

Assessment of Anti-inflammatory and Anti-Proliferative Potential of *Euphorbia hirta* Roots: An *in vitro* Analysis

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ABSTRACT

Background: The current study focuses *in vitro* anti-inflammatory and anti-proliferative evaluation of the alcoholic extract of *euphorbia hirta* roots. Anti-inflammatory potential was screened by incorporating inhibition of protein denaturation assay and anti-proliferative potential by MTT assay.

Methods: Anti-inflammatory potential of extract was evaluated by calculating the percentage inhibition of protein denaturation and anticancer activity of the alcoholic extract was assessed by evaluating the cell viability. Alcoholic roots extract of *euphorbia hirta* was treated with MCF – 7 cell lines and cell viability was determined at the wavelength of 570 nm by MTT method.

Results: The results suggested the significant anti-inflammatory potential as it effectively inhibits the protein denaturation. The alcoholic roots extract of *euphorbia hirta* also showed anti-proliferative effect against MCF - 7 cell-lines ($p \leq 0.05$) in a dose dependent manner. **Conclusion:** The root extract showed potential anti-inflammatory and anti-proliferative potential. As from

the results, it was concluded that extract had capability to constrain the propagation of cancer cells. Therefore, it appears to be a virtuous contender to provide protection against growth of tumour cells.

Key words: Euphorbiaceae, Medicinal plants, Phytoconstituents, Anticancer, Protein denaturation.

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INTRODUCTION

To save people from the grasps of ailments is a responsibility blessed and necessary from the time immemorial. Herbal plants are cast-off in management or as a counteractive effect since ancient ages.^{1,2} In India, use of the various parts of many herbal plants to treat various ailments has been in trend from ancient times.³ Genus *Euphorbia* from family Euphorbiaceae contains many important herbal medicinal plants. Plants which belongs to the family Euphorbiaceae has been found to contain compounds like flavonoids, alkaloids and triterpenoids by means of intense phytochemical screening. *Euphorbia hirta* belongs to the genus *Euphorbia* (Family - Euphorbiaceae). It is a slender-stemmed, twelve-monthly hairy plant; grow up to a length of 40 cm in height. *Euphorbia hirta* has a separate and developed primary root i.e. tap root system.⁴ In traditional indigeneous system of medicine, *Euphorbia hirta* is widely used for the management and prevention of various bronchial and respiratory diseases.^{2,5} In spite of usage in bronchial and respiratory diseases, it is also used as antipyretic, anxiolytic, antifertility and analgesic herb.² Many researchers have done phytochemical screening of *Euphorbia hirta* and it is testified to contain flavonoids like quercetin, afzelin, myricitin, euphorbin-A, euphorbin-B, shikmic acid, kaempferol and gallic acid.^{2,6}

Inflammation is a procedure in which cells of human body protect them from infection caused by outside infectious agents, like bacteria. Inflammation is generally considered to be of two types. First one is acute inflammation and second is chronic inflammation.^{7,8} Protein denaturation has been correlated with the development of inflammatory disorders like arthritis, diabetes and cancer. Herbal anti-inflammatory agents are being explored as a potential alternative to synthetic drugs depending upon the presence of various types of phytoconstituents like flavonoids, alkaloids, glycosides, terpenoids and resins.⁹

Cancer is a disease in which cells and tissues of body grow abnormally. For proper functioning of a body, a strictly regulated cell growth is required. Cancerous cells continue to divide and multiply at abnormal speed, producing tumours. Benign tumours are the tumours which do not spread elsewhere in the body. Malignant tumour are the tumours which can enter the other tissues of body as well as can carry on to the other parts of body, if not get treated on time.^{10,11} Breast cancer is the cancer which is normally found in one in four cancers detected in women. The exact reason behind the cause of breast cancer is still not identified. In contemporary medicine, the chief treatments available for cancer include chemotherapy, radiotherapy and surgery. Although many chemotherapeutic drugs are accessible, but as they produce undesirable adverse effects as well as they are extremely expensive.^{12,13} Therefore, there is a significant curiosity in finding of new herbal agents which are effective against cancer. Some chemical constituents, that are present within the plant have evidenced cyanogenetic effect on these cancer cells and have no other side effects also. So, they possibly will be reflected as prominent anti - proliferative representatives to treat cancer.^{14,15} Although, many studies are done on *Euphorbia hirta*. Most of the studies were performed with the complete plant or leaves and few of them were performed on the separated components of the plant. ² Accordingly this study was completed with an objective to evaluate the anti-inflammatory and anti-proliferative potential of *Euphorbia hirta* root extract via *in vitro* measures. *In vitro* studies are essential to make therapeutic developments. It cannot perform the whole thing which *in-vivo* studies can do, but it can perform a lot. Thus *in-vitro* research is significant across all the arenas of medical research. For assessment of anti-inflammatory activity, protein denaturation inhibition assay was performed and activity was compared with diclofenac sodium as standard. For

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anti-proliferation potential, MTT test was performed to grasp the capability of this plant for use in herbal medicine.

MATERIALS AND METHODS

Collection of Plant material

Euphorbia hirta root was collected from Palwal, Haryana, India, in the month of November, 2018. Identification and authentication was done by senior principal scientist of CSIR-NISCAIR and a plant specimen voucher was available with Raw Material Herbarium and Museum (RHMD). The root was cleaned thoroughly with distilled water to remove any type of contamination.

Extraction of Plant material by soxhlet method

The shade dried root was powdered by using dry grinder and passed through sieve. The root powder was packed properly into soxhlet apparatus and extracted successively with alcohol. This procedure was continued nonstop until the solvent in the thimble converts transparent. A semisolid product was produced when extract was evaporated in a rotary evaporator under reduced pressure and was stored in a refrigerator in airtight container below 10°C.^{16,17}

Anti-inflammatory activity

Protein denaturation inhibition assay: A test solution was made by adding 0.5 ml of Bovine albumin serum (5 % w/v aqueous solution) to 0.05 ml of extracts at various concentrations varied from 100 to 1000 µg/ml. Test control was made by using 0.05 ml of distilled water instead of extract and product control contains only different concentrations of extract and distilled water. 1N hydrochloric acid was accustomed change the pH of all the solutions to 6.4 and all the reaction mixture was incubated at 37°C for 20 min and heated at 70°C for 5 min. Once cooling the samples, the turbidity was measured using spectrophotometer at 660 nm. Diclofenac sodium was used as a standard.¹⁸ The percentage inhibition of protein denaturation was calculated using the following formula.¹⁹

$$\text{Inhibition (\%)} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100$$

In vitro Anti-proliferative Activity

Preparation of MCF - 7 Cell culture: The MCF - 7 cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM). The Medium was supplemented with 10% heat inactivated fetal bovine serum (FBS). The cell lines were subculture in cell culture flask and then incubated for 2 days at 37°C in a humidified CO₂ incubator with 5% CO₂. The cells were observed every 24 hr under an inverted tissue culture microscope.²⁰

In vitro cell cytotoxicity activity (MTT assay): MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) is reduced by mitochondrial succinate dehydrogenase to purple coloured formazan product, which reflects the normal functioning of mitochondria and cell viability. 15000 cells per well were seeded in 96-well plates at varying cell number in 100 µl of growth medium and incubated for 24 hr in a CO₂ incubator. 100 µl of the medium containing the plant extract (initially dissolved in DMSO) was added and re-incubated for 48 hr in a CO₂ incubator. MTT solution was prepared as a solution of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide 5 mg/ml, pH 7.2 in phosphate buffered saline and 200 µl of this solution was added to each well.²¹ After 2 hr contact with the MTT solution, blue crystals were formed. The suspension was transferred to a spectrophotometer cuvette and absorbance was measured at a wavelength of 570 nm.²²

Statistical analysis

The data was stated as mean ± SD. Student's t-test was adopted to assess the significance of differences between results obtained from different samples and between results from samples and controls using the Graph

Pad Prism 7 software. A probability value of less than 0.05 will be considered as significant.

RESULTS

In vitro Anti-Inflammatory Activity

Alcoholic root extract effectively inhibits the protein denaturation. Table 1, showed the significant inhibition of 66.20±1.19% and 85.03±0.73% for the root extract and diclofenac sodium respectively at 1000 µg/ml concentration.

Cytotoxicity Assay

The alcoholic root extract exhibited a time and dose dependent activity and possesses moderate anti-proliferative effect against the cancerous cell lines (Table 2).

DISCUSSION

Plants are becoming possible source for phytoconstituents with diverse pharmacological activities. Documentation of such plants of probable use in medicine is of connotation. The scientific studies to work out the actual efficacy and other limitations of the drugs would definitely extend their scope for further use, if they come out to be really operative. The presently available drugs provide only symptomatic relief with many side effects. So, the goal was to discover newer drugs from herbal resources, with lesser side effects.²⁶

In-vitro anti-inflammatory potential was examined by adopting protein denaturation method, in which diclofenac sodium was used as a standard. Alcoholic root extract effectively inhibits the protein denaturation. Pharmacological investigations clearly indicated that anti-inflammatory activity in many plants has been attributed to their flavonoid and phenol contents.²³ Several flavonoids isolated from the medicinal plants have been discovered to possess significant anti-inflammatory activity.²⁴ From the results obtained in the current study, it may be concluded that alcoholic root extract possesses anti-inflammatory potential, which is comparable to the synthetic anti-inflammatory agent diclofenac sodium.

Table 1: Inhibition of Protein Denaturation.

S.No.	Concentration (µg/ml)	% Inhibition of standard (mean ± standard deviation)	% Inhibition of sample (mean ± standard deviation)
1.	100	74.49±0.84	41.89±1.42
2.	200	77.92±1.42	45.73±0.46
3.	500	79.58±1.24	55.36±0.95
4.	1000	85.03±0.73	66.20±1.19

Values are expressed as mean ± SD. Values are significant at $p < 0.05$ ($n=6$)

Table 2: Effect of alcoholic extract of *Euphorbia hirta* roots on MCF-7 cell viability.

Concentration (µg/ml)	% cell viability (mean ± standard deviation)
0.01	88.61±.18
0.1	87.29±.20
1.0	81.43±.10
10	61.57±.16
100	48.08±.30

Values are expressed as mean ± SD. Values are significant at $p < 0.05$ ($n=6$)

Anti-proliferative potential (*in-vitro*) of alcoholic root extract of *Euphorbia hirta* was evaluated by using MTT assay on MCF-7 cell lines. The alcoholic root extract exhibited a time and dose dependent response. As for *euphorbia hirta*, anti-proliferative action of roots via MCF-7 cell lines was reported for first time. Plant extracts anticancer effect is mainly found to be due to the presence of different phytochemical constituents present in the extract such as flavonoids.¹⁵ As the initial phytochemical investigation of the extract suggested the presence of chemical constituents like flavonoids in the extract and the effect of flavonoids in reducing cancer risk has been already reported in various studies.^{2,15,25} Results obtained from the present study, concluded that alcoholic root extract possesses prominent anti-inflammatory and moderate anti-proliferative potential.

CONCLUSION

From the results obtained, alcoholic root extract could be concluded to possess anti-inflammatory potential. Conjointly anti-proliferative potential (*in-vitro*) was also assessed by means of MTT assay on MCF-7 cell lines.²⁶ The alcoholic root extract exhibited a time and dose dependent response. Biochemical mediators involved in these biological processes need to be investigated to assess the specific mechanisms of the extract tested and also to identify the possible lead molecules involved during the biological process.

Thus, in future, further studies are needed to isolate and identify the active constituents responsible for such activities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **FBS:** Fetal Bovine Serum; **MTT:** 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, **MCF-7:** Human breast cancer, **SD:** Standard deviation.

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