



In Vitro Anti Cancer Activity of Ethanolic Extract of Eclipta Alba (L.)

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ABSTRACT

Cancer is a group of diseases involving uncontrolled and abnormal growth of cells with potential to invade (or) spread to other parts of body. The aim of present study is to evaluate the invitro anti cancer screening of ethanolic extract of whole plant of Eclipta alba against HL-60 cell lines by SRB (sulphorhodamine) assay, besides this property it has many other therapeutic uses. The plant with methanol is kept in closed vessel for seven days and the obtained extract is subjected to preliminary phytochemical analysis and in vitro anti cancer screening, these test reveals the presence of secondary metabolites and LD₅₀ concentration of extract was >80 µg/ml respectively i.e) the extract can inhibit the growth of cancer cells rather than killing them. For killing the cancerous cells very high concentration of extract is required.

Key words: Eclipta alba, Anti-cancer activity, SRB assay, HL-60 cell lines.

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INTRODUCTION

Eclipta alba [L] is an annual herbaceous plant, commonly known as false daisy. It is an erect (or) prostrate, much branched, roughly hairy, rooting at the nodes, the leaves are opposite, sessile and lanceolate. It is also known as *Bhringaraj* and *Karisilakanni*. The genus name comes from the Greek word meaning “Defecient”, with reference to the absence of the bristles and owns on the fruits. Main active principles consist of coumestans like wedelolactone, dimethyl wedelolactone, furanocoumarins, olenane and taraxastane glycosides. It is adaptable to changing habitat and also found in some temperate parts of the world, prefers warm climate at a temperature range of 25 - 35°C for its good growth and yield. It is a cosmopolitan weed by western colonization activity (Duthie, 1905). *Eclipta* is said to be the best drug for the treatment of liver ailments such as cirrhosis, infective hepatitis and other conditions involving hepatic enlargement. Medicinal value of *Bhringaraj* has been discussed in the “*Bhavaprakash*”- the ancient Indian classic fresh juice of leaves is rubbed on the scalp for promoting growth of hair. *Bhringaraj* is bitter, hot fattening alterative anthelminticum and alexipharmic. It is useful in mental disorders, anaemia, headaches, earaches, insomnia, dysentery, fevers and as a mild laxative^[1-3]. *Bhringaraj* works as an antiseptic, astringent, oral mouthwash for infected gums and loose teeth. It also helps to maintain and rejuvenate teeth, bones, sight, hearing and memory. Externally it can also use or minor cuts or injuries, athlete foot, eczema.

Scientific Classification

It belongs to Kingdom of Plantae, Clade of Angiosperms, Asteraceae^[4] family. Its genus is *Eclipta* and species is *alba*. Its binomial name is *Eclipta alba* L.

Eclipta alba is geographically distributed in Himalayas, in moist and cool places of Punjab, Orissa, Bihar, West and south India.^[5] *Eclipta alba* has antihepatotoxic properties- it significantly counteracted CC14-induced inhibition of hepatic microsomal drug metabolising enzymes^[6], immunomodulatory activities, analgesic and anti-inflammatory activity^[7], anti-diabetic, anti-cancer^[8], anti hyperlipedemic properties^[9], anti oxidant properties^[10], anti microbial activity, miscellaneous activity.

MATERIALS AND METHOD

The plant material *Eclipta alba* was collected from the botanical gardens of Andhra university, Visakhapatnam, India in august 2017. The plant species was authenticated by Dr.M.Ramaiah, Associate professor and Head, Department of pharmacognosy, Hindu College of Pharmacy, Guntur, India.



Figure 1: Diagram of Eclipta alba plant

Extraction Process

The dried powdered plant material (1000gms) was allowed to contact with solvent methanol in a closed vessel and then allowed to macerate with occasional shaking for seven days. Strain the liquid, press the marc, mix the liquids and finally clarifying by filtration. The extract thus obtained was concentrated under vacuum (50°C) by using rotary evaporator, dried completely and weighed. The extract thus collected was subjected to preliminary phytochemical analysis and invitro anticancer screening. (Yield = 52.24%)

Preliminary Phytochemical Analysis

The extract was prepared and tested for preliminary phytochemical analysis which is used to identify the presence of various secondary metabolite. Identified secondary metabolites are carbohydrates, proteins & amino acids, steroids and triterpenoids, glycosides, flavonoids, tannins, etc, are given in Table1.

Table 1: Phytochemical analysis of Ethanolic Plant extract

S. No.	Compound	Ethanolic Extract
1.	Carbohydrates	+
2.	Proteins & amino acids	+
3.	Steroids &Triterpenoids	+
4.	Glycosides	+
5.	Flavonoids	+
6.	Alkaloids	-
7.	Tannins	+
8.	Saponins	-

‘+’Present, ‘-’ Absent

***In-Vitro* Anticancer Screening against HL-60 Cell lines**

Crude methanolic plant extract was prepared by cold maceration technique and then further extract was subjected to preliminary phytochemical studies and in-vitro anticancer screening by SRB assay.

Chemicals

Test drugs: Ethanolic extract of *Eclipta alba* (at 10, 20, 40 and 80 µg/ml doses) mentioned in Table 2.

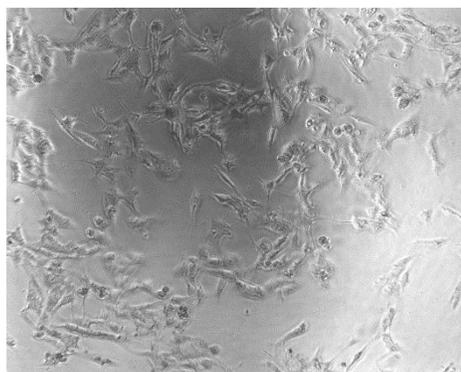
Table 2: % Control Growth of Ethanolic extract of *Eclipta alba* on HL60 cell lines

Human Leukemia Cell Line HL60																
% Control Growth																
Drug Concentrations ($\mu\text{g/ml}$)																
	Trail- I				Trail- II				Trail- III				Average Value			
	10 $\mu\text{g/}$ Ml	20 $\mu\text{g/}$ ml	40 $\mu\text{g/}$ ml	80 $\mu\text{g/}$ ml	10 $\mu\text{g/}$ ml	20 $\mu\text{g/}$ ml	40 $\mu\text{g/}$ ml	80 $\mu\text{g/}$ ml	10 $\mu\text{g/}$ ml	20 $\mu\text{g/}$ ml	40 $\mu\text{g/}$ ml	80 $\mu\text{g/}$ ml	10 $\mu\text{g/}$ ml	20 $\mu\text{g/}$ ml	40 $\mu\text{g/}$ ml	80 $\mu\text{g/}$ ml
EEEE	97.9	52.1	15.0	4.5	94.3	55.1	13.8	2.7	81.0	50.5	16.6	5.9	91.1	52.6	15.2	4.4
ADR	-7.3	-31.3	-46.6	-49.0	-4.7	-34.5	-34.5	-41.7	1.6	-12.7	-17.4	-26.8	-3.5	-26.2	-32.8	-39.2

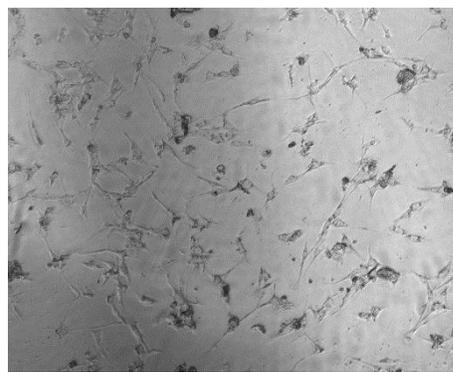
Standard drug: Adriamycin

Cell lines: human leukemia cell lines (HL60 cell lines)

Cell culture: Human Leukemia Cell lines (HL60 cell lines) used in this study were procured from Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Mumbai. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO₂ incubator. Photomicrographs of growth morphology of Human Leukemia Cell Line HL60 was shown in Figure 2.



STANDARD



SAMPLE

Figure 2: Photomicrographs of growth morphology of Human

Leukemia Cell Line HL60 (Standard & Sample)

Sulphorhodamine (SRB) assay^[11]

The monolayer cell culture was trypsinized and the cell culture was adjusted to 5×10^3 cell/ml. To each well of the 96 well microtiter plate, 0.1ml of the diluted cell suspension was added. After 24hrs, when a partial monolayer had formed. Supernatant was flicked off, the monolayer was washed once with medium and 100 μ l of different concentrations of extract were added to the cells in microtiter plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, microscopic examination was carried out and observations were recorded every 24 hr. After 72 hrs, 25 μ l of 50% TCA (Trichloroacetic acid) was gently added to the wells in such a way that it formed a thin- layer over the extract. The plates were incubated at 4°C for 1hr. They were flicked and washed 5 times with water to remove traces of the medium, extract and serum and air dried. They were stained with SRB (0.4% prepared in 1% acetic acid) for 30 mins. The unbound dye was then removed by rapidly washing 4 times with 1% acetic acid. The plates were then air dried. Tris base [tris (hydroxymethyl) aminomethane] (10mM, 100 μ l) was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 min. The absorbance was measured

using a micro plate reader at a wavelength of 540nm. The percentage control growth, GI50 (conc. of drug or test extract needed to inhibit cell growth by 50%), TGI and LC₅₀ were calculated from dose response curves for each cell line.

RESULTS AND DISCUSSION

Anticancer activity of ethanolic extract of *Eclipta alba* was conducted with appropriate positive control (Adriamycin) which yielded results on HL60 cell lines. The extract shows good inhibitory activity but the inhibitory effect of this extract was observed to be a little bit weaker than that shown by standard drug Adriamycin. The results were shown in the Table 3.

Table 3: LC50, TGI and GI50 Drug concentrations (µg/ml) of Ethanolic extract of *Eclipta alba* on HL60 cell lines

Name of the extract	Drug concentrations (µg/ml) calculated from graph		
	LC50	TGI	GI50
EEEA	>80	70.2	30.5
ADR	71.1	30.7	<10

From the results, it is clear that LD₅₀ concentration of extract was greater than 80 µg/ml i.e, the extract is more preferably inhibiting the growth of cancer cells rather than killing the cells as the extract requires very high concentration to kill the cancerous cells. The ethanolic extract of *Eclipta alba* showed total growth inhibition at 30.5 µg/ml against HL 60 cell lines.

If GI 50 value of extracts is ≤ 20 µg/ml then it is considered to possess the anticancer activity. Here ethanolic extract of *Eclipta alba* showed result i.e.,30.5 µg/ml so it is considered as inactive on cancerous stem cells, but at the same time it was proved that a minimum of 40µg/ml concentration is sufficient to produce GI 50. Therefore, from the above results it was clear that the plant *Eclipta alba* was having cytotoxic activity against the HL-60 cell lines and supports the folkloric usage of the plant and confirmed that the studied plant possesses the constituents (steroids, flavonoids, glycosides, carbohydrates and tannins) with cytotoxic properties that can be used for developing novel anticancer agents.

GI50: Growth inhibition of 50 % (GI50) calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, drug concentration resulting in a 50% reduction in the net protein increase

TGI: Drug concentration resulting in total growth inhibition (TGI) will calculate from

$$Ti = Tz$$

LC₅₀: Concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of 50% cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$.

ADR: Adriamycin (Doxorubicin), Positive control compound.

GI50 value of $\leq 10^{-6}$ (i.e. 1 μ mole) or $\leq 10\mu$ g/ml is considered to demonstrate activity in case of pure compounds. For extracts, GI50 value $\leq 20\mu$ g/ml is considered to demonstrate activity.

EEEE: Ethanolic extract of *Eclipta alba*.

CONCLUSION

In vitro anticancer screening of ethanolic extract of whole plant of *Eclipta alba* against HL-60 cell lines by Sulphorhodamine (SRB) assay reveals that the plant having cytotoxic activity against the HL-60 cell lines and supports the folkloric usage of the plant. Preliminary phytochemical analysis of methanolic extract of whole plant of *Eclipta alba* reveals the presence of steroids, flavonoids, glycosides, carbohydrates and tannins. The presence of above constituents in alone or in combination might be responsible for the observed anticancer activity.

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