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Preliminary Phytochemical Screening, *Invitro* Antioxidant Activity and HPTLC Finger Printing Profile Of *Eclipta prostrata*

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ABSTRACT: The objective of the present study is to carry out the preliminary phytochemical screening, *Invitro* anti-oxidant activity and HPTLC finger printing analysis of *Eclipta prostrata*. The various extracts were subjected to carry out preliminary phytochemical screening by standard procedure and *Invitro* antioxidant activity by DPPH method. HPTLC finger printing analysis of *Eclipta prostrata* using Automatic TLC applicator Linomat 5 with nitrogen flow (CAMAG Switzerland) in methanol extract. Preliminary phytochemical screening indicates the presence of various secondary metabolites like alkaloids, flavonoids, terpenoids, tannins etc. *Invitro* antioxidant by DPPH method showed significant activity with % inhibition as 70.86% at 200 µg/ml. HPTLC finger printing profile of selected medicinal plant showed 10 peaks with R_f values starts from 0.19-0.95. The present investigation of *Invitro* antioxidant activity proved to exhibit significant activity which is comparable against standard drug vitamin C. HPTLC finger printing profile act as a diagnostic tool for proper selection and identification of herbal drug.

KEYWORDS: *Eclipta prostrata,* HPTLC, DPPH, Inhibition, Terpenoids.

I. INTRODUCTION

In India traditional health care system are widely used to treat mankind with lesser side effects. Among the various medicinal plants some plants are systematically investigated and some are still to get explored. The need of the hour is perfect standardization of plant materials. Various pharmacopoeias describe only about certain physicochemical parameters, hence modern instrumentation like HPLC and HPTLC may be useful for standardization of herbs, as it exerts good resolution in the estimation of active principles with good precision and accuracy in lesser time. *Eclipta prostrata* is small evergreen herb called as false daisy. It is cylindrical and grayish root and it also possesses white florets. It is widely distributed across the world Literature reported for the presence of various activities like antimicrobial, analgesic and immune modulatory property[1]. The present work is undertaken to establish preliminary phytochemical screening, *Invitro* antioxidant by DPPH method and HPTLC finger printing profile of *Eclipta prostrata* in methanolic extract

II. MATERIALS AND METHODS

Plant material : The plant materials were collected in the month of September freshly from local market and voucher specimen is stored in our herbarium for future reference.

Preparation and extraction of plant materials : The aerial parts of selected medicinal plant were dried under shade and cut into small pieces and coarsely powdered. Successive solvent extraction was carried out using cold maceration based on increasing polarity. All the extracts were stored in desiccator.

Preliminary screening [2,7]
Preliminary screening of aerial parts of *Eclipta prostrata* was carried out as per standard procedure.

Invitro Antioxidant Activity : By using DPPH method free radical scavenging activity was determined for *Eclipta prostrata*. The reaction mixture contains 0.3mM DPPH (1ml) in 100% methanol and test extract (3ml) of various concentrations (10-1000µg/ml) shake thoroughly at frequent intervals. At room temperature the reaction mixture was allowed to stand for 30 minutes. By using UV visible spectrophotometer, the absorbance was measured at 517nm.

HPTLC fingerprint of *Eclipta prostrata* [3-6]

Sample Preparation : 50mg of the *Eclipta Prostrata* extract was weighed and dissolved in 1ml of methanol. 2µl and 4µl of the sample was applied using automatic TLC applicator Linomat 5.

Chromatographic Conditions [8-14]

The following chromatographic conditions were used for determination of HPTLC fingerprinting. Using reactivated silica gel $60F_{254}$ chromatography was performed on 5X10 cm plate. By using HPTLC plate the sample were applying as 8mm wideband in Automatic TKC Applicator Linomat 5 nitrogen flow (CAMAG Switzerland). A constant application rate of 150nL/s was used. Densitometric scanning was performed on CAMAG scanner III. The plates were pre-washed by methanol for 5 minutes prior to chromatography[15]. After running the trial, the mobile phase was fixed by varying polarity.10 ml of mobile phase was used. HPTLC finger printing was carried out using linear ascending development using Twin glass chamber which was previously saturated with mobile phase[16-17]. The plates were dried by hair dryer. The developed plates were scanned at appropriate wavelength using CAMAG TLC scanner 3 and are photo-documented using CAMAG REPROSTAR 3.

Chromatographic condition

Sample	: *Eclipta prostrata*
Sample prepared in	: Methanol
Stationary phase	: Silica gel GF_{254}
Mobile phase	: Toluene: ethyl acetate: Formic acid (7:2:1)
Scanning wavelength	: 254 nm
Sample concentration	: Extract (50mg/ml)
Applied volume	: Track 1(2 µl) and Track 2 (4 µl)
Development mode	: Ascending mode

III. RESULTS

Preliminary phytochemical screening of *Eclipta prostrata* indicated the presence of secondary metabolites. At various concentrations, *invitro* antioxidant was carried out in methanolic extract and it showed better activity which is comparable with standard drug vitamin C. The results from HPTLC profile indicated R_f value which starts from 0.13-0.83.The chromatograms are given below.

Table 1: Preliminary Phytochemical Screening

Chemical Test	Pet.ether extract	Chloroform extract	Ethyl acetate	Methanol
Alkaloids	-	+	-	+
Carbohydrates	-	-	-	-
Glycosides	-	+	+	+
Proteins	-	-	+	+
Amino acids	-	-	+	+
Flavonoids	+	+	+	+
Phenolic compounds	+	+	+	+
Tannins	+	+	+	+
Terpenoids	+	+	+	+
Steroids	-	-	-	-

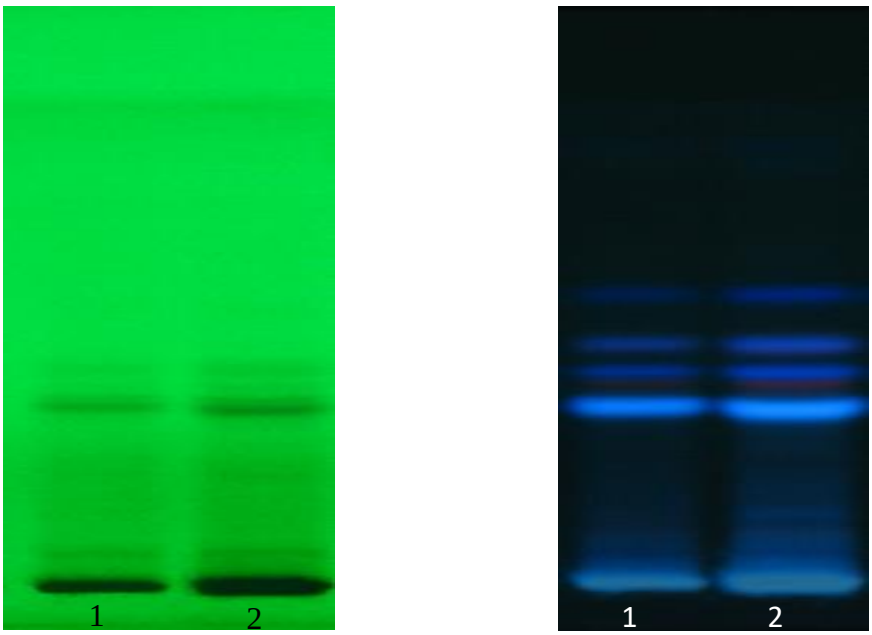
Phytochemical screening test was carried out for petroleum ether, chloroform, ethyl acetate and methanol extracts. Except steroids and carbohydrates other compounds like, alkaloids, glycosides, amino acids, flavonoids, phenolic compounds, tannins and terpenoids were identified in the methanolic extract.

Table 2: DPPH Radical Scavenging Assay

Conc. (µg/ml)	% inhibition	
	Methanolic extract	Standard Vitamin C
10	28.71 ±0.16	39.85±0.24
50	44.79 ± 0.20	55.41±0.32
100	61.75± 0.26	69.22±0.41
200	70.86± 0.35	80.55±0.12
400	72.61 ± 0.15	81. 92±0.24
800	70.84±0.13	78.05±0.58
1000	69.84±0.42	80.22±0.45

Antioxidant activity was carried out by using DPPH radical scavenging assay method. 10, 50, 100, 200, 400, 800 and 1000 µg/ml concentration of methanolic extract were tested for % inhibition. Vitamin C is used as standard. The maximum percentage inhibition were observed in the concentration of 400 µg/ml (72.61 ± 0.15) and 200 µg/ml (70.86 ± 0.35) in the methanolic extract as compared to standard which shown in table: 2.

Figure: 1 HPTLC finger printing images in 254 nm and 366 nm.



Lane 1 – 2µl extract of *Eclipta prostrata*
Lane 2 – 4µl extract of *Eclipta prostrata*

Figure: 2 Track 1 – 2µl

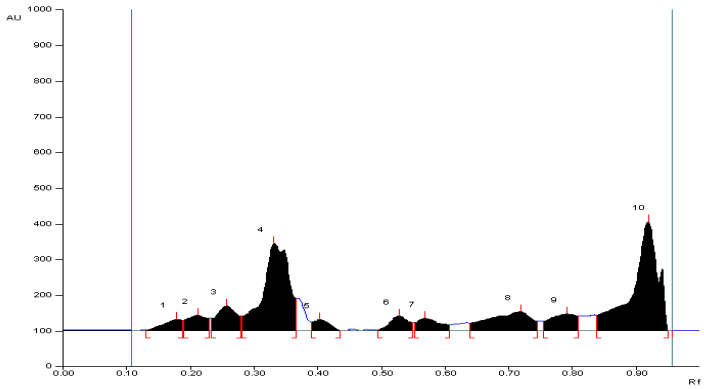


Figure: 3 Track 2 – 4µl

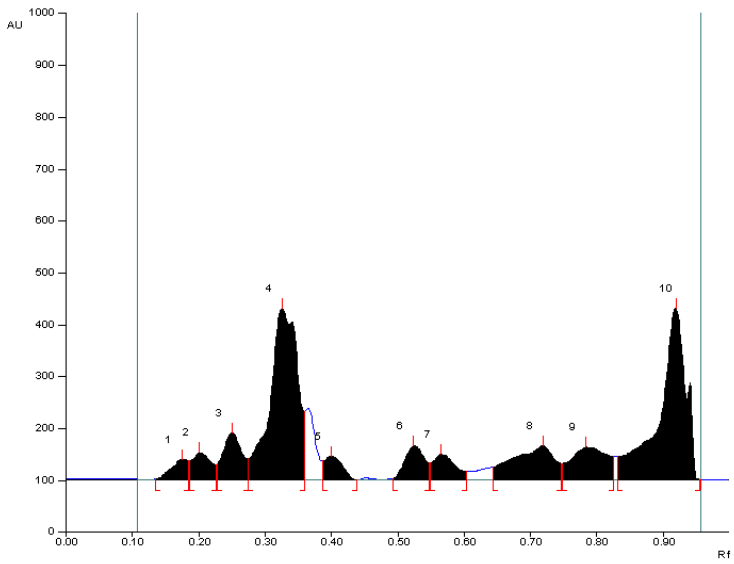


Table 3: Peak table of *Eclipta prostrata*

Track	Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	1	0.13	1.4	0.18	32.1	3.57	0.19	28.7	849.8	2.63	unknown *
1	2	0.19	28.7	0.21	42.9	4.77	0.23	34.1	1226.1	3.79	unknown *
1	3	0.23	34.2	0.26	69.3	7.7	0.28	40.1	1983.8	6.13	unknown *
1	4	0.28	40.2	0.33	243.9	27.09	0.37	90.3	9272.9	28.67	unknown *
1	5	0.39	24.6	0.4	31.3	3.48	0.44	0.4	736.4	2.28	unknown *

1	6	0.49	2.4	0.53	41.2	4.57	0.55	22.4	1095.2	3.39	unknown *
1	7	0.55	21.4	0.57	34.2	3.8	0.61	15.9	1124	3.47	unknown *
1	8	0.64	21	0.72	53.2	5.91	0.74	25.8	3182.6	9.84	unknown *
1	9	0.75	26.2	0.79	46.8	5.2	0.81	40.4	1785.4	5.52	unknown *
1	10	0.84	43.5	0.92	305.2	33.91	0.95	0.1	11092.5	34.29	unknown *
2	1	0.14	1.7	0.17	40	3.53	0.19	36.7	948.9	2.31	unknown *
2	2	0.19	36.9	0.2	52.4	4.62	0.23	29.4	1381.7	3.37	unknown *
2	3	0.23	30.1	0.25	91.1	8.03	0.27	40.3	2373.8	5.78	unknown *
2	4	0.28	40.5	0.33	330.6	29.15	0.36	132.1	12684.4	30.91	unknown *
2	5	0.39	36	0.4	45.7	4.03	0.44	0.4	1157	2.82	unknown *
2	6	0.49	2.5	0.52	65.5	5.77	0.55	31.9	1786.1	4.35	unknown *
2	7	0.55	32	0.56	48.9	4.31	0.6	16.6	1528.4	3.72	unknown *
2	8	0.64	25.2	0.72	66	5.82	0.75	31.2	3737.4	9.11	unknown *
2	9	0.75	31.4	0.78	62.8	5.54	0.82	45.4	3180.7	7.75	unknown *
2	10	0.83	44.7	0.92	331.1	29.2	0.95	0.4	12264.4	29.88	unknown *

In HPTLC finger printing method was used to identify the number of compounds present in the methanolic extract. In this methods there are two lane was used. Lane 1 – 2µl extract of *Eclipta prostrata* and lane 2 – 4µl extract of *Eclipta prostrata* were taken for analysis. In both Lane 1 & 2 there are 10 peaks each were identified and the end R_f value starts from 0.19 to 0.95.

IV. CONCLUSION

The present investigation of invitro antioxidant activity proved to exhibit significant activity which is compared with vitamin C as standard. HPTLC finger printing profile act as a diagnostic tool for proper selection and identification of herbal drug. Future study may be extended to isolate active constituents which may be responsible for treating various diseases and also to quantify the active constituents present in selected medicinal plant.

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