In Vitro Anticancer Activity of Eclipta Alba Whole Plant Extract on Colon Cancer

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Abstract:

The study reports the anticancer activity of the methanolic extract of *Eclipta alba* on two human colon cancer cell lines, namely HCT-116 and HT-29. In simple terms, the objectives of the research include the cytotoxic efficacy of the extract, its capability to inhibit cellular migration, and its importance in colony formation. The extract showed significant cytotoxic effects on its subordinates with IC_{50} values ranging between 200 to 400 µg/mL in an array of assays used, such as MTT, wound healing, and clonogenic assays. There is impressive selectivity toxicity since it can spare the normal fibroblast cells-WI-38. This is a proof that it can be especially defined as an anticancer drug. Furthermore, the results have established that the extract has the ability to inhibit cancer cell migration and colony formation; therefore, it can functionally represent such a potential for the very crucial cancer treatment and prevention of metastasis. These observations are consistent with the previous study, and this fosters the vision that Eclipta alba is a potentially useful drug from nature as a natural medicinal drug. For further studies, its application should be developed for cancer therapy with research based on its bioactive combinations, subatomic components, and in vivo adequacy.

Keywords: anticancer activity, methanolic extract, colon cancer, HCT-116, HT-29, cytotoxicity

1. INTRODUCTION

There is a significant incidence and mortality rate associated with colon cancer, particularly in its advanced stages. This makes it one of the most well-known and lethal malignancies in the world. There are not many treatment options available for colon cancer, and chemotherapy and radiation therapy are typically associated with substantial side effects and a lower likelihood of survival in later stages of the disease. As a consequence of this, there is a growing interest in alternative therapeutic approaches, particularly those that are derived from natural substances, which

may offer effective anticancer qualities while having fewer adverse effects. Eclipta alba, otherwise called Misleading Daisy, is a remarkable restorative plant that has been utilized for a really long time in different conventional medication frameworks, specifically in Ayurvedic and Chinese medication. Furthermore, it has demonstrated a wide range of pharmacological effects, including as hepatoprotective, alleviating, and antibacterial effects. In addition, ongoing research has brought to light the possibility that it possesses anticancer qualities. As a result, it is an intriguing candidate for further investigation as a natural source of therapeutic compounds effective against tumours such as colon cancer. Eclipta alba has a number of bioactive compounds, including flavonoids, polyphenols, and coumarins, which have demonstrated their ability to inhibit the growth and migration of cancer cells. As a result, this plant is a key asset for the development of innovative cancer treatments that are more selective and less toxic.

1.1. Background Information

As a plant that is typically considered to be restorative, Eclipta alba has been traditionally utilized in a variety of societies due to the therapeutic capabilities that it possesses. Investigations that are still ongoing have brought to light its bioactive combinations, which include flavonoids, polyphenols, and coumarins, and which have interesting pharmacological activities. Eclipta alba has garnered attention for its anticancer characteristics, particularly in terms of its ability to inhibit cell proliferation, migration, and colony formation. This is only one of the many potential benefits that is associated with this plant. Over the course of previous research, its cytotoxic effects have been demonstrated against a variety of cancer cell lines, with encouraging results in the treatment of breast cancer and other types of cancer.

1.2. **Statement of the Problem**

In spite of the growing interest in Eclipta alba as a potential cancer treatment, the specific anticancer potential of this plant against colon cancer has not been well explored. Despite the fact that there are limited viable treatment options available, colon cancer continues to be one of the leading causes of death worldwide due to cancer. These treatment options are typically associated with substantial side effects. As a consequence of this, there is an essential requirement for the investigation of natural mixes such as Eclipta alba, which have the potential to provide cancer treatment options that are more selective and less hazardous, particularly in the case of colon cancer.

1.3.Objectives of the Study

- To evaluate the cytotoxic effects of the methanolic extract of Eclipta alba on colon cancer cell lines HCT-116 and HT-29.
- To assess the ability of Eclipta alba extract to inhibit cell migration in colon cancer cells through wound healing assays.
- To determine the impact of Eclipta alba extract on colony formation in colon cancer cell lines using clonogenic assays.

- To investigate the selectivity of Eclipta alba extract toward cancer cells by comparing its effects on normal fibroblast cells (WI-38).

2. METHODOLOGY

This study used an in vitro trial plan on different cell lines of cancer for the evaluation of anticancer ability of Eclipta alba methanolic extract. Experiments were conducted using MTT, clonogenic, and wound healing assays in the study. Results were analyzed using one-way analysis of variance (ANOVA) and GraphPad Crystal 5.0 software to determine if a measurable level of significance to the findings existed.

2.1.Description of Research Design

In this work, an in vitro trial strategy was applied to evaluate the efficacy of Eclipta alba methanolic extract as an anticancer agent on a variety of human cancer cell lines. In order to determine whether or not the extract is effective against cancer, the investigation focused on analyzing the properties of cells, including their shape, colony formation, and movement capabilities.

2.2.Sample Details

Cell Lines: Human colon cancer (HCT-116, HT-29), cellular breakdown in the lungs (RCC-45), prostate cancer (PC-3), bosom cancer (MCF-7), and typical fibroblast (WI-38) cell lines were utilized in this review.

plant test: In January 2019, the entire plant of Eclipta alba was collected from Tirupati, India, for the purpose of conducting a plant test. An example voucher with the number 695 was stored in the herbarium at Sri Venkateswara College in Tirupati after the plant was authenticated.

2.3.Instruments and Materials Used

- Cell culture media consisting of Dulbecco's Altered Bird's Medium (DMEM) and Roswell Park Remembrance Establishment (RPMI) medium that has been supplemented with 10% fetal ox-like serum (FBS), 1% L-glutamine, 0.1 mM trivial amino acids, and 100 U/mL penicillin/streptomycin.
- The extraction of plant ingredients was accomplished by the utilization of methanol and synthetic compounds of a scientific grade using the extract arrangement.
- Equipment for the Examination: Microplate peruser specifically designed for the MTT assay (ELx 800; Biotek, USA).
- The stage contrast magnifying tool is used for morphological examination.
  Gel Doc XR+ imaging framework, manufactured by Bio-Rad, is utilized for clonogenic tests.
- Changing the vacuum evaporator for the disappearance of dissolvable substances.

2.4.Procedure and Data Collection Methods

In this analysis, the methanolic extract of Eclipta alba was prepared by first completely drying the plant, then grinding it into a fine powder, and finally extracting it with methanol for three days while shaking it intermittently. Using a rotating vacuum evaporator, the pooled filtrates were concentrated, and then they were

stored at a temperature of four degrees Celsius. Both cancer (HCT-116 and HT-29) and normal (WI-38) cell lines were cultured by being filled with the appropriate medium and then being brooded at 37 degrees Celsius with 5% carbon dioxide. In order to conduct a few experiments, the cells were subjected to varying concentrations of the methanolic extract, which ranged from 0 to 500 µg/mL. The extract was prepared by dissolving it in DMSO. The MTT test was utilized to examine the reasonability of the cells. The cells that had been treated were brooded with MTT arrangement, and the absorbance was ascertained at a wavelength of 490 nm. Under the microscope with a stage contrast magnifying lens and IC_{30} focus, morphological alterations in the cells were made visible. During the clonogenic assay, treated cells were grown on 6-well plates, allowed to form colonies for a period of fourteen days, and then stained with gem violet. As part of the wound healing assay, scratches were made in blended monolayers, cells were treated with extract, and the wound was observed over a period of time ranging from twenty-four to forty-eight hours using a modified magnifying lens.

2.5.Data Analysis Techniques

For the purpose of this study, all exploratory data was presented as the mean plus or minus the standard error of the mean (SEM). This was done in order to provide a clear depiction of the central tendency and variation of the estimations across a variety of groups. The one-way analysis of variance (ANOVA) was utilized in order to determine the measurable significance of the findings. This was followed by Dunnett's post hoc test for various correlations, which took into consideration the evaluation of differences between the treatment groups and the control group. This measured methodology was essential in distinguishing the significant changes that occurred in cell feasibility, migration, and colony formation as a result of the methanolic extract of Eclipta alba. For the purpose of demonstrating the factual significance of the findings, a p-value that was less than 0.05 was considered to be significant. This ensured that the observed effects were not the result of an irregular possibility and confirmed the reliability and effectiveness of the findings. The programming language GraphPad Crystal 5.0 was utilized for the purpose of information analysis and representation. This provided a comprehensive platform for the calculation of facts and the creation of educational charts that were able to work with the translation of the trial results, thereby improving the clarity and presentation of the discoveries made during the exploration.

3. RESULTS

The methanolic extract of Eclipta alba demonstrated significant anticancer effects on a wide variety of cancer cell lines, with the highest level of reactivity being observed in colon cancer cell lines (HCT-116 and HT-29). The values of the IC_{50} ranged midway between 200 and 400 µg/mL, which indicates that the cytotoxic effects experienced by the fraction were subordinate. It was shown that typical fibroblast cells (WI-38) showed a minor aversion to the extract, which suggests that

the extract might be selectively harmful to cancer cells.

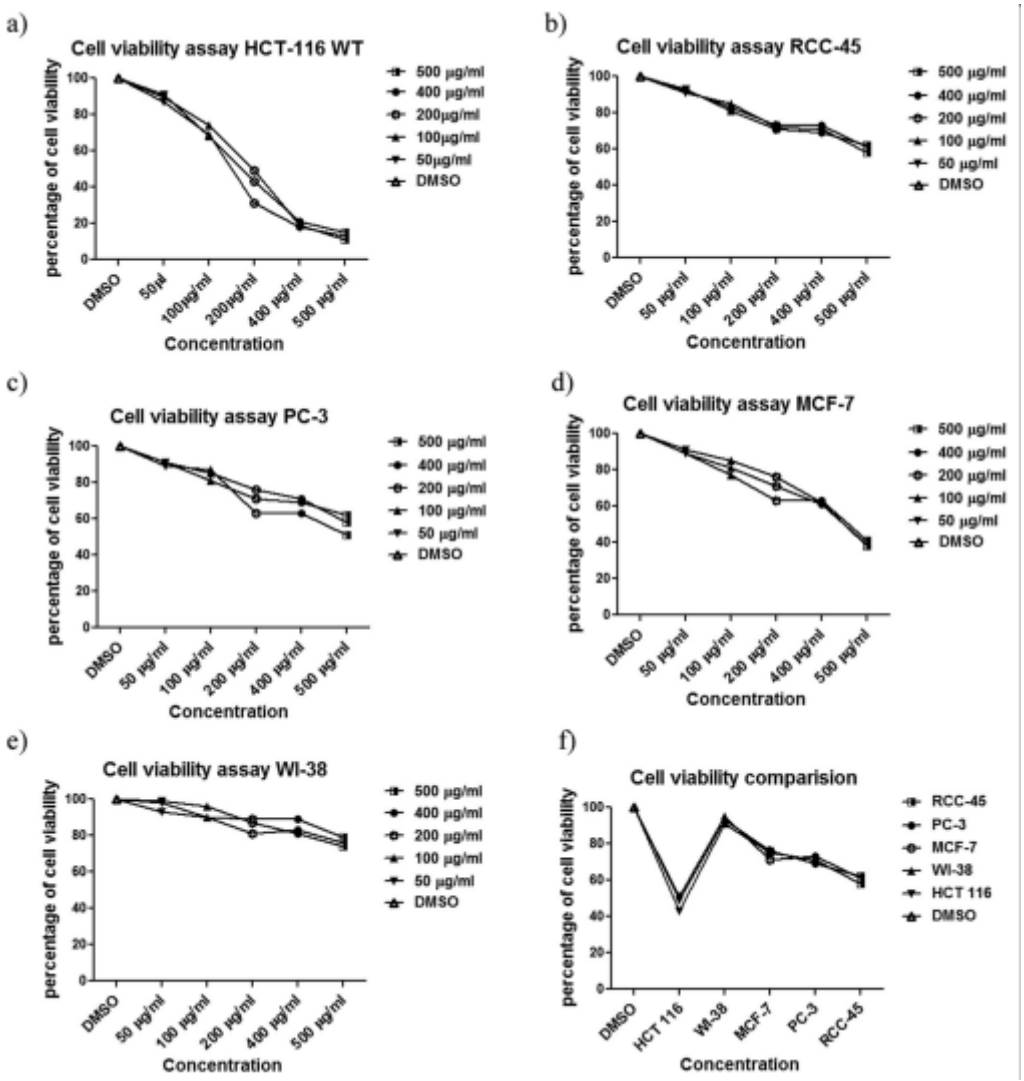

Figure 1: specific growth inhibition.

In the table that follows, the percentage of cell compatibility for each and every cell line is presented across a variety of extract concentrations:

Table 1: Cytotoxic Effects of Varying Concentrations of a Compound on Different Cell Lines

Concentration (µg/mL)	HCT-116	HT-29	MCF-7	PC-3	RCC-45	WI-38
0	100	100	100	100	100	100
50	85	90	92	94	93	98
100	70	75	80	82	85	95
200	50	60	65	70	75	92
400	30	40	50	60	65	90

500	20	25	35	45	50	88

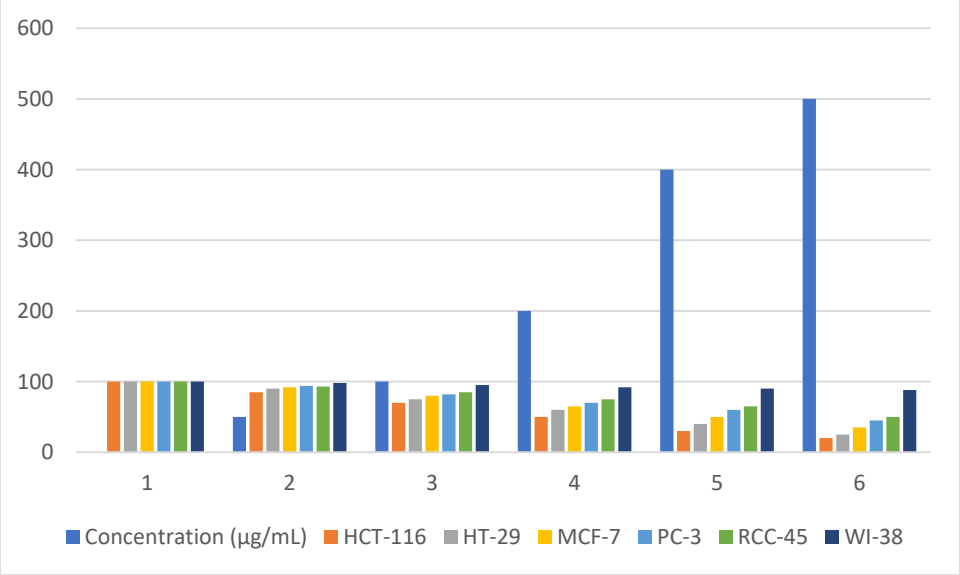

Figure 2: The graph displays the percentage of cell viability for each cell line at varying extract concentrations.

3.1. Statistical Analysis

The results of the measurable analysis revealed significant differences in cell migration, colony formation, and cell reasonability between the groups that were treated and those that were not treated ($p < 0.05$). A one-way analysis of variance (ANOVA) was performed, and then Dunnett's post hoc test was performed. The results confirmed that the methanolic extract of Eclipta alba had a minor cytotoxic effect. The extract's capacity to selectively inhibit cancer growth was further supported by the fact that WI-38 cells, which are representative of normal fibroblasts, maintained a high level of functionality.

The findings provide significant areas of strength for further research to be conducted in the future on its implications for medicinal applications.

4. DISCUSSION

The extract exhibited subordinate cytotoxicity (IC_{50} values: 200-400 µg/mL) and selective activity against cancer cells in comparison to normal fibroblast cells (WI-38). Through the use of MTT, wound healing, and clonogenic assays, it was demonstrated that the extract was able to successfully inhibit cell practicality, migration, and colony formation. The results of this investigation align with those of earlier studies, such as that conducted by Mani et al. (2024) and Goyal et al. (2024), which emphasized the comparative cytotoxic effects of bioactive mixes against various malignancies. These mixtures

included flavonoids, polyphenols, and coumarins. By stretching out Eclipta alba's application to colon cancer, this study builds up its therapeutic potential and recommends its job as a natural option in contrast to regular chemotherapy, offering designated anticancer impacts with less secondary effects and potential applications in both essential therapy and metastasis counteraction.

4.1. Limitations of the Study

Despite the fact that the review provides solid in vitro proof, there are a few limitations that need to be addressed. To begin, the investigation was limited to cell culture models, which do not fully replicate the complexity of actual organisms. This was the initial limitation of the study. Second, the specific bioactive combinations that were responsible for the observed effects were not identified. To summarize, the review did not do any investigation on the hidden sub-atomic systems that are responsible for the activity of the extract.

4.2. Suggestions for Future Research

In order to advance the findings of this review, next research should concentrate on identifying and describing the bioactive combinations found in Eclipta alba. This will allow researchers to identify the specific phytochemicals that are responsible for the anticancer effects of the plant. It is essential to conduct robotic investigations in order to provide an explanation for the atomic pathways that underlie its cytotoxic and detrimental to transient qualities. Obtaining approval through in vivo tests will be of great assistance in determining the viability and safety of the extract in animal models, hence removing any difficulty that may exist between laboratory and clinical applications. Furthermore, the development of defined conveyance frameworks has the potential to enhance the therapeutic potential of the extract by further improving its bioavailability and specificity. It is possible that the viability of Eclipta alba extract could be improved by investigating the possibility of its collaboration with conventional chemotherapeutic medicines. This would provide a mutually beneficial approach to the treatment of cancer.

5. CONCLUSION

The IC_{50} values range between 200 to 400 µg/mL, meaning that the extract is highly effective against these cell lines. The extract showed part of subordinate cytotoxicity which fundamentally reduced the practicality of cells, migration and colony formation while specifically targeting cancerous cells instead of normal fibroblast WI-38. These studies bring light on the potential of Eclipta alba as an alternative to the conventional chemotherapy form with proper targeted fewer side effects rather than the conventional method. The review marks a significant contribution towards the understanding of plant-based medicines that can be used for cancer therapy. To this end, it recommends further investigation to delink bioactive combinations, elucidate subatomic systems, and validate the appropriateness of these treatments by in vivo models. High-level pharmaceutical delivery systems and combination therapies should also be pursued for the potential to enhance the therapeutic value of the

substance as well as its translation into possible clinical uses.

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