ejpmr, 2020,7(1), 274-278

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IN VITRO STUDY OF AN ETHANOLIC EXTRACT OF *ECLIPTA ALBA* HASSK. FOR HEPG2 CELL LINE

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Article Received on 28/10/2019 **Article Revised on 18/11/2019** **Article Accepted on 08/12/2019**

ABSTRACT

For developing bridge way between Ayurveda and modern researches with advance technologies in Ayurveda we tried here an ethanolic extract of *Eclipta alba* against hepatic cancer. *Eclipta alba* has potent anti-cancer activity and therefore 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 beneficial for the researcher planning 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 and effective anticancer drugs and will further the discovery of their mechanism of action. We have used an ethanolic extract of *Eclipta alba* Hassk. for phytochemical analysis, TLC, HPLC analysis to test active chemical components in it. All tests proved that an ethanolic extract of *Eclipta alba* Hassk. containing many active chemical components, therefore our experiment showed the positive results for an ethanolic 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 79.33%. 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, ethanolic extract, (Sulforhodamine B) srb assay.

1. INTRODUCTION

Cancer is the second largest cause of death which killed 9 million in 2015 and will 11.5 million in 2030 (WHO 2007). Scientists are going to focus 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. Ayurveda becomes a ray of hope for hepatocellular carcinoma. Plants have been a prime source of extremely effective predictable drugs for the treatment of many forms of cancer.[1] The careful and cautious use of the Ayurvedic plant will definitely prove to be beneficial in hepatic cancer management. 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 ethanolic extract of it for our experiment. In Ayurveda, *Bhringraj* has special attention because of its multidimensional applications and in modern science, it is an important research drug due to its proven qualities.[3] Out of different extraction methods here we select the soxhlet extraction method for an ethanolic 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. With the help of modern technologies of biotechnology, we can put our best results of the medicinal plant in front of the world. It is the need for time 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 improve the pharmacokinetics of medicinal plants. Ethan-pharmaceutical uses shows that *Eclipta alba* has tremendous external and internal applications. It is the need for time to think 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 an ethanolic extract of *Eclipta alba* Hassk was evaluated against hepatic cancer cell line-HepG2. In Ayurveda *Eclipta alba* Hassk is having very important medicinal value. In our study, we focus the use of an ethanolic extract of *Eclipta alba* Hassk, on the liver cancer cell line. As many shreds of evidences 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 of it on hepatic disorders like jaundice.[3] *Bhringraj* having Latin name *Eclipta alba* Hassk, belonging to family Asteraceae. It is the important medicinal plant among the Asteraceae family because of it's widely used in medicinal purposes.

2. AIM OF THE STUDY

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

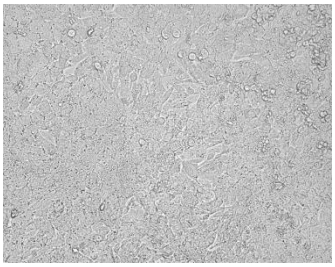
3. MATERIAL AND METHOD

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 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.



1. HepG2 cell line

3.3. PREPARATION OF AN ETHANOLIC 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. Ethanol was used for the extraction of the *Eclipta alba* with the help of the soxhlet apparatus. 10gm of *Eclipta alba* in 150ml an ethanol solution was used for extraction.

2. Preparation of an Ethanolic extract of *Eclipta alba*

3.4. PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACT OF *Eclipta alba*[4],[5]

An ethanolic 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 AN ETHANOLIC EXTRACT OF *Eclipta alba*

The collected fractions of an ethanolic 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 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 AN ETHANOLIC EXTRACT OF *Eclipta alba*[6]

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 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 ethanolic 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,

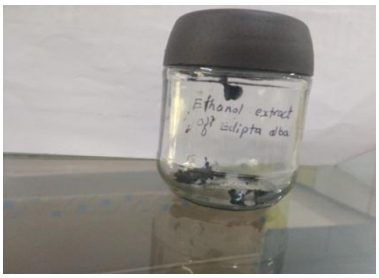
an aliquot of frozen concentrated (1mg/ml) was thawed and diluted to 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml with complete medium containing test article. Aliquots of extract (10µl) were mixed to appropriate microtiter wells containing 90µl of medium and final extract concentrations of 25µg/ml, 50µg/ml, 75µg/ml, were obtained. The plates with 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.[7]

4. RESULTS AND DISCUSSION

4.1. ETHANOLIC EXTRACT

10gm of *Eclipta alba* in 150ml an ethanol solution results in 0.9 gm an ethanolic extract during this 8.1gm was the residual part. As a result, we can say that 10% of an ethanolic extract obtained from 10gm of a powered form of *Eclipta alba*. The yield of extract was 10% (w/w).



3. Ethanolic extract of *Eclipta alba*

4.2. PHYTOCHEMICAL ANALYSIS

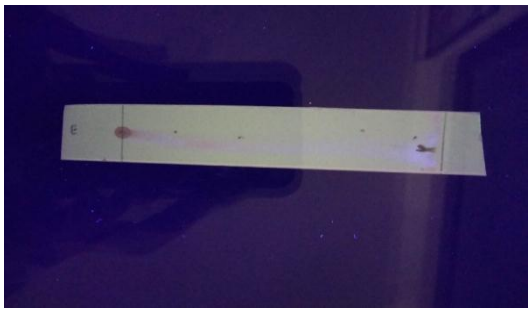
While studying the phytochemical analysis we found that phenol, tannin, quinones, and coumarin were present in an ethanolic extract of *Eclipta alba*.

Table no. 1: Phytochemical analysis of an ethanolic 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	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 3 different spots and Rf values of the spots were - 0.91, 0.38, 0.16. That proved that in the ethanolic extract of *Eclipta alba* had 3 active chemical constituents.



4. TLC of an ethanolic extract of *Eclipta alba*

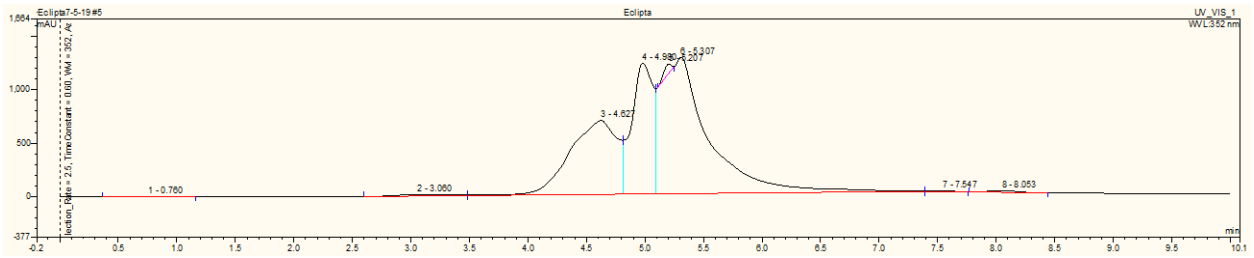
4.4. HPLC ANALYSIS

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

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

No.	Ret.Time	Area	Type	Height	Rel.Area
	Min	mAU*min		mAU	%
1	0.76	0.3953	BMB	1.177	0.03
2	3.06	6.4352	BM	13.805	0.53
3	4.627	320.6729	M	691.505	26.61
4	4.98	258.3326	M	1225.586	21.43
5	5.207	7.0085	Ru	82.094	0.58
6	5.307	606.7108	Mb	1274.641	50.34
7	7.547	1.5228	bMB	9.127	0.13
8	8.053	4.1982	BMB	15.709	0.35
	Total	1205.276		3313.644	100

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



5. HPLC chromatogram of ethanolic 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 and 3 were included. Experimental readings were optical densities for given concentrations at 565 nm wavelength.

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

Concentrations ⇨	25µg/ml	50µg/ml	75µg/ml
Experiment ⇩	O.D.	O.D.	O.D.
1.	0.376	1.114	0.341
2.	0.348	0.371	0.270
3.	0.364	0.369	0.213
Average	0.362	1.608	0.274
% cell growth	10	44.01	7.5
% growth inhibition	90	56	92

In vitro study of methanolic extract of *Eclipta alba* shows positive results against HepG2 cell line, percentage of cell viability (growth) are 10%, 44.01%, 7.5% and percentage of growth inhibition are 90%, 56% and 92 for 25µg/ml, 50µg/ml, 75µg/ml concentrations respectively. The average percentage of growth inhibition is 79.33%.

5. STATISTICAL ANALYSIS

Table 4: Statistical analysis.

	x1	x1* x1	x2	x2*x2	x3	x3*x3	
	0.38	0.14	1.11	1.24	0.34	0.12	
	0.35	0.12	0.37	0.14	0.27	0.07	
	0.36	0.13	0.37	0.14	0.21	0.05	
Summations	1.09	0.39	1.85	1.51	0.82	0.23	
	1.18		3.44		0.68		
	0.42	1.73	1.77	0.38	0.67	0.06	10.71
	Cx	**SSr**	**1.35**	**SSw**	**MSSa**	**MSSw**	**F Ratio**
			SSA				

Source of variance	df	Ss	mss	F ratio
Among groups	2	0.38	0.67	10.71
Within Groups	6	1.35	0.06	10.71
Total	8			

Here we calculated the valuation of three concentrations 25µg/ml, 50µg/ml, 75µg/ml of an ethanolic extract of *Eclipta alba* against HepG2 cell line where triplicates 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 6 (k-1). The sum of square (SS) value among the group is 0.38 and SS within the group is 1.35. The mean of the sum of square (MSS) value among the groups is 0.67 and MSS within the groups is 0.06. Thus, calculated the F- ratio is 10.71 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 6.

6. CONCLUSION

The present study revealed that an ethanolic 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 an ethanolic extract of *Eclipta alba* against the HepG2 cell line.

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