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In vitro study of *methanolic* extract of *Eclipta alba Hassk.* for *hepg2* cell line

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ABSTRACT:

Synthetic anticancer drugs, apart from their high cost are well known for their hazardous side effects. So identification of new anticancer herbal medicine, which should not be cost effective only, but should be in possession of good anti-cancer effects also, is the need of the day. Here we are presenting such an Ayurvedic herb which is used since centuries for the treatment of diseases of diverse origin. *Eclipta alba* Hassk., also called as *Bhrungraj* is very important medicinal herb in different medicinal formulations. In Ayurveda *Bhrungraj* is well known drug especially for hair growth. Many researches from modern world have proved *Bhrungraj* as a prominent liver-corrective in last 40 years. In the ancient science of life, *Bhrungraj* is described in previous researches as in possession of regeneration of hepatic cells.

Here we are presenting all aspects about *Bhrungraj* in terms of qualitative and quantitative values and we have also tried to prove the anticancer activity of it. As mentioned earlier, it is traditionally used as a hepatoprotective agent. We have used the methanolic extract of *Eclipta alba* Hassk. for phytochemical analysis, TLC, HPTLC analysis to test active chemical components in it. Extract showed presence of many active chemical components which were responsible for its anticancer activity. In vitro study we used the methanolic extract of *Eclipta alba* Hassk. for the evaluation of its effects on HepG2 (Human liver cancer cell line). 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. 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

77.36%. 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 set up of different extract studies of *Bhrungraj* for its anticancer activity.

KEY WORDS : *Eclipta alba* Hassk., HepG2, (Sulforhodamine B) srb assay.

1. INTRODUCTION:

Scientists all over the world for more than a century have searched a way to cure cancer through more surgery, more radiation, more chemotherapy and, more immunotherapy without any outstanding success. The problem is like an iceberg. Here is a chance for the traditional knowledge system to overcome the challenge. But how? Can Ayurveda give better treatment for cancer and prevention too? Is this great effort works to eradicate every patient's cancer? Every patient is uniquely made up of a complex body that needs different thinking of the medical regime. According to WHO, cancer is the second leading cause of death globally and was responsible for an estimated 9.6 million deaths in 2018 worldwide. Globally, about 1 in 6 deaths are due to cancer. Liver cancer is the fourth leading cause of cancer-related mortality in the world [1]. It has been established that the prognosis rate of patients with liver cancer is very poor, and the 5-year survival rate of untreated liver cancer is only 12% [2]. Herbal resources can be taken as the best option for therapeutic as well as a preventive major in the patients of liver cancer.

The challenge for Ayurvedic fraternity against this deadly disease is to develop a proper anti-cancer drug by improving the pharmacokinetics of medicinal herbs. Throughout the world, ancient medicinal herbs have established a significant role in the common man's health care system. The need for the time is to think about the absolute and perfect approach with medicinal plants. A Multi-facet approach for the study of a particular medicinal plant can be a key to the prevention and cure of diseases. In this research article, we have evaluated the anticancer activity of *Eclipta alba* Hassk, against hepatic cancer, in which methanolic extract of *Eclipta alba* Hassk was evaluated against hepatic cancer cell line-HepG2. Ayurveda has already described various important attributes of *Eclipta alba* Hassk, which is also known as *Bhrungraj*. In Nighantu Aadarsh Vd. Bapalal has mentioned that *Bhrungraj* is very useful in maintaining metabolic activities of the liver and by this, it can cure different types of liver diseases [3]. *Bhrungraj* itself means shining like Peacock, as the peacock is having different attractive colors, the same way *Bhrungraj* shows it's various shades of qualities. Due to the hazardous side effects of modern chemical drugs, people are being attracted to herbal medicinal preparations. *Bhrungraj*, with Latin name *Eclipta alba* Hassk., belongs to family Asteraceae, which is the largest family of flowering plants (Angiosperms), and distributed throughout the world. Among all the plants of Asteraceae family ***Eclipta alba* Hassk**., is having special importance due to its medicinal uses, which gives remarkable results and have obtained special status among the Asteraceae family.

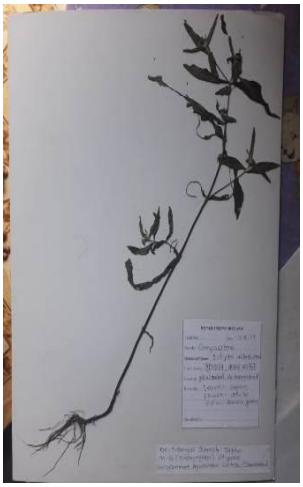
2. AIM OF THE STUDY:

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

3. MATERIAL AND METHOD:

3.1.PLANT MATERIAL:

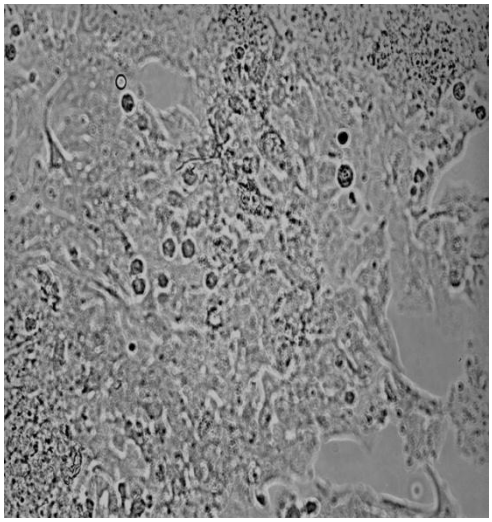
Eclipta alba was collected from Aurangabad and the sample was authenticated at Head of the Botany Department, Dr. Babasaheb Ambedkar Marathwada University Aurangabad, India. Specimen sample of *Eclipta alba* Hassk. has been allotted a voucher sample accession number 0660 and kept at the medicinal plant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



1. **Authentification of *Eclipta alba***

3.2. CELL LINE CULTURING:

HepG2 cell line is used for study which was purchased from the National Centre for Cell Science (NCCS) Pune. HepG2 cell line is human liver cancer cell line. It is 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.



2. **HepG2 cell line**

3.3. PREPARATION OF METHANOLIC EXTRACT OF *Eclipta alba*:

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

3. Preparation of Methanolic extract of *Eclipta alba*

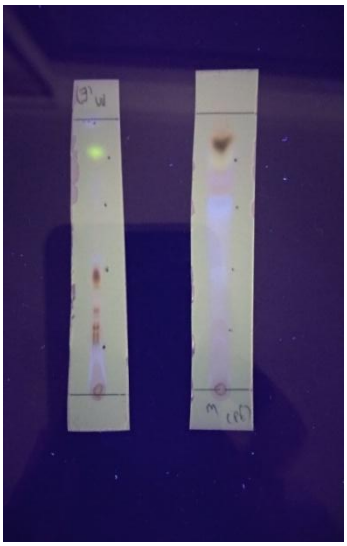
3.4. PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF *Eclipta alba*[4]-[5]

The methanolic 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 coumarins.

3.5. TLC ANALYSIS OF METHANOLIC EXTRACT OF *Eclipta alba*:

The collected fractions 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 contain 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.

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



4. TLC of methanolic extract of *Eclipta alba*

3.6. HPLC ANALYSIS OF METHANOLIC EXTRACT OF *Eclipta alba*:[6]

For obtaining HPLC chromatogram, chromatographic conditions were optimized with the mobile phase and flow rate. Methanol, water, acetic acid (95:5:0.04) as mobile phase in isocratic elution with a flow rate 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 methanolic 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, 75µg/ml and 100µg/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 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 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 extract wells relative to control wells. Tabulate the results and calculate the percentage of viability.

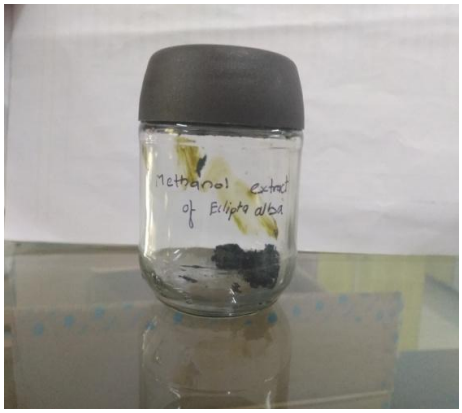
% cell growth (viability) = Absorbance sample/Absorbance negative control or untreated x 100

% growth inhibition = 100 - % cell growth [7]

4. RESULTS AND DISCUSSION:

4.1. METHANOLIC EXTRACT-

10gm of *Eclipta alba* in 150ml methanol solution results in 1.1 gm methanolic extract during this 8.2gm is the residual part. As a result, we can say that 11 % of the methanolic extract obtained from 10gm of a powered form of *Eclipta alba*. The yield of extract was 11% (w/w).



5. Methanolic extract of *Eclipta alba*

4.2. PHYTOCHEMICAL ANALYSIS-

While studying the phytochemical analysis we found that phenol, tannin, and quinones, and coumarin were present in the methanolic extract of *Eclipta alba*.

Sr	Phytochemica	Test	Resul

. no.	ls		t
1.	Carbohydrate	Fehling's test	-
2.	Phenols	$Fecl_3$ test	+
3.	Flavonoids	NH_3 test	-
4.	Alkaloids	Wagner's test	-
5.	Steroids	Salkowski's test	-
6.	Tannins	Lead acetate test	+
7.	Saponins	Frothing test	-
8.	Glycosides	Nitroprussi de test	-
9.	Quinones	-	+
10.	Amino acids	Ninhydrin test	-
11.	Coumarin	UV light test	+

Table no.1. Phytochemical analysis of methanolic extract of *Eclipta alba*

Above result shows that, there is presence of active secondary metabolites in methanolic extract of *Eclipta alba*.

4.3.TLC ANALYSIS-

TLC analysis shows 4 different spots and Rf values of the spots are - 0.18, 0.43, 0.66, 0.83. That proves that in methanolic extract of Eclipta alba has 4 active chemical constituents.

4.4.**HPLC ANALYSIS-**

HPLC analysis shows the following result in which five chemical compounds were observed at retention time shown in below table.

No.	Ret.Time	Area	Type	Height	Rel.Area
	min	mAU*min		mAU	%
1	3.12	3.0063	BM	4.968	0.89
2	4.64	27.2931	Ru	65.033	8.08
3	5.287	294.273	M	339.078	87.1
4	7.52	4.2976	M	13.418	1.27
5	8	8.9792	MB	13.321	2.66
Total:		337.8492		435.819	100

Table No.2. HPLC analysis of methanolic extract of *Eclipta alba*

A chromatogram for the methanolic extract of *Eclipta alba* shown in the following graph. **The** HPLC chromatogram of standard active chemical compounds has been shown in Fig. No. 1[8] and HPLC chromatogram for the methanolic extract of *Eclipta alba* is shown in Fig. No. 2

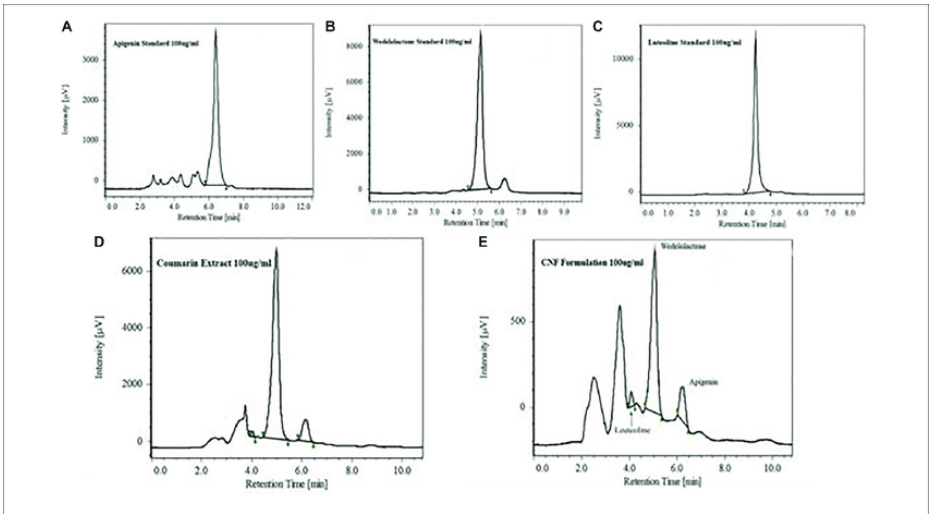


Fig.No. 1- HPLC chromatogram showing major peak of standard apigenin (A), standard wedelolactone (B), standard luteolin (C), wedelolactone in coumarin fraction (D), and CNF (E).

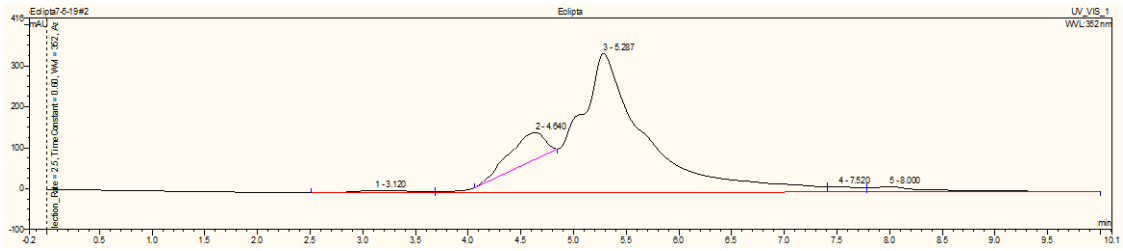


Fig. No.2. HPLC chromatogram of methanolic extract of *Eclipta alba*

4.5. CELL VIABILITY SRB ASSAY–

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

Concentrations ⇨	25µg/ml	50µg/ml	75µg/ml
Experiment ⇩	**O.D.**	**O.D.**	**O.D.**
1.	0.769	0.493	1.187
2.	0.941	0.513	1.127
3.	0.789	0.556	0.540
Average	0.833	0.520	0.951
% cell growth	24.55	15.33	28.03
% growth inhibition	75.45	84.67	71.97

Table No.3. Optical density readings of srb assay

In vitro study of methanolic extract of *Eclipta alba* shows positive results against HepG2 cell line, percentage of cell viability (growth) are 24.55%,

15.33% , 28.03% and percentage of growth inhibition are 75.45%, 84.67% and 71.97 for 25µg/ml, 50µg/ml, 75µg/ml concentrations respectively. The average percentage of growth inhibition is 77.36%.

5. STATISTICAL ANALYSIS:

Statistical analysis of our study is done with the help of statistician expert. Here we applied ANNOVA test for statistical analysis of our data, calculated data is as shown in following table:

	x1	**x1* x1**	**x2**	**x2*x2**	**x3**	**x3*x3**	
	0.77	0.59	0.49	0.24	1.19	1.41	
	0.94	0.89	0.51	0.26	1.13	1.27	
	0.79	0.62	0.56	0.31	0.54	0.29	
Summations	2.50	2.10	1.56	0.82	2.85	2.97	
	6.25		2.44		8.15		
	0.77	5.12	5.61	0.28	2.42	0.05	52.75
	Cx	**SSr**	**4.84**	**SSw**	**MSSa**	**MSSw**	**F Ratio**
			SSA				

Source of variance	**df**	**Ss**	**Mss**	**F ratio**
Among groups	2	0.28	2.42	52.75
Within Groups	6	4.84	0.05	52.75
Total	8			

Table 4. Statistical analysis

Here we calculated valuation of three concentrations 25µg/ml, 50µg/ml, 75µg/ml of methanolic extraction of *Eclipta alba* against HepG2 cell line where triplicates of optical densities were seen in Elisa reader. Degree of freedom among groups is 2 (n-1) and degree of freedom within the groups is 6 (k-1). Sum of square (SS) value among the group is 0.28 and SS within the group is 4.84. Mean of sum of square (MSS) value among the groups is 2.42 and MSS within the groups is 0.05. Thus, calculated F- ratio is 52.75 which is highly significant at 95% confidence and 5% level of significance. F ratio is calculated with the help of F ratio chart at degree of freedom 2 and 6.

6. CONCLUSION:

Present study revealed that methanolic extract of *Eclipta alba* shows anticancer activity against
HepG2 cell line due to presence of active chemical compounds present in it. Presences of active chemical constituents are proved with the help of phytochemical analysis, TLC and HPLC analysis. 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 support the anticancer activity of methanolic extract of Eclipta alba against HepG2 cell line.

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