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Research Article

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

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**ABSTRACT**

It is the need of the day to identify the new anticancer herbal drug, which not only in possession of good anticancer effects but also cost effective. Here we are presenting such an anticancer Ayurvedic herb which is used since the centuries for the treatment of different diseases of diverse origin. *Eclipta alba* Hassk., also called as *Bhringraj* is very important medicinal herb in many medicinal formulations. Though it is commonly used for hair growth, many evidences found its hepatoprotective activity.

Here we are presenting all aspects about *Bhringraj* in terms of qualitative and quantitative values and we have also tried to prove the anticancer activity of it for hepatic cancer. We have used the aqueous extract of *Eclipta alba* Hassk. for phytochemical analysis, TLC, HPLC 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 aqueous 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 68.74%. 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 *Bhringraj* for its anticancer activity.

**KEYWORDS:** *Eclipta Alba*, *Bhringraj,* Cell viability SRB assay, HepG2 Cell Line, Anticancer activity.

## INTRODUCTION

Medicinal plants and their Phytoconstituents are being increasingly used as corresponding treatment for cancer. The many researches and clinical studies proved that the beneficial effects of herbal medicines on treat and prevent the cancer. Herbal medicines were most commonly used group of treatments, increasing in utilize from 5.3% before the diagnosis of cancer to 13.9% after the diagnosis of cancer.[1] Medicinal herbs are considered by common people to be safe, having less side effects and likely to having low dependency.[2] Due to the harmful side effects of modern chemical drugs, people are being attracted to herbal medicinal preparations. Many ethno-pharmaceutical evidences showed that different medicinal herbs in the form of fresh juice can be best option for live diseases, *Bhringraj* (*Eclipta alba* Hassk.) is one of them. It is belonging to family Asteraceae. It is widely used by many tribes in India for multipurpose medicinal uses. Herbal resources can be taken as the best option for therapeutic as well as a preventive major in the patients of liver diseases. Different researches also proved that *Bhringraj* is having important pharmacological actions, which are proved by modern technology that's why many Ayurvedic formulations are made up of *Bhringraj* as a main component. Here we planned to study anticancer activity of it against hepatocellular carcinoma.

In present days it is challenge to world's scientist to invent better medicine for cure and prevent the cancer. In our research study we use the herbal medicinal plant *Bhringraj* having Latin name *Eclipta alba* Hassk. and hepatic liver cancer cell line HepG2. Many of the Phytoconstituents in *Eclipta alba* having well known chemical constituent like wedelolactone, beta sitosterol, etc. There are approximately 53 to 55 chemical compounds present in it. [3] In Ayurveda *Bhringraj* has special attention for its *Keshya* (hair rejuvenation) action and is very commonly used in hair oils. But here we considered

in detail about its hepatoprotective activity of it. It is not possible to get fresh plant material all the time so, different extractions can be used for medicine uses. Different extraction solvents show presence of different active chemical compounds. We have evaluated and experimented different extracts of *Bhringraj* like ethanol, methanol, petroleum ether. In this research article we studied aqueous extract of *Bhringraj* for presence of different Phytoconstituents through phytochemical analysis, TLC, HPLC. After that with the help of srb assay we performed In vitro study of it. During study of different extracts of *Bhringraj*, every time we got better and positive results. Here purpose of our study is to put therapeutic and preventive herbal medicine option against hepatic carcinoma. *Bhringraj* itself means shining like Peacock, as the peacock is having different attractive colors, the same way *Bhringraj* shows its various shades of qualities.

## AIM OF THE STUDY

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

## MATERIAL AND METHOD

### 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.

### 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.

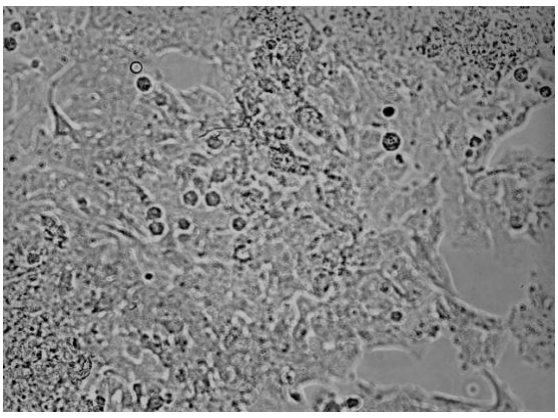

**Fig 1: HepG2 cell line**

### Preparation of an Aquous Extract of *Eclipta alba*

First of all fresh sample of *Eclipta alba* was dried at room temperature for 8-10 days, the dried whole plant was then powdered with the help of an electric blender. Distilled water ($H_2O$) used for the aqueous extraction of the *Eclipta alba* with the help of the soxhlet apparatus. 10gm of *Eclipta alba* in 150ml Distilled water solution was used for extraction.

**Fig 2: Preparation of an aqueous extract of *Eclipta alba***

### Phytochemical Analysis of an Aqueous Extract Of *Eclipta alba*[4,5]

An aqueous 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 etc

### TLC Analysis of an Aqueous Extract of *Eclipta alba*

The collected fractions of an aqueous 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.

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

**HPLC Analysis of an Aqueous Extract of *Eclipta alba* [6]**

For obtaining the HPLC chromatogram of an aqueous extract of *Eclipta alba*, 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.

**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 micro titer plates in 100µL at plating densities. Cell inoculated and micro titer 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]

**RESULTS AND DISCUSSION**

**Ethanolic Extract**

10gm of *Eclipta alba* in 150ml of distilled water solution results in 1.5 gm an aqueous extract during this 6.9gm was the residual part. As a result, we can say that 15 % of an aqueous extract obtained from 10gm of a powered form of *Eclipta alba*. The yield of extract was 15% (w/w).


**Fig 3: An aqueous extract and residue of *Eclipta alba***

**Phytochemical Analysis**

While studying the phytochemical analysis we found that phenol, Flavonoids, Tannins, Quinones, Amino acids and coumarin were present in an ethanolic extract of *Eclipta alba*.

**Table 1: Phytochemical analysis of an aqueous extract of *Eclipta alba***

| S.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 | + |

**TLC Analysis**

TLC analysis shows 2 different spots and Rf values of the spots were - 0.81, 0.54. That proved that in the ethanolic extract of *Eclipta alba* had 2 active chemical constituents.

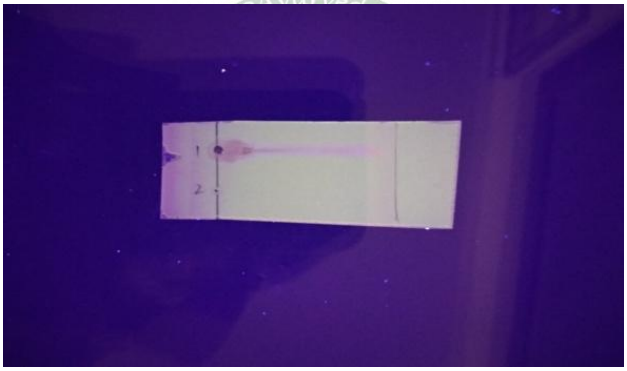

**Fig 4: TLC of an aqueous extract of *Eclipta alba***

**HPLC Analysis**

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

**Table 2: HPLC analysis of aqueous extract of *Eclipta alba***

| No. | Ret.Time | Area | Type | Height | Rel.Area |
|---|---|---|---|---|---|
| | Min | mAU*min | | mAU | % |
| 1 | 0.627 | 0.3235 | BMB | 0.978 | 5.08 |
| 2 | 2.667 | 0.0001 | BMB | 0.005 | 0 |
| 3 | 2.767 | 0.0001 | BMB | 0.004 | 0 |
| 4 | 4.333 | 1.5544 | BMB | 4.529 | 24.43 |
| 5 | 4.933 | 0.8733 | BM | 3.337 | 13.72 |
| 6 | 5.38 | 2.8717 | M | 3.291 | 45.13 |
| 7 | 8.02 | 0.1632 | Mb | 0.242 | 2.56 |
| 8 | 9.427 | 0.5774 | bMB | 0.864 | 9.07 |
| | Total | 6.3638 | | 13.251 | 100 |

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

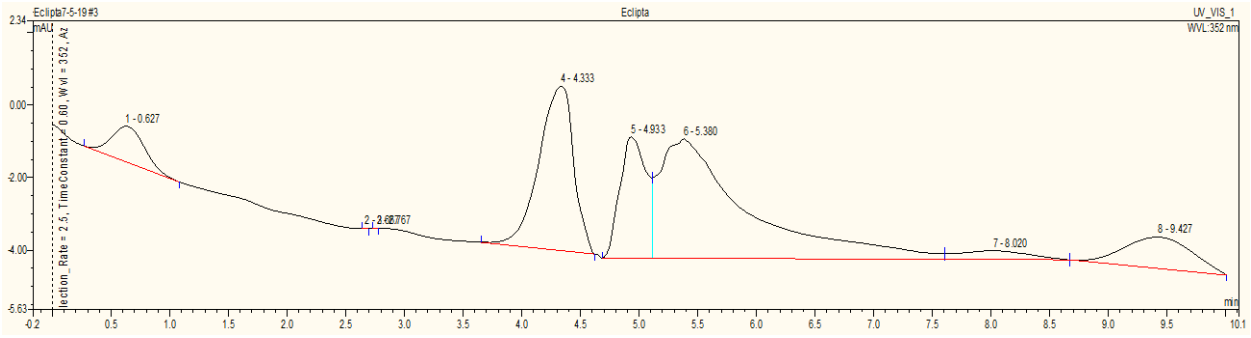


**Fig 5: HPLC chromatogram of an aqueous extract of *Eclipta alba***

**Cell Viability SRB Assay**

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

**Table 3: Optical density readings of SRB assay**

| **Concentrations ⇨** | 25µg/ml | 50µg/ml | 75µg/ml | |
|---|---|---|---|---|
| **Experiment ⇩** | **O.D.** | **O.D.** | **O.D.** | **O.D. (Media)** |
| 1. | 0.109 | 0.329 | 0.547 | 0.918 |
| **2.** | 0.168 | 0.168 | 0.291 | 2.919 |
| **3.** | 0.121 | 0.155 | 0.637 | 0.048 |
| **4.** | 0.128 | 0.132 | 0.903 | 0.048 |
| **Average** | 0.1315 | 0.196 | 0.594 | 0.983 |
| **% cell growth** | 13.37 | 19.93 | 60.47 | - |
| **% growth inhibition** | 86.63 | 80.07 | 39.53 | - |

In vitro study of an aqueous extract of *Eclipta alba* shows positive results against HepG2 cell line, percentage of cell viability (growth) are 13.37%, 19.93%, 60.47% and percentage of growth inhibition are 86.63%, 80.07% and 39.53% for 25µg/ml, 50µg/ml, 75µg/ml concentrations respectively. The average percentage of growth inhibition is 68.74%.

**Statistical Analysis**

**Table 4: Statistical analysis**

| | **x1** | **x1* x1** | **x2** | **x2*x2** | **x3** | **x3*x3** | |
|---|---|---|---|---|---|---|---|
| | 0.11 | 0.01 | 0.33 | 0.11 | 0.55 | 0.30 | |
| | 0.17 | 0.03 | 0.17 | 0.03 | 0.29 | 0.08 | |
| | 0.12 | 0.01 | 0.16 | 0.02 | 0.64 | 0.41 | |
| | 0.13 | 0.02 | 0.13 | 0.02 | 0.90 | 0.82 | |
| **Summations** | 0.53 | 0.07 | 0.79 | 0.18 | 2.38 | 1.61 | |
| | 0.28 | | 0.61 | | 5.65 | | |
| | 0.31 | 1.55 | 1.64 | 0.22 | 0.66 | 0.02 | 27.49 |
| | **CX** | **SSR** | **1.35** | **SSW** | **MSSA** | **MSSW** | **F Ratio** |
| | | | **SSA** | | | | |

| Source of variance | df | Ss | Mss | F ratio |
|---|---|---|---|---|
| **Among groups** | 2 | 0.22 | 4.50 | 27.49 |
| **Within Groups** | 9 | 1.33 | 0.02 | 27.49 |
| **Total** | 11 | | | |

Here we apply F-test for statistical analysis. We calculated the valuation of three concentrations 25µg/ml, 50µg/ml, 75µg/ml of an aqueous 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 0.22 and SS within the group is 1.33. The mean of the sum of square (MSS) value among the groups is 4.50 and MSS within the groups is 0.02. Thus, calculated the F- ratio is 27.49 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.

## CONCLUSION

The present study revealed that an aqueous 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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