ISSN 2063-5346 *Section A-Research paper*

In-Vitro Antioxidant and Cytotoxicity Activity of *Euphorbia hirta*Petroleum Ether, Chloroform Extracts

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ABSTRACT

Introduction: One of the most prevalent disorders that affect people is hepatic disease. Numerous studies have been done to determine whether herbal treatments for hepatitis illnesses are beneficial.**Aim:** Antioxidant activity (DPPH) and cytotoxicactivity of *Euphorbia hirta*L. petroleum ether, chloroform extract against human liver cancer cell lines.**Methodology:** *Euphorbia hirta*L. was collected from Salem, Tamil Nadu, India. The air-dried whole plant was crushed, and 500 g of the powder, petroleum ether andchloroform were used for continuous extraction by the Soxhlet device.Using the DPPH assay, *Euphorbia hirta* L.'s antioxidant capacity was investigated. The anticancer efficacy of *Euphorbia hirta*L.petroleum ether and chloroform extract was investigated in human liver cancer cell lines (HepG2).**Results:** Phytochemical examination confirmed that *Euphorbia hirta* extracts have different biologically active phytoconstituents like flavonoids, phenols,sterols, and alkaloids.*Euphorbia hirta*petroleum ether and chloroform extract have significantly shown antioxidant activity and cytotoxicity compared to the negative control. Antioxidant activity of *Euphorbia hirta*L. petroleum ether and chloroform extract showed an antioxidant activity percentage of 68.75% (500 µg), 71.72 (500 µg) and that of ascorbic acid showed an antioxidant activity percentage of 65.5% (200 µg) 70.15% (200 µg) with DPPH.MTT assay was used to test the antitumor activity of *Euphorbia hirta* L. petroleum ether and chloroform extract against human liver cancer (HepG2) cells. The IC_{50} value was calculated by plotting cell viability versus extract concentration. The MTT assay at 24 hr determined the inhibitory

concentration of the complex at 50% cell destruction, HepG2 IC_{50}, as 200 µg and 150 µg of *Euphorbia hirta* L. petroleum ether and chloroform extract, respectively.**Conclusion:** Petroleum ether and chloroform extract from the whole plant *Euphorbia hirta* L. contain active phytoconstituents. There is strong antioxidant activity in the extracts and good cytotoxicity activity against HepG2 human liver cancer cell lines because of the presence of phytoconstituents. The study suggested treating liver disease by using *Euphorbia hirta* L. whole plant extract.

KEYWORDS: *Euphorbia hirta*L., Petroleum ether extract, Chloroform extract, *In-Vitro*antioxidant, Cytotoxicity.

INTRODUCTION

Phytochemistry is the study of plant chemicals. By utilizing the curative properties of therapeutic plants, phytotherapy serves as a means of treating and improving several ailments. Medicinal plants contain bioactive, organic molecules known as phytochemicals. [1, 2].

Traditional herbal remedies are used to cure a variety of acute and chronic illnesses with little to no hazardous side effects. They have played a significant part in health systems around the world. Numerous health issues, including hypertension, cancer, diabetes mellitus, wound healing, asthma, pharyngitis, and tuberculosis, can be treated naturally with herbal plants. Plants with high amounts of alkaloids, flavonoids, tannins, and polyphenols have been used to treat ailments [3].

The liver is the human body's most crucial organ when it comes to metabolic processes. It has a significant ability to synthesize helpful principles and detoxicate harmful substances. Therefore, hepatotoxic substances that cause liver damage have serious implications [4].

Euphorbia hirta L. belongs to the genus Euphorbia and the family Euphorbiaceae. Reddish, hairy, 40 cm tall annual plant. Opposing leaves are elliptic-oblong to oblong-lanceolate, subacute, and 1-2.5 cm long. 1-2 mm yellow, three-celled, hairy capsules [5].It is widely used as an essential medicinal plant for treating diseases like non-communicable and communicable [6]. The present research conducted antioxidant activity and *In-Vitro* hepatocellular cytotoxicity of petroleum and chloroform extract of *Euphorbia hirta*L.

MATERIAL AND METHODS

Plant collection, extraction

Botanist Dr A. Balasubramanian of the ABS Botanical ConservationResearch and Training Centre inSalem, Tamil Nadu, authenticated the *Euphorbia hirta*L plant. This species was collected in Salem, Tamil Nadu, India. The whole plant wasair-dried at room temperature for 10 to 14 days. The air-dried whole plants were crushed, and 500 g of the powder was then used to continuously extract 1000 ml of petroleum ether and chloroform in a Soxhlet apparatus.

Preliminary phytochemical examination of the whole plant of *Euphorbia hirta*L. extracted using petroleum ether and chloroform.

Following the normal protocol, petroleum ether and chloroformextract of *Euphorbia hirta* L were employed for phytochemical screening investigations.

***In-Vitro*antioxidant activity (DPPH) of *Euphorbia hirta*L. petroleum ether and chloroform extract.**

Using the 1,1-diphenyl-2-picryl hydrazyl (DPPH) method, the free reactive oxygen species of the petroleum ether and chloroform extractof the whole plant were examined. The stock solution was created by dissolving 24 mg of DPPH in 100 mL methanol. Methanol was used to filter the DPPH stock solution, and the result was a useful mixture with an absorbance of roughly 0.973 at 517 nm. 100 µL of *Euphorbia hirta* Lpetroleum ether and chloroform extractand 3 mL of DPPH working solutions were mixed in a test tube. Three mL of DPPH solution in 100 mL of methanol is frequently provided as a standard. The tubes were then left in total darkness for 30 minutes. Therefore, the absorbance was calculated at 517 nm [7, 8].

$$\% \text{ of antioxidant activity} = [(A \text{ control} - A \text{ test}) \div A \text{ control}] \times 100$$

***Euphorbia hirta* L. petroleum ether and chloroform extract cytotoxicity.**

Cell culture maintenance

HepG2 was collected from the NCCS cell repository in Pune, India. 10% FBS was added to DMEM to keep the cell line alive. The media was sterilised with 100 µg/mL penicillin and streptomycin. Cell lines were maintained at 37°C with 5% CO2 for 24 hours.

MTT assay

*Euphorbia hirta*L. petroleum ether and chloroform extract cytotoxicity on human liver cancer(HepG2)cells were carried out as described by Nemati *et al* [11], Uğur*et al*[12] with modification. The viability experiment used HepG2 cells. The cells were counted with a hemocytometer, diluted in DMEM to 1104 cells/mL, and seeded in 96 well plates for 24 h. After treating HepG2 cells, each well received the control and 0 to 500 µg/mL Euphorbia

hirta L. petroleum ether and chloroform extract. HepG2 cells were incubated at 37°C in 95% air/5% CO2 for 24 hours. After incubation, the *Euphorbia hirta* L. petroleum ether and chloroform extract-containing cells were flushed with fresh culture media, MTT (5 mg/mL in PBS) dye was added, and the cells were incubated for another 4 h at 37°C. Multi-well plate reader assessed cell viability at 540 nm. The percentage of stable cells was compared to the control. The half-maximal inhibitory concentration (IC50) and optimum dosages were calculated.

Proliferation inhibition (%) = (Optical density of control-Optical density of test) X 100

*Euphorbia hirta*L. petroleum ether and chloroform extract dose-response curve showed 50% less cytotoxicity than control cells. Double-checked all experiments[13].

AO/EB staining was employed to measure Euphorbia hirta L. petroleum ether and chloroform extract apoptosis.

Fluorescence microscopy of apoptosis. 100µL acridine orange and 100µL ethidium bromide were dissolved in PBS to make a 200µL dye mixture. HepG2 cells were plated at 5 x 104 per well and incubated for 24 hrs. After being treated with *Euphorbia hirta* L. petroleum ether and chloroform extract for 24 hr, cells were detached, washed with cold PBS, and stained with AO/EB at RT for 5 min. Examining stained cells under a 40x fluorescent microscope. Number of apoptosis-like cells was estimated as a function of total field cells.

RESULTS

Phytochemical examination of *Euphorbia hirta*L. whole plant was extracted using petroleum ether and chloroform.

*Euphorbia hirta*L extracts in petroleum ether and chloroform were subjected to a preliminary phytochemical examination, which identified the presence of alkaloids, phenolic compounds, flavonoids, and proteins. The petroleum ether extracts contained sterols and alkaloids.

Table 1: Phytochemicals in *Euphorbia hirta*L. whole plant petroleum ether and chloroform extract

S. No.	Test	Petroleum ether extract *Euphorbia hirta*L	Chloroform extract *Euphorbia hirta*L
1.	Carbohydrate	-	-
2.	Alkaloids	-	+
3.	Terpenoids	+	+
4.	Flavanoids	-	+
5.	Saponins	-	-

6.	Cardiac glycoside	-	-
8.	Anthraquinone glycoside	-	-
8.	Steroids	+	+

+ (present), - (absent)

Antioxidant activity of *Euphorbia hirta*L. whole plant petroleum ether and chloroform.

At 517 nm, free-radical DPPH interacts with an odd electron (purple color).The reaction between DPPH and a free-radical scavenger antioxidant produces DPPH, which has a lower absorbance than DPPH due to the lower hydrogen content. This radical form exhibits decolourization compared to the DPPH state as the number of electrons gathered rises.

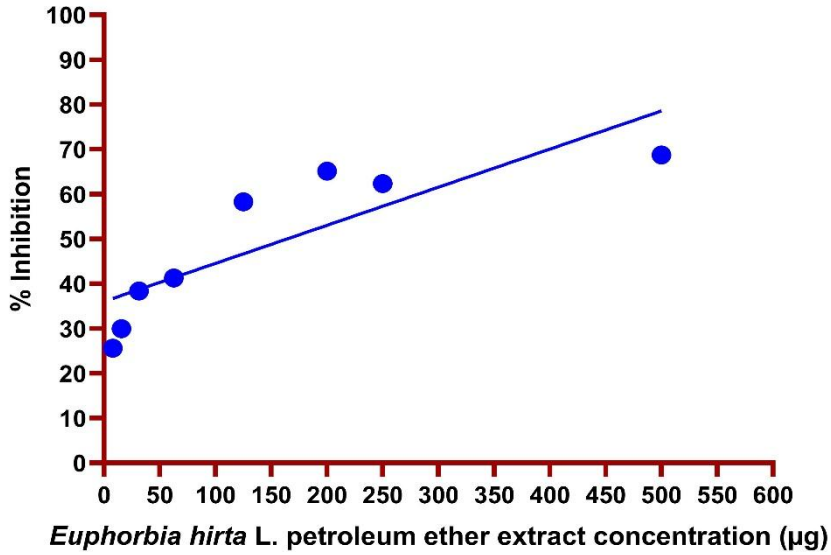

Figure 1: Antioxidant activity of *Euphorbia hirta*L. petroleum ether extract by DPPH assay

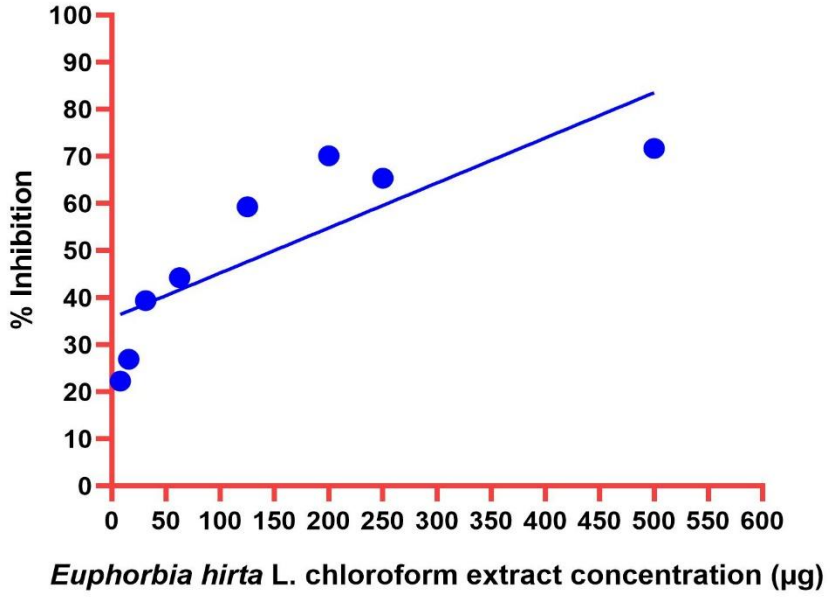

Figure 2: Antioxidant activity of *Euphorbia hirta*L. chloroform extractby DPPH assay

Fig 1and 2 shows that the antioxidant activity of *Euphorbia hirta*L. petroleum ether and chloroform extract showed an antioxidant activity percentage of 68.75% (500 µg), 71.72 (500 µg) and that of ascorbic acid showed an antioxidant activity percentage of 65.5% (200 µg)70.15% (200 µg) with DPPH.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The cytotoxicity activity of the *Euphorbia hirta*L. petroleum ether and chloroform extractwastested inhuman liver cancer cell lines (HepG2). The IC_{50} was found by making a graph that compared the cell viability to the concentration of the complex. The inhibitory concentration of the complex at 50% cell destruction, HepG2$IC_{50,}$ was determined from the MTT assay at 24 hr as 200 µg and 150 µg of *Euphorbia hirta*L. petroleum ether and chloroform extract (Fig 3 and 4).

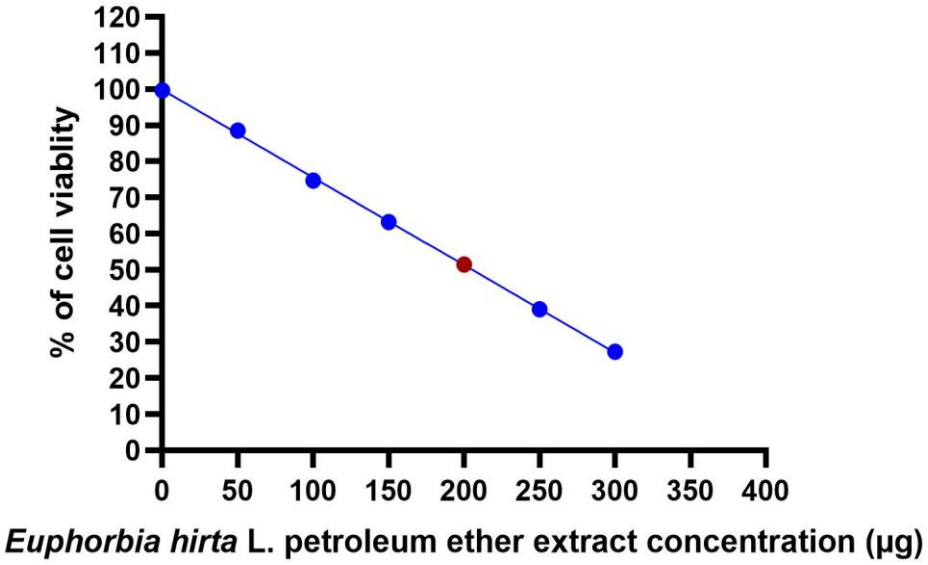

Fig 3: *Euphorbia hirta* L. petroleum ether extract kills HepG2 cells.

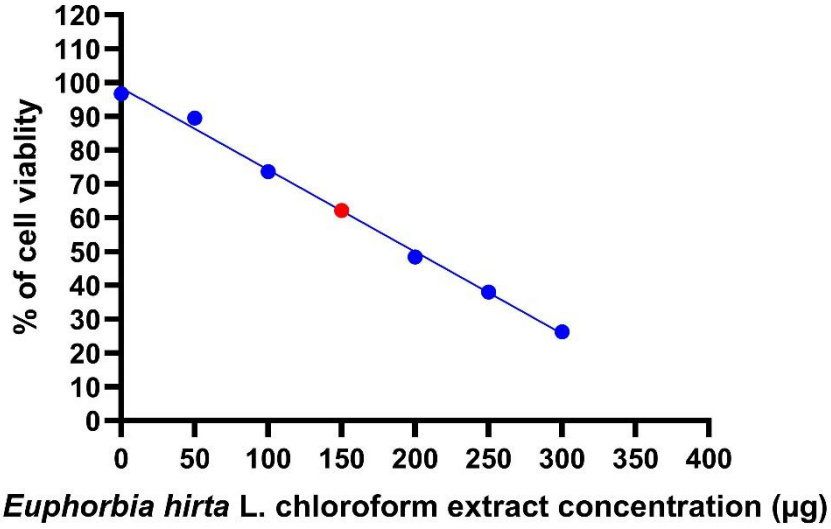

Fig 4: Chloroform extractfrom *Euphorbia hirta*L. has cytotoxicity against HepG2 cell lines

Fluorescent staining for apoptosis

To study HepG2 cell structural changes, an examination of fluorescence staining was done using acridine orange/ethidium bromide (AO/EB) staining (Figure 6). AO/EB staining was used to study the apoptotic features of HepG2cells induced by *Euphorbia hirta*L. petroleum ether and chloroform extract. Cell viability and membrane integrity are predicted by the AO/EB staining fluorescence pattern.Live cells are permeable to AO, which causes a green fluorescence, whereas dead cells are frequently permeable to EB, resulting in an orange-red fluorescence.It is well established that experimentally treated cell lines displayed

four types of cytological and structural alterations in the AO/EB-stained nuclei.Green fluorescence is characteristic of live cells that have nuclei that are highly structured. Orange-green fluorescence is emitted by early apoptotic cells that have undergone nuclear condensation. Fluorescence ranging from orange to red is seen in late apoptotic cells that have heavily condensed or fragmented chromatin. mainly necrotic cells are present.

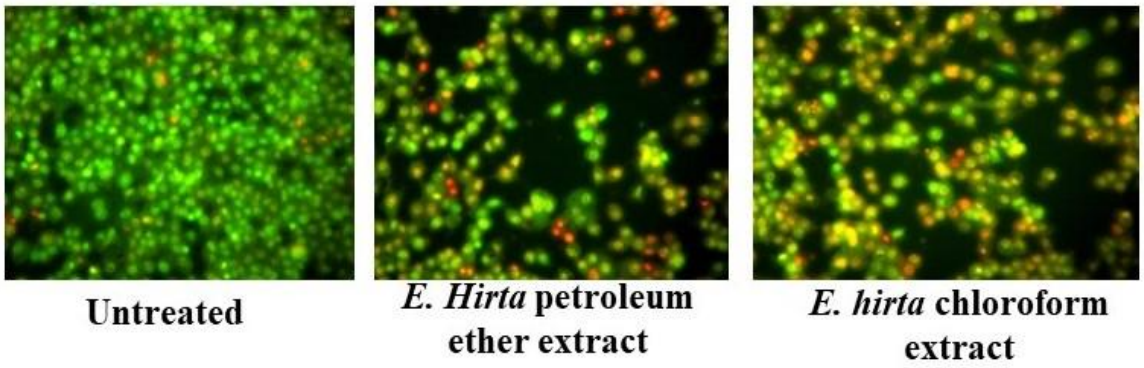

Fig 5:A: HepG2control cells, B: *Euphorbia hirta*L. petroleum ether treated HepG2cells (24 hr), C: *Euphorbia hirta*L. chloroform extract treated HepG2cells (24 hr).

DISCUSSION

Since ancient times, plants and their preparations have been used as medicines. Folk medicine practitioners utilize various herbal mixtures with various philosophies and cultural roots to treat various conditions [14]. The Indian ancient Vedic literature, Ayurveda, is the science of wellness and good health. It is a collection of conventional and cultural medical philosophies. Ayurveda-based modern drug research programmes have widespread support in the current healthcare system. Natural chemicals originating from plants are less hazardous to healthy cells and more tolerated; thus, current medication discovery is interestingto them [15].

Numerous phytochemicals have been identified as the active components in these plant species, which have been found to inhibit the growth and progression of tumours in cancer patients. Numerous pathways exist via which phytochemicals have anticancer effects [16]. They target improperly expressed molecular factors, reduce oxidative stress, control cell growth factors, block angiogenesis of malignant tissue, and cause apoptosis in addition to selectively killing fast-dividing cells [17]. For instance, certain polyphenols, and flavonoids, induce apoptosis, which has anticancer properties [18] through antioxidant mechanisms, curcumin, thymol, rosmarinic acid, alpha-carotene, quercetin, rutin, allicin, gingerol, and coumarin all combat cancer [19].

It has been discovered that *E. hirta* extracts have anticancer properties. *E. hirta* extract significantly decreased the production of prostaglandins I2, E2, and D2. The *E. hirta* extract also prevents the infection of crops such as rice, wheat, and maize.*E. hirta* methanolic extract possesses antifungal and antibacterial properties. Warm leaves that have been mashed with coconut oil and turmeric are applied to itchy soles. Like Surma, the latex of *E. hirta* is applied to the lower eyelids to treat eye ulcers. Nematocidal action is present in the root exudate against young meloidogyne incognita [20].Free radicals have been implicated in several communicable and non-communicable diseases, which have a negative impact on human health. These free radicals are produced by the body's metabolic process. Exogenous antioxidant intake can aid the body's efficient scavenging of free radicals. At the moment, antioxidants are getting a lot of attention, especially those that can defend the body from the purported negative effects of free radicals as well as the oxidation of lipids and other dietary components [21].

*Euphorbia hirta*Lwhole plant petroleum ether extract and chloroform extract have the phytoconstituents confirmed by preliminary phytochemical analysis (Table 1) (Alkaloids, Terpenoids, Flavanoids, steroids etc.). *Euphorbia hirta*Lwhole plant petroleum ether and chloroform extract show good antioxidant activity. *Euphorbia hirta*Lwhole plant petroleum ether and chloroform extract cytotoxicity activity conducted against human liver cancer(HepG2) cells. The plant extract has good cytotoxic activity. Due to active phytoconstituents, the cytotoxicity activity of *Euphorbia hirta*Lwhole plant petroleum ether and chloroform extract. The phytoconstituents will interact with the cell membrane of the cancer cells, which can also interact the DNA damage, mutation and apoptosis (Fig.6).

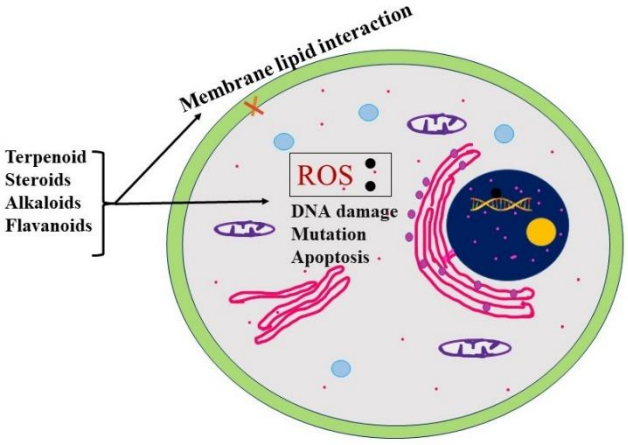

Fig 6: Possible mechanism of anticancer activity of*Euphorbia hirta*L. phytoconstituents

CONCLUSION

The most significant aspect of phytoconstituents is their biological activity. However, it is vital to choose the most promising component by looking at several parts of that plant to gain an entire picture of any plant's phytochemical content and biological activities.The present study conducted antioxidant activity (DPPH) and cytotoxicity (human liver cancer cells -HepG2) study of petroleum ether and chloroform extract of hole *Euphorbia hirta*L. whole plant.Petroleum ether and chloroform extract of *Euphorbia hirta*L. whole plant has active phytoconstituents. Due to phytoconstituents, the extract is having antioxidant and anti-cancer activity in HepG2 cell lines. The above study recommended that *Euphorbia hirta*L. whole plantbe used to treat liver dysfunction, and further study is required to understand the anticancer mechanism of action.

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CONFLICT OF INTEREST: Nil

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