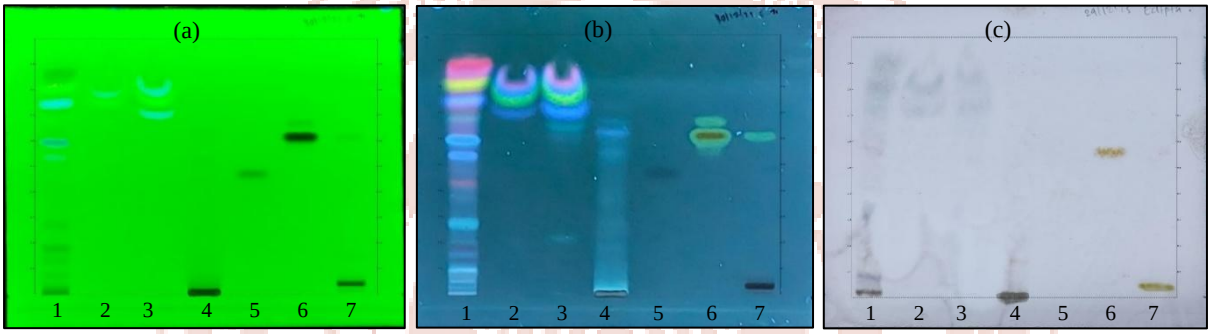

Figure 2: HPTLC chromatogram of *E. alba*: (a) 254nm; (b) 366nm and (c) anisaldehyde-H_2SO_4 derivatization, with tracks (1) *E. alba* methanolic extract; (2) *E. alba* petroleum ether extract; (3) *E. alba* toluene extract; (4) *E. alba* water extract; (5) gallic acid; (6) quercetin and (7) rutin.

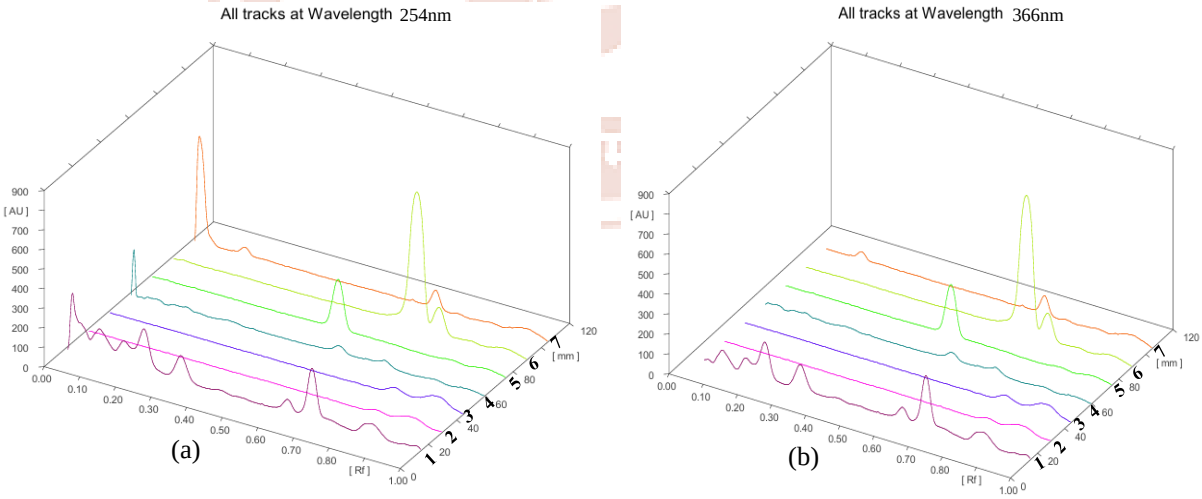

Figure 3: HPTLC Densitograms of *E. alba* samples and standards, all tracks at wavelength (a) 254nm and (b) 366nm with track (1) *E. alba* methanolic extract; (2) *E. alba* petroleum ether

extract; (3) *E. alba* toluene extract; (4) *E. alba* water extract; (5) gallic acid; (6) quercetin and (7) rutin.

Table 2. HPTLC analysis of *Eclipta alba* methanolic extract

Rf value	Detection	Max. Height (%)	Area (%)	Visual Appearance
0.02	254nm	22.25	18.35	Light black
0.04*	366nm	2.48	0.84	Dull white
0.08	254nm	-	-	Light black
	366nm	5.86	5.15	Whitish blue
0.10	254nm	11.41	14.54	-
	366nm	-	-	Fluorescent blue
0.14	254nm	-	-	Grey
	366nm	6.62	4.47	Light blue
0.17	254nm	9.45	9.46	Black
0.19	366nm	12.69	9.77	Light blue
0.22	254nm	16.33	17.47	-
0.28	254nm	-	-	Light black
	366nm	10.25	8.16	Fluorescent blue
0.33	254nm	10.07	10.39	-
0.38	366nm	0.64	0.39	-
0.43	254nm	1.06	1.14	-
	366nm	-	-	Pink
0.54	254nm	-	-	Bluish green
	366nm	7.57	6.30	Pale blue
0.60*	254nm	-	-	Bluish-black
	366nm	20.01	18.31	Cyan
0.62	254nm	4.93	4.07	-
0.65	366nm	-	-	Bluish-pink
0.68	366nm	3.17	2.63	Greenish-blue
0.69	254nm	19.68	17.86	-
0.71	366nm	-	-	Light pink
0.75	254nm	-	-	Cyan
	366nm	12.80	14.76	Blue
0.81	254nm	-	-	Green
	366nm	-	-	Bright yellow
0.86	254nm	4.83	6.72	Light black
	366nm	17.91	29.21	Pink fluorescent
0.89	366nm	-	-	Pink-red fluorescent

*Matched Rf with standard compounds (Rutin Rf 0.04 and Quercetin Rf 0.61)

HPTLC profiling revealed the separation of four compounds in petroleum ether and five in toluene extracts at 366nm respectively (Figure 2 and 3; Table 3 and 4). In previously

reported study the petroleum ether extract resolved on TLC shown three and four compounds were detected in UV and fluorescent white light respectively by Sahu et al (2021).

Table 3. HPTLC analysis of *Eclipta alba* petroleum ether extract

Rf value	Detection	Max. Height (%)	Area (%)	Visual Appearance
0.70	366nm	23.78	19.32	Blue
0.75	254nm	-	-	Green
	366nm	-	-	Green
0.79	254nm	-	-	Bluish green
	366nm	76.22	80.68	Blue
0.81	254nm	36.08	38.44	-
	366nm	-	-	Pink
0.91	254nm	63.92	61.56	-

Table 4. HPTLC analysis of *Eclipta alba* toluene extract

Rf value	Detection	Max. Height (%)	Area (%)	Visual Appearance
0.64	366nm	-	-	Light green
0.70	254nm	-	-	Blue
	366nm	48.86	37.60	Greenish blue
0.74	366nm	-	-	Green
0.76	254nm	-	-	Green
0.79	254nm	-	-	Light blue
	366nm	51.14	62.40	Blue
0.81	254nm	40.21	31.21	Green
	366nm	-	-	Pink
0.93	254nm	59.79	68.79	-

Water extract of *E. alba* nine and three peak generated in 254nm and 366nm respectively by CAMAG Scanner (Table 5). However, all peaks are not detected through visual appearance.

Table 5. HPTLC analysis of *Eclipta alba* water extract

Rf value	Detection	Max. Height (%)	Area (%)	Visual Appearance
0.05	254nm	7.03	2.52	-
0.08	254nm	8.91	6.75	Light black
0.13	254nm	7.57	5.33	-
0.16	254nm	10.96	7.87	-
0.28	254nm	6.89	9.34	-
0.55	366nm	-	-	Light blue
0.59	254nm	25.29	31.96	-

0.63	366nm	100	100	Blue
0.67	366nm	-	-	Light blue
0.72	254nm	20.83	24.75	-
0.86	254nm	6.54	8.27	-
0.93	254nm	5.98	3.21	-

Conclusions

Secondary metabolites detected were alkaloids, flavonoids, steroids, cardiac glycosides, saponins, tannins, phenolics and carbohydrates. However, carotenoids and reducing sugars were absent or minimal in all extracts. Quantitative analysis of methanolic extract shown tannin in highest concentration, followed by flavonoid and phenolic respectively. HPTLC analysis of methanolic extract has higher number of compounds separation. These results indicate the successful HPTLC fingerprinting developed for dry aerial plant powder of *E. alba* served as pharmacognosy tool for raw material confirmation. The results show the benchmark data for holistic phytochemical analysis that can serve as standard for the pharmaceutical industries.

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