

ANTIPSORIASIS, ANTIOXIDANT, AND ANTIMICROBIAL ACTIVITIES OF AERIAL PARTS OF *EUPHORBIA HIRTA*

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ABSTRACT

Objectives: The aim of the study was curing antipsoriasis through *Euphorbia hirta*. The antipsoriasis activity was done by the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay method.

Methods: Aerial parts were shade, dry for 2 days, make into coarse powder and soaked in methanol for 72 h. The supernatant liquid was filtered by Whatman filter paper and condensed in a hot plate at 50°C. Dark gummy mass obtained. The study identified the antioxidant, antimicrobial, and antipsoriasis activities of methanol extract of aerial parts of *E. hirta*.

Results: The IC₅₀ value of methanol extract of aerial parts was found to be 72.20 µg/mL, 97.88 µg/mL, 55.88 µg/mL, and 36.31 µg/mL by 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay, superoxide radical scavenging assay, phosphomolybdenum reduction assay, and ferric (Fe³⁺) reducing power assay. The antipsoriasis activity was done by the MTT assay method. The maximum cell death was 88.37% observed at 0.781 µg/mL concentration and IC₅₀ was 12.20 µg/mL concentrated.

Conclusion: The results of the present investigation reveal the antipsoriasis activity of the extracts of *E. hirta* against bacteria and viruses, the psoriasis causing organisms. The methanol extract of *E. hirta* shows antioxidant, antimicrobial, and antipsoriasis activity and can be used to formulate a potential therapeutic agent for psoriasis.

Keywords: *Euphorbia hirta*, Antioxidant, Antimicrobial, Anticancer; HaCaT, 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay.

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INTRODUCTION

Euphorbia hirta belongs to the family Euphorbiaceae. Medicinal plants are used to cure various diseases in India since ancient times. The indigenous system of the medicine, namely Ayurvedic, Siddha, and Unani, has been in existence for several centuries. Some drugs from Ayurveda approaches modern diseases have already reached the marketplace [1]. In modern medicine, plants occupy a very important place as the raw material for some important drugs. Synthetic drugs are effective in controlling different diseases, but these synthetic drugs are out of reach of millions of people. It is estimated that around 70,000 plant species had been used for medicinal purposes. The plant herbs provide the starting material for synthesis of conventional drugs. Medicinal plants have curative actions due to the presence of complex chemical composition. India recognizes more than 2500 plant species having medicinal value, Sri Lanka around 1400, and Nepal around 700 [2]. This review intends to provide an overview of chemical composition and pharmacological actions of plant *E. hirta*.

E. hirta is commonly known as asthma plant (or) snakeweed. The common name is Amman pacharisi. It is a medicinal rhizomatous herb distributed in southern the Western Ghats of India and also in the Northern east Coast of Tamil Nadu [3]. *E. hirta* has a long history as a medicinal herb in China. Different formulations are used, including crude drug, decoction, infusion, lotion, and powder. It plays a very important role of traditional Chinese medicine, especially in the folk medicine because of its wide range of biological and pharmacological products [4].

METHODS

Collection of plant material

The aerial parts of *Euphorbia hirta* were received from agricultural land (Periyapalayam), Chennai, Tamil Nadu, India. The plant was

authenticated by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy and Research Centre, Chennai-45.

TAXONOMY

Kingdom: Plantae
 Division: Angiosperms
 Class: Eudicots
 Subclass: Rosids
 Order: Malpighiales
 Family: Euphorbiaceae
 Species: *E. hirta*
 Genus: Euphorbia
 Binomial name: *Euphorbia hirta*

Preparation of the plant extract

The aerial parts were dried in shadow for 2 days, at room temperature. The sample was soaked in 500 mL of methanol at the room temperature. The supernatant was filtered using filter paper and allowed for condensation using a hot plate until the extract reaches a semi-solid state.

Antioxidant assays

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of methanol extract of the aerial parts of *Euphorbia hirta* has evaluated through free radical scavenging effect on DPPH radical. The determination was based on the method proposed by Akowuah *et al.* [5]. 1 mL of 0.1 mM of DPPH in the methanol solution was mixed with 1 mL of (20–120 µg/mL) sample extracts. The mixture has completely added and kept in the dark for 30 min. The control was prepared by adding 1 mL of DPPH and 1 mL methanol. The absorbance was calculated at 517 nm using a spectrophotometer. Percentage of

DPPH radical scavenging activity was calculated as follows:

$$\% \text{ of DPPH radical inhibition} = \left(\frac{\text{Control} - \text{Sample}}{\text{Control}} \right) * 100$$

Superoxide radical scavenging assay

Superoxide radical, known to be very harmful to cellular components as a precursor of the more reactive oxygen species, contributes to tissue damage and many diseases [6]. In a biological system, its toxic role can be eliminated by superoxide dismutase. The radicals may also play an important role during the peroxidation of unsaturated fatty acids and other potential susceptible substances. Superoxide is biologically quite toxic and is deployed by the immune system to kill invading microorganisms. It is an oxygen-centered radical with selective reactivity.

It also produced by a number of enzyme systems in auto-oxidation reactions and by non-enzymatic electron transfers that univalent reduces molecular oxygen. The biological toxicity of superoxide is due to its capacity to inactivate iron-sulfur cluster-containing enzymes, which are critical in a wide variety of metabolic pathways, thereby liberating free iron in the cell, which can undergo Fenton chemistry and generate the highly reactive hydroxyl radical. It can also reduce certain iron complexes such as cytochrome c. Superoxide anions are a precursor to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage. It has been implicated in several pathophysiological processes due to its transformation into more reactive species such as hydroxyl radical that initiates lipid peroxidation. Furthermore, superoxide has been observed to directly initiate lipid peroxidation. In addition, it has been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical. Superoxide anion plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA. Superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of free radicals and oxidizing agents. Superoxide anion derived from dissolved oxygen by riboflavin/methionine/illuminate system and reduces NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT2+) to produce the blue formation which is calculated spectrophotometrically at 560 nm.

The reaction mixture in various concentrations of *E. hirta* 50 mM of phosphate buffer (pH 7.8), 1.5 mM of riboflavin, 12 mM of EDTA, and 50 mM of NBT, added in that sequence. The reaction was started by illuminating the reaction mixture for 150 s. Immediately after illumination, the absorbance was calculated at 590 nm and IC₅₀ was calculated. Ascorbic acid has used as positive control.

% of superoxide radical cation inhibition

$$= \left(\frac{\text{Control} - \text{Sample}}{\text{Control}} \right) * 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of methanol extract of aerial parts of *Euphorbia hirta* has assessed as described by Basma et al. [7]. The extracts in dilution from 10 to 60 µg/mL were added to the reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulfuric acid (600 mM). The reaction mixture was incubated in a water bath at 90°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Increased the absorbance of the reaction mixture indicates the increase in phosphomolybdenum reduction.

% of Phosphomolybdenum radical inhibition

$$= \left(\frac{\text{Sample} - \text{Control}}{\text{Sample} - \text{Control}} \right) * 100$$

Ferric (Fe³⁺) is reducing power assay

According to the method of various concentrations of the methanol extract of the aerial parts of *E. hirta* (10–60 µg/mL) in 1.0 mL of methanol were reacted with phosphate buffer (2.5 mL) and potassium ferricyanide (2.5 mL) [8]. The mixture was incubated at the 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 mL), they were adding to the mixture, which have then centrifuged at the 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled H₂O (2.5 mL) and a freshly produced ferric chloride solution (0.5 mL). The absorbance was calculated at 700 nm. A blank was produced without mixing extract. Ascorbic acid was used as standard. Increased the absorbance of the reaction mixture indicates the increase in reducing power.

$$\% \text{ of Fe}^{3+} \text{ radical inhibition} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} * 100$$

Antimicrobial activities

Petri plates carrying 20 ml Mueller-Hinton agar medium [9] were seeded with a bacterial culture. Wells of approximately 5 mm were bored using a good cutter and a sample of 50, 75, and 100 µg/ml concentrations was mixed [10]. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assessed by measuring the diameter of the inhibition zone formed around the well. Tetracycline was used in the positive control [11].

Thin-layer chromatography (TLC)

TLC was carried out for aerial parts of *E. hirta* in Merck TLC aluminum sheets, silica gel 60 F254 (20 cm×20 cm), and preloaded plates. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. The chromatogram was developed with a mixture of suitable solvent system. The spots were visualized with UV light at 356 nm. The R_f values of the colored spots were measured. The ratio in which distinct bands appeared was optimized and R_f values were calculated [12].

Calculation of R_f value:

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Antipsoriasis activity on HaCaT cell line

3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay method

Cell viability has measured by the conventional MTT reduction assay method was described Mossman with slight modification. Briefly, HaCaT cells were seeded at the density of 5×10³ cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% fetal bovine serum. Then, the culture supernatant was removed, and RPMI containing various concentrations (0.781–100 µg/mL) of test compound was added and incubated for 48 h. After the treatment, cells are incubated with 10 µL of MTT (5 mg/mL) at 37°C for 4 h and then with dimethylsulfoxide (DMSO) at the room temperature for 1 h. The plates were read at 595 nm on a scanning multiwell spectrophotometer [13].

RESULTS AND DISCUSSION

DPPH' radical scavenging assay

In this day study, the antioxidant activity of methanol extract of the aerial parts of *E. hirta* was investigated using the DPPH radical scavenging assay. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH

accepts an electron or hydrogen donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively calculated from the changes in absorbance. The methanol extract of aerial parts of exhibited a significant dose-dependent inhibition of DPPH activity. The percentage of inhibition was found to be 69.59 at 120 µg/mL (Table 1). The IC₅₀ value of the methanol extract of the aerial parts of *E. hirta* was 77.52 µg/mL.

Superoxide radical scavenging assay

Superoxide radical scavenging activity was based on the capacity of the sample to inhibit blue formazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Superoxide is an oxygen-centered radical with selective reactivity. Although it is a relatively weak oxidant, superoxide exhibits limited chemical reactivity can generate more dangerous species, including singlet oxygen and hydroxyl radicals, which cause the peroxidation of lipids. These species are produced by a number of enzyme systems.

Superoxide can also reduce certain iron complexes such as cytochrome C. Superoxide anions are thus precursors to active free radicals that have potential for reacting with biological macromolecules and thereby inducing tissue damage. Furthermore, superoxide has been observed to directly initiate lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly through scavenging of superoxide anion radical. Superoxide radicals are

Table 1: DPPH radical and superoxide radical scavenging activities of methanol extract of the *Euphorbia hirta*

S. No	Concentration (µg/ml)	% of inhibition	
		DPPH at 517 nm	Superoxide at 590 nm
1	20	13.06±0.91	13.09±0.91
2	40	30.63±2.14	28.38±1.98
3	60	41.44±2.90	41.94±2.93
4	80	55.40±3.87	47.61±3.33
5	100	65.76±4.60	51.09±3.51
6	120	69.59±4.89	60.98±4.26

DPPH: 1,1-diphenyl-2-picrylhydrazyl

Table 2: Phosphomolybdenum reduction and Fe³⁺ reducing power assays of methanol extract of *Euphorbia hirta*

S. No	Concentration (µg/mL)	% of reduction	
		Phosphomolybdenum reduction at 695 nm	Fe ³⁺ reducing power at 700 nm
1	10	32.96±2.30	18.87±1.32
2	20	36.69±2.52	31.42±2.19
3	30	40.85±2.85	37.04±2.59
4	40	46.77±3.27	50.08±3.50
5	50	46.82±3.27	53.17±3.72
6	60	53.68±3.75	54.06±3.78

Table 3: R_f values of compounds separated by thin-layer chromatography of *Euphorbia hirta*

Spots observed	R _f value (UV 234 nm)
1	0.10
2	0.24
3	0.32
4	0.54
5	0.72
6	0.81

UV: Ultraviolet

normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents.

Superoxide anions derived from dissolved oxygen by the riboflavin-light-NBT system will reduce NBT in this system. In this method, superoxide anion reduces the yellow dye (NBT²⁺) to produce the formazan, which is measured spectrophotometrically at 590nm. Antioxidants are able to inhibit the blue NBT formation. The decrease of absorbance at 560nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The maximum superoxide reduction of leaves was 60.98 at 120 µg/mL concentration. The experiment demonstrated high antioxidant activity, the IC₅₀ was 97.88 µg/mL and was compared with standard ascorbic acid.

Phosphomolybdenum reduction assay activity

The total antioxidant activity of methanol extract of the *E. hirta* was measured spectrophotometrically by phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum absorbance was 53.68 at 60 µg/mL concentration. It was compared with the standard (0.359) ascorbic acid.

Ferric (Fe³⁺) is reducing power activity

The reducing power of Fe³⁺ to Fe²⁺ by the methanol extract of the *E. hirta* was studied and showed reduction ability in a dose-dependent manner. The maximum absorbance was 54.06 at 60 µg/mL and was compared with the standard (0.289) ascorbic acid (Figs. 1 and 2, Table 2).

TLC

TLC Thin layer chromatography (TLC) was carried out for the methanol extract of *E. hirta* of TLC aluminium sheets silica gel 60 F 254 (20x20 cm). Natural of substance to be separated, i.e., acidic, basic, and amphoteric.

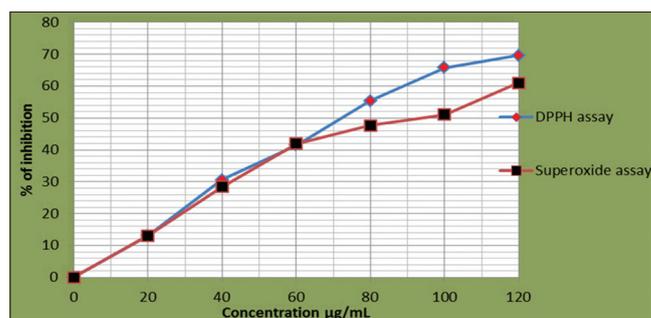


Fig. 1 Quantitative analysis of 1,1-diphenyl-2-picrylhydrazyl and superoxide radical scavenging assay of methanol extract of aerial part of *Euphorbia hirta*. Both assays were decreasing assay

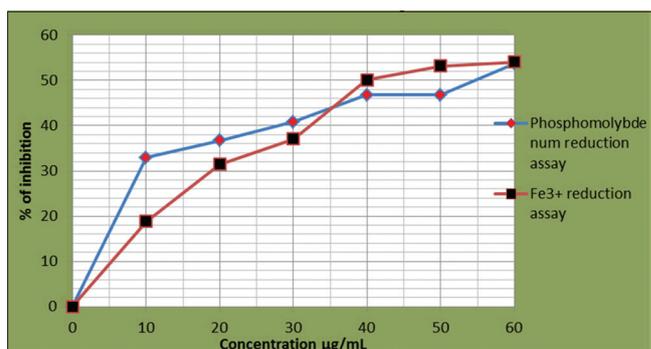


Fig. 2 Quantitative analysis of phosphomolybdenum reduction and Fe³⁺ reducing power assays of methanol extract of aerial parts of *Euphorbia hirta*. Both of the assays were increasing in the graph

Table 4: Antimicrobial activity of aerial parts of *Euphorbia hirta*

Organism	Zone of inhibition (mm)				Standard
	250 µg/mL	375 µg/mL	500 µg/mL	600 µg/mL	
<i>Escherichia coli</i>	13	16	21	23	10
<i>Staphylococcus aureus</i>	16	18	21	23	10
<i>Bacillus subtilis</i>	13	16	19	16	27
<i>Shigella flexneri</i>	15	19	22	24	10
<i>Proteus vulgaris</i>	14	16	19	25	10
<i>Micrococcus luteus</i>	15	19	21	24	10

Table 5: Antipsoriasis (MTT) of methanol extract of aerial parts of the *Euphorbia hirta*

S. No	Concentration (µg/mL)	Cell death (%)
1	0.781	88.37
2	1.562	77.13
3	3.125	70.96
4	6.25	61.35
5	12.5	48.18
6	25	40.33
7	50	34.23
8	100	23.77
9	250	16.26

MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

To see whether a compound is liable to react chemically with adsorbent (or solvent) or not. The separated compounds in TLC are shown in Fig. 3 (Table 3).

Antibacterial activity

Antimicrobial activity was screened against. *Melica minuta* extracts and the extract showed good antibacterial activity [14]. The methanol extract of aerial parts of the *E. hirta* shown maximum inhibition of 16 mm against *Bacillus subtilis* and maximum inhibition of 23 mm against *Staphylococcus aureus* and maximum inhibition of 23 mm against *Escherichia coli* and inhibition of 24 mm against *Shigella flexneri* and maximum inhibition of 25 mm against *Proteus vulgaris* and maximum inhibition of 24 mm against *Micrococcus luteus* [15]. The methanol extract of *E. hirta* inhibited the growth organism with the values of 250, 375, 500, and 625 µg/mL concentrations (Table 4 and Fig. 4).

Antipsoriasis activity

MTT assay

The fruit and leaf extracts of *Solanum erianthum* showed inhibitory effect on cancer cell lines [16] in *in vivo* studies. The cytotoxic activity of methanol extracts of *E. hirta* on the HaCaT cell line result. The cell viability assay was measured by the MTT reduction assay method as described by Mossman with slight modification. After treatment, the cells were incubated with MTT (10 µL, 5 mg/mL) at 37°C for 4 h and then with DMSO at room temperature for 1 h. The plate was read by a scanning multiwell spectrophotometer at 595 nm. The MTT assay is a colorimetric assay for assessing cell metabolic activity. The MTT *in vitro* cell proliferation assay is most widely used assay for evaluating the preliminary antipsoriasis activity [17]. This assay gives an indication of the whole cell cytotoxicity, however, to determine the exact molecular target further assays need to be perform enzyme activity that plays the key role in a number of physiological processes and their inhibitors have been found to exhibit antipsoriasis activity. The MTT assay involves in the conversion of the water-soluble MTT to an insoluble formazan. The formazan is then solubilized, and the concentration was determined by optical density at 570 nm. The maximum HaCaT cell death was 83.74±1.20 at 250 µg/mL concentration of methanol extract of aerial parts of the *E. hirta*, and the IC₅₀ was 12.20 µg/mL concentration.

$$\% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

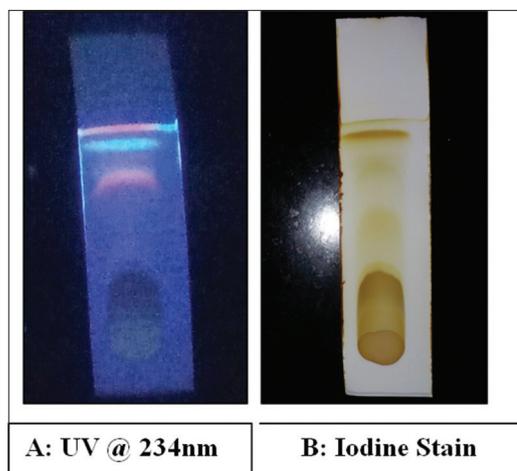


Fig. 3 Thin-layer chromatography (TLC) (natural substance separated in TLC was acidic, basic, ethyl acetate, methanol, iodine, and toluene) aerial parts of *Euphorbia hirta*

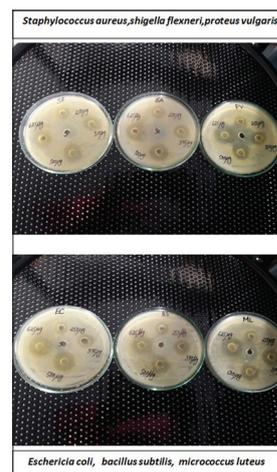


Fig. 4 Antimicrobial activity of methanol extract of aerial parts of *Euphorbia hirta*. The zone formation in the Petri plate was inhibited the against the microorganism

All data were reported as mean ± standard deviation. Results of methanol extract of aerial parts of *E. hirta* were tabulated in Table 5 (Figs. 5 and 6).

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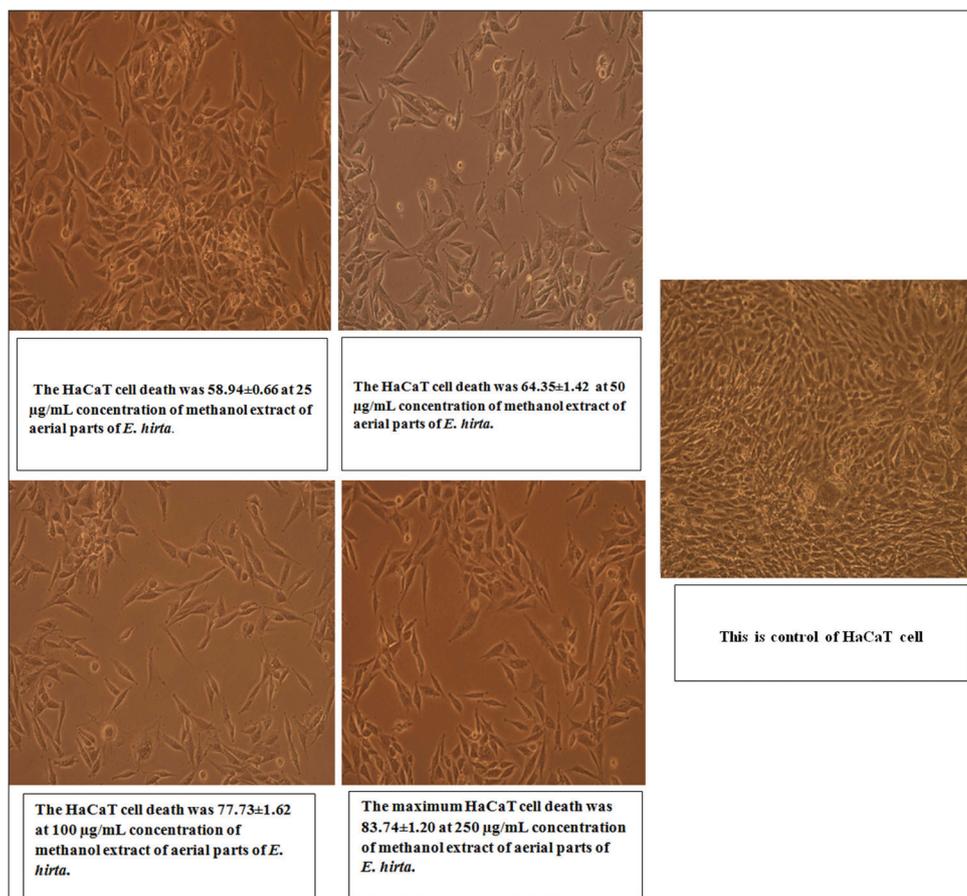


Fig. 5 Antipsoriasis (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay) activity of aerial parts of *Euphorbia hirta*

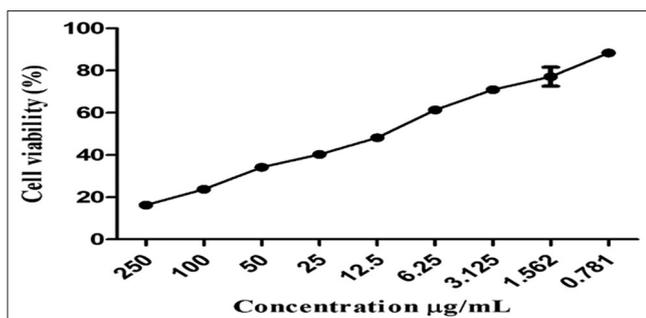


Fig. 6 Antipsoriasis (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay) the cell viability for aerial parts of *Euphorbia hirta*

CONFLICTS OF INTEREST

All the authors declare that there are no conflicts of interest.

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