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IN VITRO STUDY OF PETROLEUM ETHER EXTRACT OF *ECLIPTA ALBA* Hassk. FOR HepG2 CELL LINE

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ABSTRACT

With advanced technologies we can find new bridge between Ayurveda and modern researches, for that we tried here petroleum ether extract of *Eclipta alba* against hepatic cancer. Due to having potent anti-cancer activity in *Eclipta alba*, it can be successfully used in cancer management both as mainline treatment and an adjuvant medication with minimum side effects. With the help of this paper the *Eclipta alba* which will prove to be helpful for the researcher development clinical trial and it can be used in the clinical practices for treating hepatic cancer in near future. Future research on such a topic would help to identify safe, pure and effective anticancer drugs and will further the discovery of their mechanism of action. We have used petroleum ether extract of *Eclipta alba* Hassk. for phytochemical analysis, TLC, HPLC analysis to test active chemical components in it. All tests proved that an petroleum ether extract of *Eclipta alba* Hassk. containing many active chemical components, therefore our experiment showed the positive results for an petroleum ether extract of Eclipta alba Hassk. against hepatic cancer. The srb assay results were used to evaluate the anti-cancer activity of the extract. The effects of whole plant extract on cancer cell line were studied and evaluated. The percentage of cell growth and cell viability were calculated from tabulated result values of srb assay. The experiment revealed that the average percentage of growth inhibition was 55.06%. Cell viability srb assay also showed significant growth inhibition, at the same time statistical

analysis of srb assay also proved significant results. The research performed here is very useful for setting up of different extract studies of *Bhringraj* for its anticancer activity.

KEYWORDS: *Eclipta alba* Hassk., HepG2, petroleum ether extract, (Sulforhodamine B) srb assay.

1. INTRODUCTION

According to WHO, Cancer is the second largest cause of death which killed 9 million in 2015 and will 11.5 million in 2030. Scientists are going to spotlight on both synthetic as well as natural sources for research and development on the new anticancer drug. Recently plants are widely used for developing anticancer drugs due to their active chemical constituents. Plant-derived products can be used in cancer treatment and it may reduce adverse side effects. Currently, a few plant products are being used to treat cancer and become a promising anticancer agent.[1] Ayurveda becomes a ray of hope for hepatocellular carcinoma. The careful and cautious use of the medicinal plant will definitely prove to be favorable results in hepatic cancer management. For a better understanding of the prevention and treatment of HCC by herbal active compounds and herbal composite formulas researches proved that the herbal compounds and different herbal formulas against HCC are effective and safe for the prevention and treatment of HCC.[2] Here we choose the medicinal plant *Bhringraj* that is *Eclipta alba* Hassk. against hepatic cancer for in vitro study. We used the petroleum ether extract of it for our experiment. In Ayurveda, *Bhringraj* has special attention because of its multidimensional applications and in modern science, it is a significant research drug due to its proven qualities.[3] Out of different extraction methods here we select the soxhlet extraction method for petroleum ether extract of *Eclipta alba*. For inventing new molecules from the medicinal plant we have to study different extraction methods and by doing TLC, HPLC, HPTLC we can find active chemical constituents. Further that we can study deeply the constituents with the help of modern technologies of biotechnology. We can put our best results of the medicinal plant in front of the world through which we can prove that Ayurveda has solutions for life threatening diseases like cancer. It is the need for science to introduce the actions of herbal drugs in the language of modern pharmacology. This research done by us is a small example of significant miraculous results of *Eclipta alba* in the field of Ayurveda.

For rising proper anticancer drug we have to understand the pharmacokinetics and pharmacodynamic of medicinal plants. Ethan-pharmaceutical uses shows that *Eclipta alba*

has tremendous external and internal applications. It is the need for time to believe about medicinal plants and their applications with a modern scientific approach. Here we can find the key to medicine for life-threatening diseases like cancer. This study we put in front of you is about evaluating the anticancer activity of *Eclipta alba* Hassk, against hepatic cancer, in which petroleum ether extract of *Eclipta alba* Hassk was evaluated against hepatic cancer cell line-HepG2. In Ayurveda *Eclipta alba* Hassk is having very important medicinal value because of common availability and effective results. In our study, we focus on the use of petroleum ether extract of *Eclipta alba* Hassk, on the liver cancer cell line. As many shreds of evidence are found about the use of *Bhringraj* as a single drug for liver disorders treatment. While studying *Bhringraj*, many tribes and local communities are found to frequently use it on hepatic disorders like jaundice at the same time many pharmacological activities are proved by researches.[3] *Bhringraj* having Latin name *Eclipta alba* Hassk, belonging to family Asteraceae. It is the significant medicinal plant among the Asteraceae family because of its widely used in therapeutic purposes. Previously we have successfully conducted an in vitro study and concluded that an ethanolic extract of *Eclipta alba* Hassk shows significant results against the HepG2 cell line.[4]

2. AIM OF THE STUDY

The present study was designed to assess and establish the role of petroleum ether extract of *Eclipta alba* as an anti-cancer agent using the HepG2 cell line.

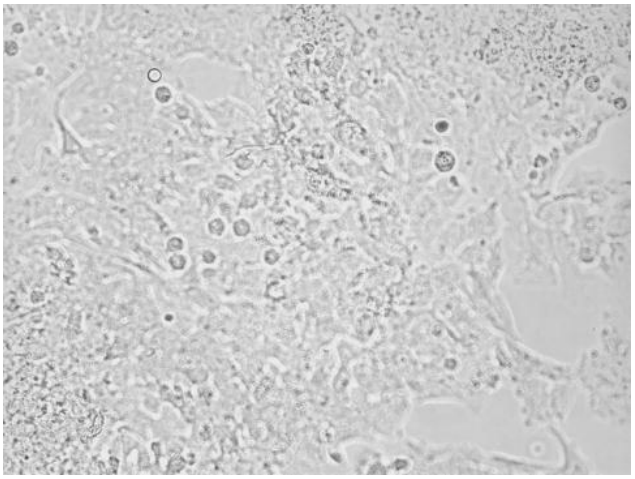
3. MATERIAL AND METHOD

3.1. Plant Material

Eclipta alba Hassk. was collected from Phulambri, District Aurangabad and the sample was authenticated at Head of the Botany Department, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, India. Specimen sample of *Eclipta alba* Hassk. has been allotted a voucher sample accession number 0660 and kept at the medicinal plant repository of the institute.

3.2. Cell Line Culturing

HepG2 cell line was used for study which was purchased from the National Centre for Cell Science (NCCS) Pune. HepG2 cell line was a human liver cancer cell line. It was cultured in medium (MEM)E, (Eagle's Minimum Essential Media) containing 10% FBS (Foetal Bovine Serum). Culturing media was used of Hi-Media Lab. Mumbai, Maharashtra, India.



HepG2 cell line.

3.3. Preparation of Petroleum Ether Extract of *Eclipta Alba*

Fresh sample dried at room temperature for 8-10 days, the dried whole plant was then powdered with the help of an electric blender. Petroleum ether was used for the extraction of the *Eclipta alba* with the help of the soxhlet apparatus. 10gm of *Eclipta alba* in 150ml petroleum ether solution was used for extraction.

Preparation of petroleum ether extract of *Eclipta alba.*

3.4. Phytochemical Analysis of Petroleum Ether Extract of Eclipta alba[5],[6]

Petroleum ether extract of *Eclipta alba* was screened for the presence of various Phytoconstituents using standard procedures. The phytochemical study was studied for the Carbohydrate, phenols, flavonoids, alkaloids, steroids, tannins, saponins, glycosides, quinones, amino acids and coumarin.

3.5. TLC Analysis of Petroleum Ether Extract of *Eclipta Alba*

The collected fractions of petroleum ether extract of *Eclipta alba* Hassk. were further evaluated for Thin Layer Chromatography for that TLC plate (Merck, India) was used. The solvent system for TLC is Benzene: Chloroform (1:1). This was used as the mobile phase. The plate was soaked gently in the TLC jar containing the above solvent. Solvents were moved until they reached the upper edge. Then the plate was removed from the jar and allowed to dry, spots were noted Rf values calculated according to the following equation.

$$\text{Retention factor} = \frac{\text{The distance of the spot sample movement}}{\text{The distance of the spot solvent movement}}$$

3.6. HPLC Analysis of Petroleum Ether Extract of *Eclipta Alba*[7]

For obtaining the HPLC chromatogram, chromatographic conditions were optimized with the mobile phase and flow rate. Methanol, water, acetic acid (95:5:0.04) as a mobile phase in isocratic elution with a flow rate of 0.6ml/min provided better peak and shape resolution. The analysis was performed with a running time of 10 min. detector wavelength was 352nm and the injection volume is 10ul.

3.7. Cell Viability Srb Assay

Sulphorhodamine B (SRB) assay kit was used of Hi-Media cell culture Laboratories, Mumbai, Maharashtra, India. It was employed for screening of anticancer activity of petroleum ether extract of *Eclipta alba*. Using the Human hepatoma cell line (HEPG2). The Cell line was cultured in medium (MEM)E containing 10% fetal bovine serum, 2mM L-glutamine and inoculated into 96 well microtiter plates in 100µL at plating densities. Cell inoculated and microtiter plates were incubated at 37°C, 5% CO2, 95% air and 100% relative humidity for 24h prior to the addition of extract. During extract addition, an aliquot of frozen concentrated (1mg/ml) was thawed and diluted to 25µg/ml, 50µg/ml, and 75µg/ml with complete medium containing test article. Aliquots of extract (10µl) were mixed to appropriate microtiter wells containing 90µl of medium and final extract concentrations of 25µg/ml, 50µg/ml, 75µg/ml, were obtained. The plates with plant extract concentrations were incubated at standard conditions for 48 hours and the assay was terminated by the addition of cold TCA (Trichloride Acetic Acid). 50µl of cold 30% (w/v) TCA (final concentration, 10% TCA) was added to fix the cells in situ and incubated for 60-70 minutes at 4°C. The supernatant fluid was discarded; plates were washed five times with tap water and air-dried. In each well SRB solution (50µl) at 0.4% (w/v) in 1% acetic acid was added and it was

incubated for 20 minutes at room temperature. One staining is completed, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air-dried and the bound stain was subsequently eluted with a 10 mM trizma base and absorbance was observed on ELISA reader (Thermo Fisher Scientific Company, Maharashtra, India) at a reference wavelength of 565nm with 610nm. The percentage of growth was calculated on a plate-by-plate basis for plant extract wells relative to control wells. Tabulate the results and calculate the percentage of viability.

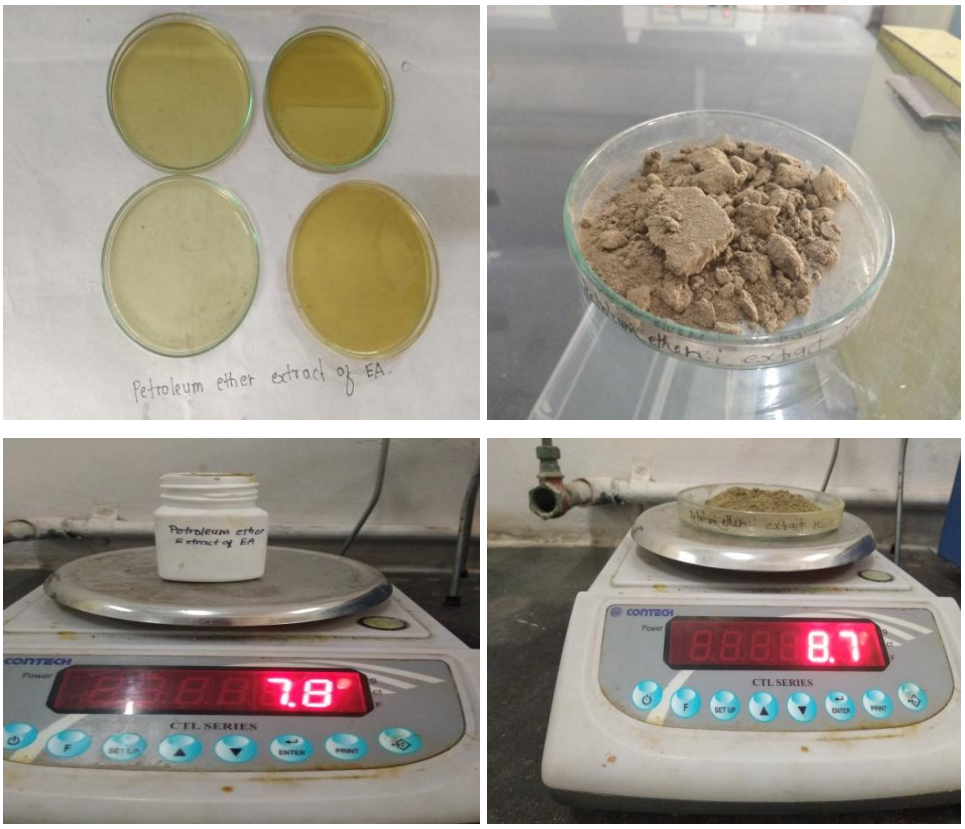
% cell growth (viability) = Absorbance sample/Absorbance negative control or untreated x 100

% growth inhibition = 100 - % cell growth.[8]

4. RESULTS AND DISCUSSION

4.1. Petroleum Ether Extract

10gm of *Eclipta alba* in 150ml petroleum ether solution results in 0.5 gm (bottle with extract weight =7.8-bottle weight=7.3gm = 0.5gm) petroleum ether extract during this 8.7gm was the residual part. As a result, we can say that 5% of petroleum ether extract obtained from 10gm of a powered form of *Eclipta alba*. The yield of extract was 5% (w/w).



Petroleum ether extract and residue of *Eclipta alba*

4.2. Phytochemical Analysis

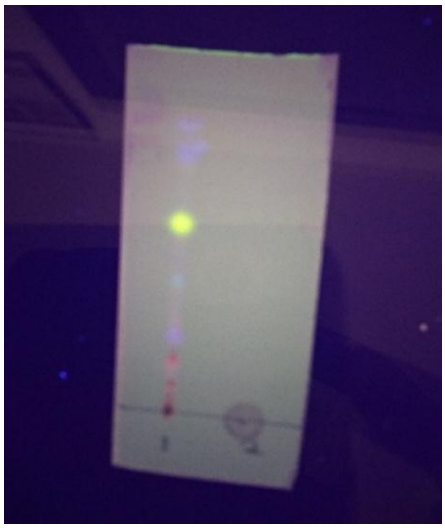
While studying the phytochemical analysis we found that alkaloid, saponin, terpenoids, flavonoids, quinones, and coumarin were present in petroleum ether extract of *Eclipta alba*.

Table no. 1: Phytochemical analysis of petroleum ether extract of *Eclipta alba.*

Sr.no.	Phytochemicals	Test	Result
1.	Carbohydrate	Fehling's test	-
2.	Phenols	$Fecl_3$ test	-
3.	Flavonoids	NH_3 test	+
4.	Alkaloids	Wagner's test	+
5.	Steroids and terpentoids	Salkowski's test	+
6.	Tannins	Lead acetate test	-
7.	Saponins	Frothing test	+
8.	Glycosides	Nitroprusside test	-
9.	Quinones	-	+
10.	Amino acids	Ninhydrin test	-
11.	Coumarin	UV light test	+

4.3. Tlc Analysis

TLC analysis shows 6 different spots and Rf values of the spots were – 0.91, 0.8, 0.55, 0.26, 0.15, 0.08. That proved that in the petroleum ether extract of *Eclipta alba* had 6 active chemical constituents.



TLC analysis of Petroleum Ether Extract of *Eclipta Alba.*

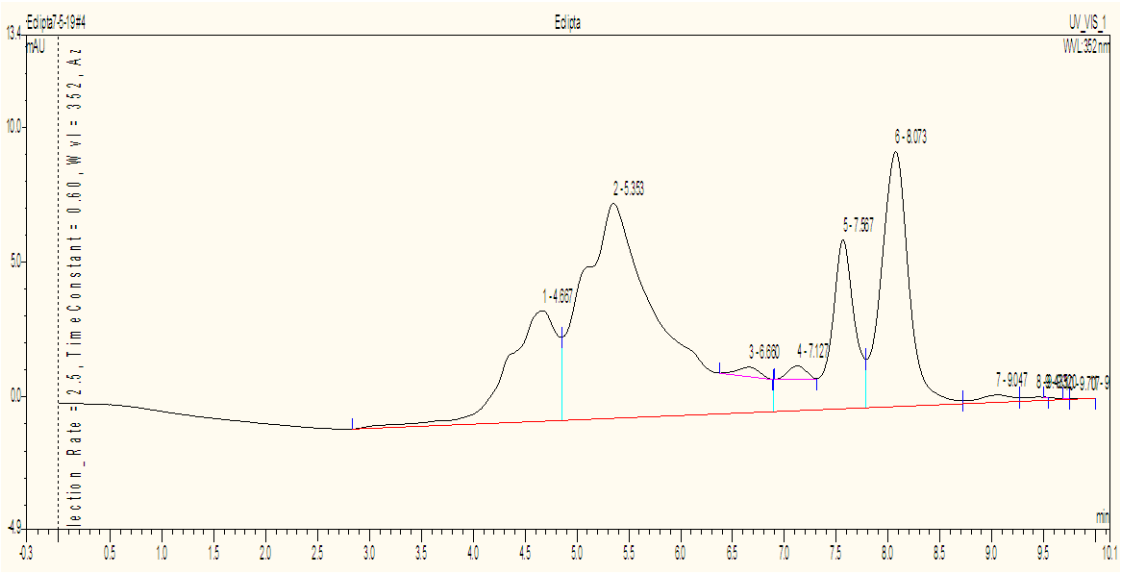
4.4. HPLC ANALYSIS

HPLC analysis of petroleum ether extract of *Eclipta alba* showed the following result in which eleven chemical compounds were observed at retention time shown in the below table.

Table No.2: HPLC analysis of petroleum ether extract of *Eclipta alba.*

No.	Ret.Time	Area	Type	Height	Rel.Area
	Min	mAU*min		mAU	%
1	4.667	2.2731	BM	4.1	15.27
2	5.353	7.2709	M	7.97	48.85
3	6.66	0.0877	Rd	0.359	0.59
4	7.127	0.099	Ru	0.492	0.66
5	7.567	2.0756	M	6.266	13.95
6	8.073	2.9218	M	9.455	19.63
7	9.047	0.1033	M	0.273	0.69
8	9.433	0.0475	M	0.136	0.32
9	9.52	0.0002	Rd	0.006	0
10	9.707	0.0002	Rd	0.005	0
11	9.94	0.0047	MB	0.018	0.03
	Total	14.8839		29.081	100

HPLC chromatogram for the petroleum ether extract of an *Eclipta alba* is shown in the following graph.



HPLC chromatogram of petroleum ether extract of *Eclipta alba*

4.5. CELL VIABILITY SRB ASSAY

The following table shows the readings of Elisa reader in which concentrations 25µg/ml, 50µg/ml and 75µg/ml in triplet form as experiments 1, 2, 3 and 4 were included. Experimental readings were optical densities for given concentrations at 565 nm wavelength.

Table No. 3: Optical density readings of srb assay.

Concentrations ⇨ / Experiment ⇩	25µg/ml	50µg/ml	75µg/ml	
	O.D.	O.D.	O.D.	O.D. (Media)
1.	0.662	0.164	0.388	0.918
2.	0.905	0.164	0.229	2.919
3.	0.897	0.398	0.051	0.048
4.	0.825	0.573	0.049	0.048
Average	0.8222	0.324	0.179	0.983
% cell growth	83.64	32.96	18.20	-
% growth inhibition	16.36	67.04	81.8	-

In vitro study of petroleum ether extract of *Eclipta alba* shows positive results against HepG2 cell line, percentage of cell viability (growth) are 83.64%, 32.96% , 18.20% and percentage of growth inhibition are 16.36%, 67.04% and 81.8% for 25µg/ml, 50µg/ml, 75µg/ml concentrations respectively. The average percentage of growth inhibition is 55.06%. Previously we have done in vitro study of ethanol, methanol and an aqueous extract by SRB assay in which we concluded that the percentage of growth inhibition was 79.33%, 77.36% and 68.74% for ethanol, methanol and aqueous extracts respectively.[4-9-10]

5. STATISTICAL ANALYSIS

	x1	x1* x1	x2	x2*x2	x3	x3*x3	
	0.66	0.44	0.16	0.03	0.39	0.15	
	0.91	0.82	0.17	0.03	0.23	0.05	
	0.90	0.80	0.40	0.16	0.05	0.00	
	0.83	0.68	0.57	0.33	0.05	0.00	
Summations	3.29	2.74	1.30	0.54	0.72	0.21	
	10.82		1.69		0.51		
	0.44	3.05	3.25	0.24	1.41	0.03	53.57
	Cx	**SSr**	**2.81**	**SSw**	**MSSa**	**MSSw**	**F Ratio**
			SSA				

6. Table 4: Statistical analysis.

Source of variance	Df	Ss	mss	F ratio
Among groups	2	0.24	4.50	53.57
Within Groups	9	2.81	0.03	53.57
Total	11			

Here we apply F-test for statistical analysis. We calculated the valuation of three concentrations 25µg/ml, 50µg/ml, 75µg/ml of petroleum ether extract of *Eclipta alba* against HepG2 cell line where quadruplets of optical densities were seen in Elisa reader. The degree of freedom among groups is 2 (n-1) and the degree of freedom within the groups is 9 (k-1).

The sum of square (SS) value among the group is 0.24 and SS within the group is 2.81. The mean of the sum of square (MSS) value among the groups is 4.50 and MSS within the groups is 0.03. Thus, calculated the F- ratio is 53.57 which, is significant at 95% confidence and 5% level of significance. The F ratio is calculated with the help of the F ratio chart at the degree of freedom 2 and 9.

5. CONCLUSION

The present study revealed that the petroleum ether extract of *Eclipta alba* shows anticancer activity against the HepG2 cell line due to presence of active chemical compounds present in it. The presences of active chemical constituents present in the *Eclipta alba* are proved with the help of phytochemical analysis, TLC and HPLC analysis. The Cell viability srb assay also shows significant growth inhibition, at the same time statistical analysis of srb assay also proved significant results. Finally, we can say that all results of our study support the anticancer activity of the petroleum ether extract of *Eclipta alba* against the HepG2 cell line.

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