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In-Vitro Studies on Antiproliferative Effect of Euphorbia Hirta L Leaves

Dr. M. Vijayapriya[1], Dr.S.Mahalakshmi [2], C. Ruth Christiya[3]
[1]Assistant Professor, Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University,
Annamalai Nagar, Chidambaram, TamilNadu, India
[2]Assistant Professor, Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University,
Annamalai Nagar, Chidambaram, TamilNadu, India
[3]Assistant Professor, PG and Research Department of Biochemistry, Rabiammal Ahamed Maideen College for Women (Affiliated to Bharathidasan University, Tiruchirappalli), Tiruvarur, TamilNadu, India.

ABSTRACT

Medicinal plants are a significant part of natural wealth. They serve as vital therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines. The use of herbal products by the majority is primarily based on the belief that herbal drugs are safe, without any side effects, accessible and available at minimal cost. However, there is need for quality assurance of the botanicals in these products in order to meet the demands of product quality and efficacy. The present investigation focus on screening of phytochemicals, UV-VIS analysis, and antiproliferative effect of *Euphorbia hirta* L leaves extracts to explore a better understanding of its pharmacological claims. The result reveals that the alkaloid, flavanoid, Phenols, steroids, saponins, carbohydrate and terpenoids were present in the leaf extracts. The results obtained from antioxidant profile of *Euphorbia hirta* L indicated that the aqueous leaf extracts exhibited significantly highest radical scavenging activity in concentration dependent manner which was compared with standard ascorbic acid. The UV-VIS profile showed different peaks ranging from 300-900nm with different absorption respectively. The UV-VIS spectrum analysis of *Euphorbia hirta* L showed the presence of phenolic compounds and flavonoids which may offer great pharmacological values. The methanolic extract of plant exhibited significant dose dependent antiproliferative activity against MG63 cell line which was ranged between 89.43% and 20.63% at concentrations of 50 µg/ml and 350 µg/ml correspondingly (24 hours). Moreover, the plant found to decrease the cell viability in dose dependent manner. The results confirm the fact that this plant posses' important bioactive constituents which may responsible for various pharmacological activities.

Keywords: *Euphorbia hirta* L, antioxidant, UV-VIS, Cell viability, MG63 cell line etc

INTRODUCTION

Human beings have depended on nature for their simple requirements as being the sources for medicines, shelters, food stuffs, fragrances, clothing, flavours, fertilizers and means of transportation throughout the ages. For the large proportions of world's population medicinal plants continue to show a dominant role in the healthcare system and this is mainly true in developing countries, where herbal medicine has continuous history of long use **(Refaz Ahmad Dar, 2017).**Medicinal plant research has increased all over the world and collected the immense potential of medicinal plants used in various traditional systems (**Bhatt et *al*, 2017).** Indian herbs are known all over the world for the medicinal properties. About 90% of the herbs and medicinal

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plants in India are collected from the forest. India forests are the source of invaluable medicinal plants and became aware of the preventive and therapeutic properties of plants and being used for human health care. These medicinal compounds are naturally gifted from the plant.

Secondary metabolites are synthesized naturally in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components making them rich sources of different types of phytochemicals. Mostly, these phytochemicals are secondary metabolites like flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenol compounds, etc **(Sanjay Kumar,2017)** These compounds extracted from different parts of plant. The amount of phytochemical compounds differs significantly from species to species and even from plant to plant, depending on the age and different ecological and climatic conditions. In current years, phytochemicals which have unknown pharmacological activities have been widely investigated as a source of phytomedicine **(Waseem Ahmed, 2017).** Numerous studies were done on many species of Euphorbia to provide phytochemistry and biological activity of the compounds. Especially, we will give the overview knowledge on the ethnomedical uses of Euphorbia L, the phytochemistry and the biological activities of the compounds which were isolated (**Hounzangbe-Adote Mawule Sylvie, 2016).**

UV-Vis spectroscopy refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived color of the chemicals involved. In this region of the electromagnetic spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state to the excited state. UV-Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds and biological macromolecules. Spectroscopic analysis is commonly carried out in solutions but solids and gases may also be studied (**Sertova *et al*,2000)**

A bone tumor is a neoplastic growth of tissue in bone. Abnormal growth found in the bone can be either benign or malignant. Bone tumors may be classified as "primary tumors", Which originate in bone and "secondary tumors" Which originate in other sites and spread to the skeleton. Carcinomas of the prostate, breasts, lungs, thyroid and kidneys are the carcinomas that most commonly metastasize to bone. Secondary malignant bone tumors are estimated to be 50 to 100 times as common as primary bone cancers. The outlook depends on the type of tumor. The outcome is expected to be good for people with noncancerous(benign)tumors, although some types of benign tumors may eventually become cancerous(malignant).With malignant bone tumors that have not spread, most patients achieve a cure, but the cure rate depends on the type of cancer, location, size and other factors (**John ,2013)** Various parts of plants were used in human diet. Currently, studies and availability of information on the phytochemical, antioxidant properties, anti-proliferative effects of many medicinal plant leaves are sporadic and lacking. This study therefore focuses on the phytochemical screening, UV-VIS analysis and antiproliferative effect of *Euphorbia hirta* L leaves.

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MATERIALS AND METHODS

COLLECTION OF SAMPLE

The fresh leaves of *Euphorbia hirta* L were collected from Tiruvarur district, Tamil Nadu in December 2019. The leaves were shade dried at room temperature and grinded o fine powder, kept in dry conditions for the further process.

PREPARATION OF EXTRACTS

AQUEOUS EXTRACTS

200 gm of the leaf powder was mixed with 1200ml of water and stirred continuously until it was reduced to one third and filtered .The filterate was evaporated to dryness. Paste form of the extract obtained was subjected to *In-vitro* studies.

ETHANOL EXTRACTS

The leaves of *Euphorbia hirta* L were used for this study. 20 g of the powdered leaves were soaked in 95% ethanol for 12 h. The extract was then filtered through Whatmann filter paper No.41 to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along was wetted with 95% ethanol. The filtrate was then concentrated and used for the further study.

QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

Chemical tests for the screening and identification of bioactive chemical constituents in the medicinal plants under study were carried out in extracts using standard procedures as described by Brindha *et al*., 1982

UV-VIS SPECTRA ANALYSIS(Sertova *et al*, 2000)

The instrument used in ultraviolet-visible spectroscopy is called a UV/Vis spectrophotomter. It measures the intensity of light after passing through a sample(I),and compares it to the intensity of light before it passes through the sample (I_0). The ratio I / I_0 is called the transmittance, and is usually expressed as a percentage (%T).The absorbance, 'A' is based on the transmittance:

$$A = - \log (\% T / 100\%)$$

The UV- visible spectrometer can also be configured to measure reflectance. In this case, the spectrophotometer measures the intensity of light reflected from a sample (I), and compares it to the intensity of light reflected from a reference material (I_0). The ratio I / I_0 is called the reflectance, and is usually expressed as a percentage(%R).

The basic parts of spectrophotometer are a light source, a holder for the sample, a diffraction grating in a monochromator or a prism to separate the different wavelengths of light and a detector. The radiation source is often a Tungsten filament (300 - 2500 nm),

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a deuterium arc lamp, Which is continuous over the ultraviolet region (190 – 400 nm), Xenon arc lamp ,Which is continuous from 160 – 2000nm or more recently, light emitting diodes (LED) for the visible wavelengths. The detector is typically a photomultiplier tube a photodiode, a photodiode array or a charge – coupled device(CCD). Single photodiode detectors and photomultiplier tubes are used with scanning monochromators, Which filter the light so that only light of a single wavelength reaches the detector at one time. The scanning monochromator moves the diffraction grating to "step-through" each wavelength so that its intensity may be measure as a function of wavelength. Fixed monochromators are used with CCDs and photodiode arrays. As both of these devices consists of many detectors grouped into one or two dimensional arrays, they are able to collect light of different wavelengths on different pixels or groups of pixels simultaneously.

Samples for UV/Vis spectrophotometry are most often liquids, although the absorbance of gases and even of solids can also be measured. Samples are typically placed in a transparent cell, known as a cuvette. Cuvettes are typically rectangular in shape, commonly with an internal width of 1 cm.(This width becomes the path length , L, in the beer-Lambert law). Test tubes can also be used as cuvettes in some instruments. The type of sample container used must allow radiation to pass over the spectral region of interest. The most widely applicable cuvettes are made of high quality fused silica or quartz glass because these are transparent throughout the UV, visible and near infrared regions. Glass and plastic cuvettes are also common, although glass and most plastics absorb in the UV, which limits their usefulness to visible wavelengths.

Cytotoxic potential analysis of *Euphorbia hirta* extracts in human bone cancer cells (Mosmann, 1983)

Source of chemical and reagents:

Dulbecco's Modified Eagle's Medium, streptomycin, penicillin-G, L-glutamine, phosphate buffered saline, 3-(4,5 dimethylthiozol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), ethylene diamine tetra acetic acid (EDTA), ethanol and dimethyl sulfoxide (DMSO), were purchased from Sigma Aldrich Chemicals Pvt. Ltd (India). All other chemicals used were of analytical grade, purchased from Hi media Laboratories Pvt. Ltd., India.

Preparation of extract *Euphorbia hirta*:

Fresh leaves of *Euphorbia hirta* were thoroughly washed using tap water and rinsed with distilled water. The leaves were dried for 5 min in an oven at 28^{o}C for one week and then pulverized to a fine powder with the aid of a Starlite blender (Model SL-999 The methanol extracts were obtained by weighing out same fraction 20 g of the pulverized powdered *leaves* of the *Euphorbia hirta* and soaking in 100 ml of the 70% ethanol for 24 hrs. The extracts were then filtered using Whatman no.1 filter paper (125 mm × 100 circles). All filtrates were air dried at 28^{o}C. And the air-dried filtrates were then reconstituted in 20 % DMSO solution and used for further experiments.

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Cell culture maintenance:

Human bone cancer MG-63 cell lines were procured from the cell repository of National Centre for Cell Sciences (NCCS), Pune, India. Dulbecco`s Modified Eagle Media (DMEM) was used for maintaining the cell line, which was supplemented with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml), and streptomycin (100 µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was maintained in a humidified environment with 5% CO_2 at 37°C.

MTT assay:

The cytotoxicity of *Euphorbia hirta leaves* extract on MG-63 cells was determined by the method of Mosmann, (1983).

Principle:

The yellow 3-4,5 dimethylthiozol-2-Yl)-2,5diphenyltetrazoliumbromide (MTT) is reduced by mitochondrial dehydrogenase of viable cells yielding a measurable purple formation product. Viable cells contain NAD (P) H-dependent reductase, which reduce the MTT reagent to formazon, with a deep purple colour. Formazon crystals are then dissolved using solubilizing solution and absorbance is measured at 500-600 nm by plate reader.

Reagents:

MTT stock solution:

MTT (50 mg) dye was dissolved in 10 mL of PBS. After vortexing for 1 min, it was filtered through 0.45 micro filters. The bottle was wrapped with aluminium foil to prevent light, as MTT was light sensitive. The preparation was stored at 4^oC.

Procedure:

Cell viability assay, MG-63 viable cells were harvested and counted using hemocytometer diluted in DMEM medium to a density of 1×10^4 cells/ml was seeded in 96 well plates for each well and incubated for 24 h to allow attachment. After MG-63 cells treated with control and the containing different concentrations of *Euphorbia hirta* leaves extract 50 to 350 µg/ml were applied to each well. MG-63 cells were incubated at 37°C in a humidified 95% air and 5% CO_2 incubator for 24 h. After incubation, the drug-containing cells wash with fresh culture medium and the MTT (5 mg/ml in PBS) dye was added to each well, followed by incubated for another 4 h at 37°C. The purple precipitated formazan formed was dissolved in 100 µl of concentrated DMSO and the cell viability was absorbance and measured 540 nm using a multi-well plate reader. The results were expressed at the percentage of stable cells with respect to the control. The half maximal inhibitory concentration (IC_{50}) values were calculated and the optimum doses were analyzed at different time period.

Inhibitory of cell proliferation (%)

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$$= \frac{\text{Mean absorbence of the control} - \text{Mean absorbence of the sample}}{\text{Mean absorbence of the control}} \text{ X } 10$$

The IC_{50} values were determined from the *Euphorbia hirta* dose responsive curve where inhibition of 50% cytotoxicity compared to control cells. All experiments were performed at least three times in triplicate.

RESULTS AND DISCUSSION

Table 1: Phytochemical qualitative analysis of *Euphorbia hirta* L

S.NO	Parameters	Aqueous Extract	Ethanol Extract
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Carbohydrates	+	+
4	Phenols	+	-
5	Glycosides	-	-
6	Saponin	-	-
7	Phytosteroids	+	-
8	Steroids	-	+
9	Tannins	-	-
10	Proteins	-	-
11	Terpenoids	-	+
12	Resin	-	-
13	Coumarins	-	-

Symbol (+) indicates Presence and (-) indicates absence of phytochemicals.

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Table 2 : UV-VIS analysis of *Euphorbia hirta* L Leaf extract

S.No	Peak(nm)	Wave length(nm)	Absorbance(AU)
1	3	304.65	0.3748
2	4	398.80	0.2974
3	5	665.75	0.1267
4	6	980.80	0.1333

Figure 1: UV-VIS analysis of *Euphorbia hirta* L Leaf extract

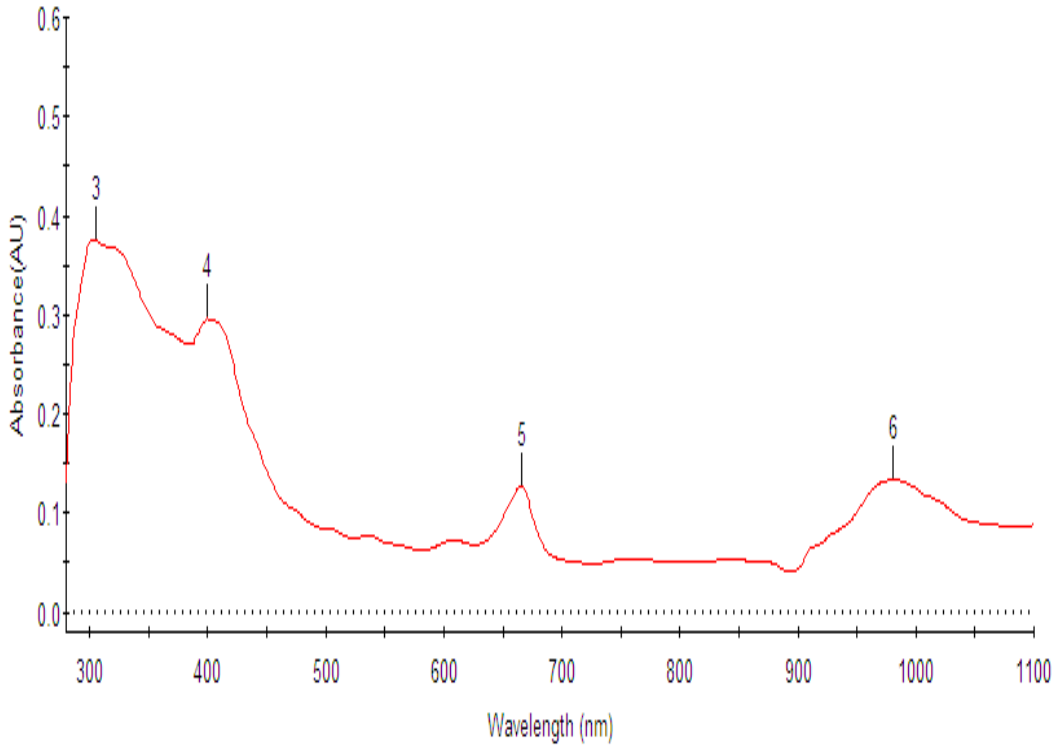

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Table 8 :Anti – Proliferative effect of *Euphorbia hirta L* on MG63 Cell line

S.NO	Concentration (µg/ml)	*Euphorbia hirta L* *Extract*
1	Control	100.03 ± 9.0
2	50	89.43 ± 8.05
3	100	76.33 ± 6.87
4	150	65.20 ± 5.87
5	200	53.21 ± 4.79
6	250	42.60 ± 3.83
7	300	31.12 ± 2.80
8	350	20.63 ± 1.86

Figure 2 : Anti - Proliferative effects of *Euphorbia hirta* L on the acivity of cytotoxicity in Bone cancer MG-63 cells

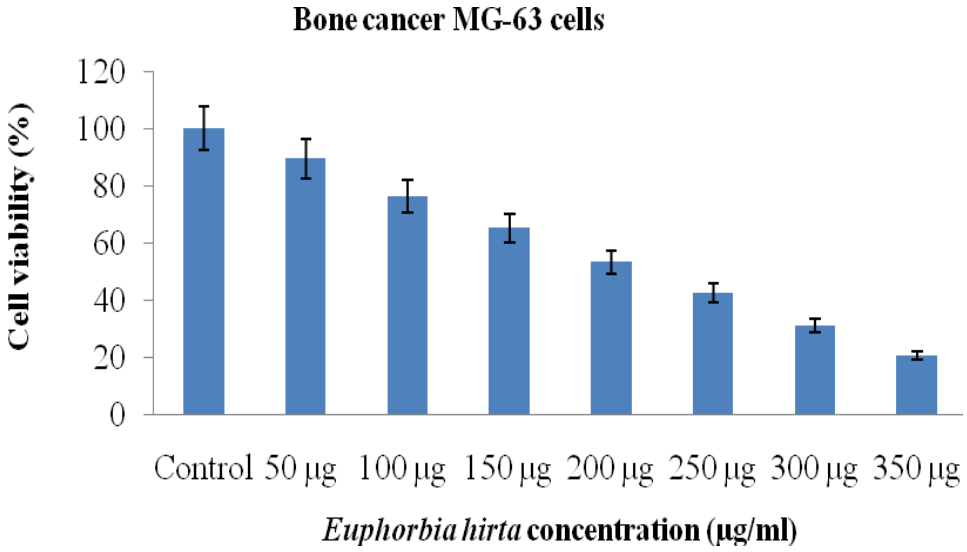

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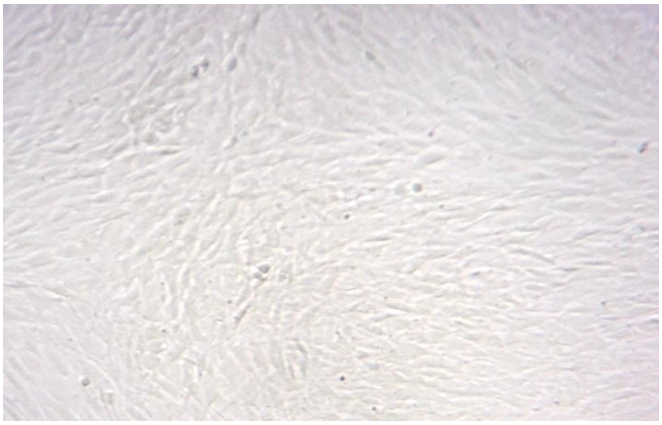
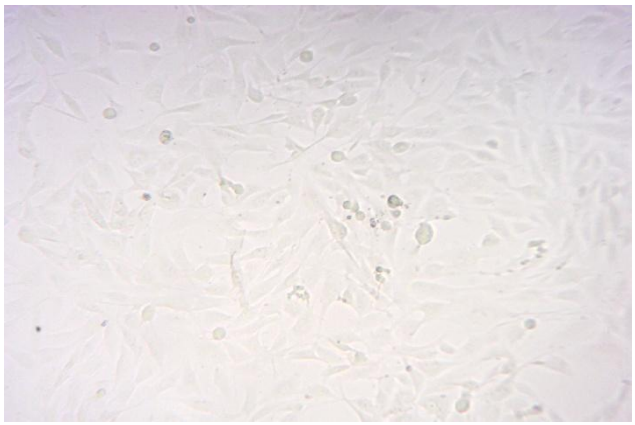
Figure 3 : Morphological changes in control and *Euphorbia hirta* treated MG-63 cells for 24h.



(a) Control at 24 hrs incubation



(b) 200 µg/ml at 24 hrs incubation

Photomicrograph represents morphological changes in MG-63 cells such as shrinkage, detachment, membrane blebbing and distorted shape induced by MG-63 treatment (200 µg/ml for 24 h) as compared with control. Control showed normal intact cell morphology and their images were captured by light microscope. The anti proliferative property of the plant extract may be attributed due to their phytoconstituents particularly phenolic and flavanoid compounds.

The results of preliminary phytochemical screening of *Euphorbia hirta* L leaves showed in table 1. The present study reveals the presence of alkaloids, flavanoids, carbohyrates phenols and phytosterols in aqueous extract. In ethanol extract the leaves of *Euphorbia hirta* L reveals the presence of alkaloid, flavanoid, saponins, carbohydrate, terpenoids and carbohydrate.

Flavonoids are water soluble phytochemical and an important plant phenolic. They show antioxidant activities and they have the property of preventing oxidative cell damage and carcinogenesis. They have anti cancer, anti inflammatory activities and a large effect in lower intestinal tract and heart disease **(Farquar *et al*, 1996).** Flavanoids present in this plant may

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attribute to its antioxidant property and prevent the cells from oxidative damage.

The aqueous and ethanol extracts of leaves shows the presence of phenols which imply that they may be used as anti-microbial agents. Generally, Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides Phenolic compounds have an electron donor capability; moreover, due to this ability, phenolic compounds are readily oxidized to form phenolate ion or quinone, which is an electron acceptor. Phenols and phenolic compounds modify the prostaglandin pathways and due to this action, they prevent platelets from clumping and have the ability to block specific enzymes that cause inflammation; antioxidant, immune enhancers, anti clotting and hormone modulators **(Okwu *et al*., 2001)**

The presence of saponins in this leaf extract, gave a justification why the extracts from these plants are used in wound healing. Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions **(Sodipo *et al*, 2000).**

Alkaloids tend to be organic and natural ingredients that have nitrogen, and are also physiologically active together with sedative and analgesic roles. They are found in reducing stress and depression symptoms. Alkaloids tend to be poisonous when taken in bulk amount due to their stimulatory effects, producing excitation associated with cell and nerve disorders **(Obochi.,2006)**. Alkaloids in *Euphorbia hirta* L leaves play some metabolic role and control the development of a living system.

The presence of Steroids in plant extract may responsible for reducing cholesterol levels, for regulating the immune response and some steroids also have immune-enhancing benefit (**Shah Qazi, 2009).** Coumarins present in *Euphorbia hirta* L are several biological activities such as antiinflammatory, anticoagulant, antibacterial, antifungal, antiviral, anticancer, antihypertensive, antitubercular, anticonvulsant, antiadipogenic, Cytochrome P450 inhibiting, antihyperglycemic **(Venugopala,2013),** antioxidant, estrogenic, dermal photosensitizing, antihelmentic, hypnotic, analgesic, hypothermic, antiulcer **(Monga, 2012)**anticlotting, hypotensive and antitumor activities **(Leal ,2000)**

Plants containing carbohydrates are known to exert a beneficial action on immune system by increasing body strength and hence are valuable as dietary supplements **(Theis & Lerdau ,2003).** Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties **(Rabi & Bishayee, 2009)**. In addition, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well **(Sultana , 2008).** It is clear from the phytochemical analysis that the *Euphorbia hirta* L are very rich in phytochemicals. These phyto constituents seemed to be the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health.

The UV-VIS analysis preformed for identification of phytoconstituents present in methanolic extract of *Euphorbia hirta* L. The UV-visible spectra were performed to identify

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the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of methanolic extract of *Euphorbia hirta* L was taken at the wavelength of 300 nm to 900 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 304.65, 398.80, 665.75, 980.80 nm with the absorption 0.3748, 0.2974, 0.1671 and 0.1333 respectively. Figure 2 shows the absorption spectrum of *Euphorbia hirta* L extract and these are almost transparent in the wavelength region of 300-900 nm. Absorption bands observed pertaining to *Euphorbia hirta* L plant extract are displayed in Table 2.

In the UV-VIS spectra the appearance of one or more peaks in the region from 300 to 900 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N, O **(Njokua, 2013)**The spectrum for *Euphorbia hirta* L extract shows two peaks at positions 304 nm, and 398 nm. This confirms the presence of organic chromophores within the *Euphorbia hirta* L extract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system.

The MG-63cells were treated with various concentration of methanolic leaf extract of *Euphorbia hirta L* (50-350 µg/ml) for 24 h and the results are expressed as a percentage of the control value in presenting as a cell cytotoxicity ratio for MG-63 cells using MTT assay. The cytotoxicity effects of methanolic extract of *Euphorbita hirta* were shown in figure 1. The cell viability of MG63 cells ranged between 89.43% and 20.63% at extract concentrations of 50 µg/ml and 350 µg/ml correspondingly in an incubation period of 24 hours. The IC_{50} value was found to be 187 µg/ml. The methanolic extract of *Euphorbita hirta* showed cytotoxicity against MG63 cells in concentration dependent manner. Exposure to increased concentration of the extract has grossly reduced the number of viable osteosarcoma cells, and their architecture was found to be disrupted. The assay detects the reduction of dimethylthiazole diphenyl tetrazolium bromide (MTT) salt to a coloured formazan product by mitochondrial enzyme succinate dehydrogenase, the intensity of the colour was measured using spectrophotometer, which measures the quantity of viable cells (**Abbas Momtazi-borojeni, 2013)**.The cell viability of MG63 cells decreased with increasing dose of the extract confirming its anti-proliferative property.

CONCLUSION

Euphorbita hirta L possesses potential of bioactive compounds which are responsible for the biological activities that is useful for natural health. Further studies should be focused on Fractionation of the active components in plant extract to take the research forward for further exploration.

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