Research Article

Evaluation of the *in vitro* Antioxidant and Cytotoxic Effects of *E. hirta* Ethanolic Extract

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Submission Date: 22-02-2024; Revision Date: 24-03-2024; Accepted Date: 16-04-2024.

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ABSTRACT

Background: Numerous studies have been carried out to investigate the efficiency of herbal remedies for hepatic illnesses, particularly hepatitis. **Aim:** This study aimed to evaluate the antioxidant activity (DPPH assay) and cytotoxicity against HepG2 human liver cancer cell lines of the ethanolic extract of *Euphorbia hirta* L., obtained from Salem, Tamil Nadu, India. **Materials and Methods:** The complete *Euphorbia hirta* L. plant was air-dried and crushed. The resulting powder (500 g) was continuously extracted with ethanol in a Soxhlet apparatus. A phytochemical study verified the existence of many biologically active components, including tannins, flavonoids, phenols, and alkaloids, in the extracts of *Euphorbia hirta* L. **Results:** The ethanolic extract of Euphorbia hirta L. was assessed for its antioxidant activity, demonstrating a noteworthy antioxidant capacity (69.55% at 500 µg), which is comparable to the antioxidant activity of ascorbic acid (63.23% at 200 µg/mL) in the DPPH assay. In addition, we examined the ability of the extract to inhibit cancer growth in HepG2 cells using an MTT assay. The IC_{50} value, which represents the concentration of *Euphorbia hirta* L. ethanolic extract necessary to destroy 50% of cells, was determined to be 200 µg over a 24 hr period. The results indicate that the ethanolic extract derived from the entire *Euphorbia hirta* L. plant contains active phytoconstituents, which exhibit strong antioxidant activity and notable cytotoxicity against HepG2 human liver cancer cell lines. **Conclusion:** This study suggests that the whole plant extract of *Euphorbia hirta* L. has the potential to be used in the treatment of liver illnesses, specifically hepatitis.

Keywords: *Euphorbia hirta* L., Ethanolic extract, DPPH antioxidant assay, Cytotoxicity, Liver cancer cell lines.

INTRODUCTION

The liver plays a pivotal role in various crucial physiological functions, including retention and absorption of digested substances.[1] Liver damage can result from inflammation and oxidative stress, which are often associated with conditions such as hepatitis virus infection and non-alcoholic fatty liver disease.[2] Conventional treatments for liver issues, such as these, may have limited efficacy and potentially severe side effects. In contrast, traditional remedies, with a history spanning centuries and employed by diverse cultures worldwide, offer alternative approaches to address liver problems, with comparatively fewer harmful effects.[3] Herbal remedies, the cornerstone of traditional medicine, have been utilised for an extensive period to manage a spectrum of ailments, encompassing conditions such as ulcers, cancer, and liver disease.[4] The exploration of phytochemistry, focusing on the chemical compounds present in plants, has provided insights into the bioactive organic compounds known as phytochemicals.

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DOI: 10.5530/ajbls.2023.13.18

Phytotherapy uses these compounds from medicinal plants to address and improve various health conditions.[5] The genus Euphorbia, which is characterised by significant morphological diversity within the plant family, has a rich traditional history of medicinal use. Euphorbia has been used for the treatment of diverse health issues, including lung infections, skin irritation, digestive disorders, inflammatory diseases, antimalarial properties, microbiological illnesses, snake or scorpion bites, pregnancy-related conditions, and sensory impairments.[6] The therapeutic success of Euphorbia has been attributed to its polycyclic and macrocyclic diterpenes, which possess pharmacological properties. In the context of the present study, we focused on free radical scavenging activity and cytotoxicity against liver cancer (HepG2) using an ethanolic extract from the whole plant of *Euphorbia hirta* L. This study aimed to explore the potential therapeutic benefits of *Euphorbia hirta* L. in combating oxidative stress and to address its cytotoxic effects on liver cancer cells.

MATERIALS AND METHODS

Materials

Ethanol, sterile water, DPPH, DMEM cell culture media, and Phosphate Buffer (PBS) were purchased from Sigma Aldrich, India. HepG2 cells purchased from the NCCS cell repository were obtained from Pune, India. *Euphorbia hirta* L. plant Salem Tamil Nadu, India.

Plant collection, extraction

The *Euphorbia hirta* L. plant (Figure 1) was verified by botanist Dr. A. Balasubramanian of the ABS Botanical Conservation Research and Training Centre in Salem, Tamil Nadu. This species was collected from Salem (Tamil Nadu, India). The entire plant was air-dried for 14 days at room temperature. In a Soxhlet system, 1000 g of powdered, air-dried entire plants were used to continuously extract 1000 mL of ethanol, and the crude extract was collected in a sterile vial and stored for further analysis.

Figure 1: *Euphorbia hirta* L. plant.

Qualitative analysis of phytochemicals extracted from the whole plant of *Euphorbia hirta* L. was performed using ethanol

The presence of the preliminary phytochemical was tested using established procedures for medicinal plant ethanolic extract solutions.[7,8] Flavonoids: A 2 mL portion of the extract was treated with a 2 mL solution of lead acetate, which had a concentration of 10%. The presence of flavonoids is indicated by yellowish-green colouration. Alkaloids: A total of 2 mL of the extract and 2 mL of Wagner's reagent were combined. The brownish precipitate indicated the presence of an alkaloid. Phenols: A 10 mg sample of plant extract was mixed with 0.5 mL of a 10% solution of lead acetate and inspected for the presence of a white residue. Tannins: 2 mL of each plant extract were mixed with a small quantity of 0.1% $FeCl_3$ solution in test tubes. The test tubes were monitored for the appearance of a brownish-green hue. The methodology was used to conduct a phytochemical investigation of the ethanolic extract of *Euphorbia hirta* L.

In vitro antioxidant activity of *Euphorbia hirta* L. ethanolic extract

The free reactive oxygen species of the ethanolic extract of the whole plant was analysed using the DPPH technique. The stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. Methanol was used to separate the DPPH stock solution, yielding a beneficial mixture with an absorbance of approximately 0.973 at 517 nm. A test tube was used to combine 100 µL of ethanolic extract from *Euphorbia hirta* and 3 mL of DPPH working solution. A standard solution is often prepared by dissolving 3 mL of DPPH solution in 100 mL of methanol. The tubes were left in absolute darkness for 30 min. Hence, the absorbance was measured at a wavelength of 517 nm, as indicated in the references. The percentage of antioxidant activity was calculated using the following formula: [(A control-A test)÷A control]×100.

Euphorbia hirta L. ethanolic extract cytotoxicity

HepG2 cells were obtained from the NCCS Cell Repository in Pune, India. Upon procurement, the cells were maintained in liquid nitrogen and subsequently subcultured in DMEM supplemented with 10% FBS. Streptomycin and penicillin 100 µg/mL were used to sterilise the media and cell lines were incubated at 37°C with 5% CO_2. Cell proliferation was assessed after 24 hr using an inverted microscope. An MTT experiment was conducted on HepG2 cells, which are human liver cancer cells, using the ethanolic extract of *Euphorbia hirta* L. These values[8,9] underwent minimal alteration,

with a sample size of 2. The cells were enumerated and dispersed in a 96 well plate (at a concentration of 1104 cells/mL) using a haemocytometer and then incubated for 24 hr. HepG2 cells were cultured for 24 hr and treated with an ethanolic extract of *Euphorbia hirta* L. at doses ranging from 500 µg/mL to 3.90625 µg/mL. Subsequently, the cells were cultured for 24 hr at 37ºC in an environment consisting of 95% air and 5% CO_2. Following incubation, the cells containing the ethanolic extract of *Euphorbia hirta* L. were washed with new culture media and then treated with MTT dye (5 mg/mL in PBS). The cells treated with the MTT dye were incubated for an additional 4 hr at 37ºC to observe their responses. Cell viability was assessed by measuring absorbance at a wavelength of 540 nm using a 96 well plate reader. The ratio of stable cells to that of the control group was evaluated. The ideal dose and IC_{50} values were then calculated. The proliferation inhibition percentage was determined by multiplying the discrepancy between the optical density of the control and the optical density of the test by 100. The ethanol extract of *Euphorbia hirta* L. demonstrated a dose-response curve, showing a 50% reduction in cytotoxicity compared to the control cells.

Apoptosis in *Euphorbia hirta* ethanolic extracts was measured using AO/EB staining

To investigate the apoptosis induced by *Euphorbia hirta* L. ethanolic extract, HepG2 cells were subjected to AO/ED staining. A solution of 100 µL acridine orange and 100 µL ethidium bromide was prepared by dissolving them in PBS, resulting in a 200 µL dye. The solution was incubated at room temperature for 5 min. The stained cells using a 40x fluorescent microscope.

RESULTS

Phytochemical screening was conducted using an ethanolic extract of *Euphorbia hirta* L.

The ethanolic extract of *Euphorbia hirta* L. was subjected to an initial analysis to determine its chemical composition. The analysis revealed the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, tannins, and proteins. The phytochemical composition of *the Euphorbia hirta* L. ethanolic extract has been investigated to shed light on its chemical constituents, providing valuable insights into its potential medicinal properties. Phytochemicals are naturally occurring compounds that have various therapeutic effects in plants. The following analysis categorises the phytoconstituents present in the ethanolic extract of *Euphorbia hirta* L. Carbohydrates, as indicated by the presence of double plus signs (++), as abundant in the ethanolic extract of *Euphorbia hirta* L. Carbohydrates serve as essential energy sources and play a crucial role in various metabolic processes within the body. Alkaloids were present in the extract and are denoted by a single plus sign (+). Alkaloids often possess pharmacological activity and have been implicated in various medicinal applications. They exhibit diverse biological effects, including analgesic and anti-inflammatory effects. The absence of sterols in the ethanolic extract suggested a lack of steroid compounds. Sterols are often associated with membrane stability and their potential benefits in cholesterol regulation have been investigated. The ethanolic extract was rich in phenolic compounds, highlighted by triple plus signs (+++). Phenolic compounds are known for their antioxidant properties, which play a crucial role in neutralising free radicals and mitigating oxidative stress. The abundance of phenolic compounds in *Euphorbia hirta* L. indicates its potential as an antioxidant agent. The absence of glycosides suggests that this particular class of compounds may not be prevalent in ethanolic extracts. Glycosides have various biological activities, and are often implicated in the medicinal properties of plant extracts. The presence of proteins and amino acids, denoted by a plus sign (+), indicates the existence of essential building blocks for cellular structures and functions. Proteins and amino acids are vital to numerous physiological processes in the body. The absence of fixed oils indicates that lipid-based components may not be prominent in the ethanolic extract. Fixed oils often contain triglycerides and fatty acids. Gums and mucilage are present, marked by a plus sign (+). These compounds are known for their soothing and demulcent properties, which make them beneficial for the management of respiratory and digestive issues. Flavonoids, with triple plus signs (+++), were abundant in the ethanolic extract. Flavonoids are versatile compounds with anti-inflammatory, antioxidant, and anti-cancer properties.[10] Their presence further underscores the potential therapeutic benefits of *Euphorbia hirta* L. Tannins, indicated by double plus signs (++), are present in the extract. Tannins have astringent properties and are often associated with wound healing and anti-diarrhoeal effects. The absence of saponins suggests that soap-like compounds are not a major component of the ethanolic extract. Saponins are known to have foaming and emulsifying properties. In conclusion, phytochemical analysis revealed a diverse array of bioactive compounds in the ethanolic extract of *Euphorbia hirta*. This botanical profile suggests potential therapeutic applications, including antioxidant and anti-inflammatory effects, supporting the traditional use

of *Euphorbia hirta* in folk medicine. Further research and clinical studies are warranted to validate and explore the full range of medicinal benefits of these phytoconstituents.

Antioxidant activity of ethanolic extract of *Euphorbia hirta* L.

Euphorbia hirta L. ethanolic extract was used for the free-radical DPPH assay (Figure 2). ROS (free radicals and reactive oxygen species) are neutralised by antioxidants, which are chemical groupings of substances. The availability of antioxidants strongly influences the development of various chronic diseases including liver disorders, aging, heart disease, anaemia, malignancy, and inflammation. Antioxidant defence against damage produced by free radicals. Sharma, Basma and his colleagues[11,12] studied antioxidant activity of the methanolic extract of *Euphorbia hirta* and showed good antioxidant activity at different concentrations.

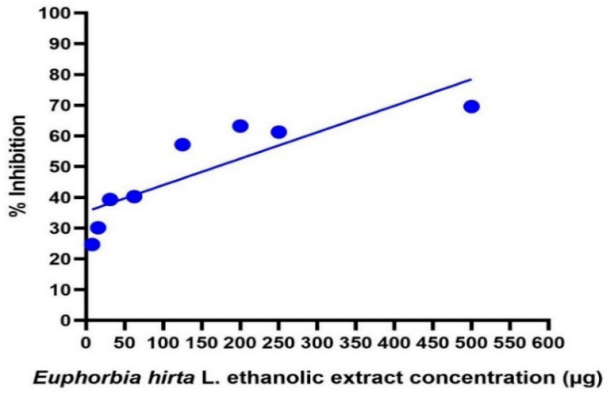

Figure 2: The presence of antioxidant activities in an ethanolic extract of *Euphorbia.*

The antioxidant activity of ascorbic acid was 63.23% (200 µg/mL), while the ethanolic extract of *Euphorbia hirta* L. also exhibited antioxidant properties. Exhibited an antioxidant activity was 69.55% (500 µg/mL) (Figure 2).

The MTT assay measures the cytotoxicity of the ethanolic extract

The cytotoxic activity of the ethanolic extract of *Euphorbia hirta* L. was assessed in HepG2 human liver cancer cell lines. By plotting cell viability under treated and untreated conditions, the inhibitory concentration required for 50% cell destruction (HepG2 IC_{50}) was determined using the MTT assay at 24 hr, revealing it to be 200 µg/mL of *Euphorbia hirta* L. ethanolic extract (Figure 3)..

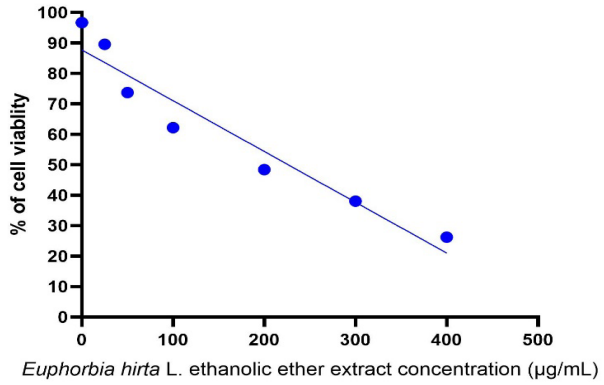

Figure 3: The ethanolic extract of *Euphorbia hirta* L. demonstrated cytotoxic effects on HepG2 cells.

Apoptosis-AO/EB staining

The apoptotic characteristics of HepG2 cells generated by the alcoholic solution derived from *Euphorbia hirta* L. were investigated using AO/EB staining (Figure 4). The AO/EB staining fluorescence pattern predicted the membrane integrity and cell survival. Dead cells are frequently permeable to ethidium bromide, which results in orange-red fluorescence, while living cells allow the passage of AO, which leads to the emission of green fluorescence.

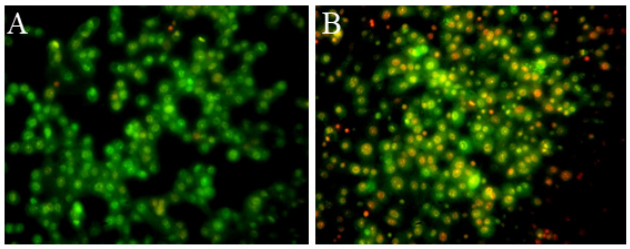

Figure 4 A: HepG2 cells in the control group were not treated with any substance. In contrast, Figure 4 B: HepG2 cells in the experimental group were treated with the ethanolic extract of Euphorbia hirta L. for 24 hr.

DISCUSSION

Phytochemical screening of the ethanolic extract of *Euphorbia hirta* L. revealed the presence of various bioactive compounds including alkaloids, carbohydrates, phenolic compounds, flavonoids, tannins, proteins, and gums/mucilage. Notably, carbohydrates were abundant (++), while phenolic compounds (+++) and flavonoids (+++) were rich in the extract, indicating potential antioxidant properties. The absence of sterols, glycosides, fixed oils, and saponins suggests their minimal presence. The extract also contained proteins and amino acids, along with gums/mucilage. Antioxidant activity testing using the DPPH assay showed the extract exhibited significant antioxidant

properties, with 69.55% activity at 500 µg/mL compared to 63.23% for ascorbic acid (200 µg/mL). Additionally, the cytotoxicity of the extract was evaluated using the MTT assay on HepG2 liver cancer cells, revealing an IC_{50} value of 200 µg/mL of extract after 24 hours of treatment, indicating cytotoxic effects. Furthermore, apoptotic characteristics were observed in HepG2 cells treated with the ethanolic extract through AO/EB staining, demonstrating changes in membrane integrity and cell survival compared to the control group.

The ethanolic extract, phytochemical analysis, antioxidant activity, and cytotoxicity studies on Euphorbia species are presented in this study. Euphorbia species have been used for the treatment of several ailments and disorders, from infectious to chronic illnesses, such as cancer. Phytochemical analysis of the *E. hirta L.* ethanolic extract revealed the presence of several phytoconstituents. The Euphorbia species having different structural variety of chemical which is responsible for the various biological activity analgesic,[13] antibacterial,[14] antidiarrhoeic,[15] gastric problems,[16] in diarrhea and constipation[17] and immunostimulant response[18] with different mechanism like the processes of cell multiplication and specialisation, programmed cell death, inhibition of excessive production of reactive oxygen species during the spread of cancer, and impacts on the formation of new blood vessels. The ethanolic extract of *E. hirta* L. was cytotoxic to liver cancer cells. The *Euphorbia hirta* L. ethanolic extract showed good cytotoxicity against cancer cell lines, with an IC_{50} value (200 µg/mL). Euphorbia hirta L. ethanolic extract IC_{50} value (200 µg/mL) can be used to protect against liver cirrhosis and liver metabolic disorders.

Studies have highlighted the presence of various bioactive compounds in Euphorbia species, including alkaloids, flavonoids, and phenolic compounds, which align with our findings. Investigations into the antioxidant and cytotoxic properties of Euphorbia extracts support our results, corroborating the potential therapeutic applications of *Euphorbia hirta* L. in oxidative stress-related disorders and cancer treatment. Further comparative analyses with different extraction methods and cell lines could provide a more comprehensive understanding of its bioactivity and therapeutic potential.[19,20]

The findings hold significant importance for both practice and research in the fields of natural medicine and cancer therapeutics. The demonstrated antioxidant and cytotoxic activities of the ethanolic extract of *Euphorbia hirta* L. suggest its potential as a natural remedy for oxidative stress-related disorders and as a promising candidate for cancer treatment. These findings could inform the development of novel therapeutic agents and guide the integration of traditional herbal medicine into mainstream healthcare practices. Additionally, the comprehensive phytochemical analysis enhances our understanding of *Euphorbia hirta* L. medicinal properties. Further research is needed to validate these findings across different populations and disease models for broader generalizability.

CONCLUSION

In the current study, the antioxidant activity and cytotoxicity HepG2 effects. Active phytoconstituents were present in the ethanolic extracts of all E. *hirta* L. plants. The phytoconstituents present in the extract exhibited antioxidant and anti-cancer properties, specifically in the HepG2 cell line. This study suggests that the entire *Euphorbia hirta* L. plant can be used to cure liver failure; however, more research is needed to fully comprehend the mechanism of anticancer activity. The biological activity of the *Euphorbia hirta* L. ethanolic extract is due to the presence of various phytoconstituents, such as phenols, alkaloids, and flavonoids.

ACKNOWLEDGEMENT

The authors express their gratitude to the Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be university), Salem for providing the necessary research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **DMEM:** Dulbecco's Modified Eagle's medium; **PBS:** Phosphate buffer; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; **AO/EB:** Acridine orange/ethidium bromide; **FBS:** Fetal Bovine Serum.

SUMMARY

This study evaluated the antioxidant activity and cytotoxicity of the ethanolic extract of *Euphorbia hirta* L., a plant found in Salem, Tamil Nadu, India. The extract showed strong antioxidant activity (69.55% at 500 µg/mL) and notable cytotoxicity against HepG2 human liver cancer cell lines. The IC_{50} value was 200 µg over a 24 hr period. The study suggests that the whole plant extract of *Euphorbia hirta* L. has potential for treating liver illnesses, particularly hepatitis.

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Cite this article: Palanisamy S, Srinivas K, Rao MVB. Evaluation of the *in vitro* Antioxidant and Cytotoxic Effects of *E. hirta* Ethanolic Extract. Asian J Biol Life Sci. 2024;13(1):138-43.