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Ethanolic leaf extract of *Euphorbia hirta* against HT29 Cell line

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ABSTRACT

Euphorbiaceae is that the family of Euphorbia hirta plant. It's distributed throughout the place ladmass, they found in waste places on the roadsides. The plant components area unit wide utilized in ancient system of medicines, within the treatment of metabolism diseases, epithelial duct disorders, wound healing, respiratory organ disorders, urinogenital disorders, tumors, lactation in ladies etc. The current study, ethanolic extract of genus Euphorbia hirta has been aimed to analyse the phytochemical analysis, TLC (Thin Layer Chromatography), MTT ASSAY, acridine orange-ethidium bromide fluorescent staining methodology. The results analysis for phytochemical analysis might include the presence of terpenoids, saponins, steroids, alkaloids, tannins, flavonoids, betacyanin, comarins respectively. For TLC steroids, alkaloids and terpenoids where present in spot look for Rf value was calculated 0.90, 0.88, 0.95 respectively. MTT results shows the IC50 value of EH sample is 112.20% and also the 5-FU sample become 2.79% respectively. Then the AO/EtBr dual staining the results might include the red color become an dead cells, the yellow color become an ethanolic extract of EH treated sample, and also the green color become an viable cells. When these are the results might analysed against the Human Colorectal Adenocarcinoma were investigated using HT29 Cell line. For the future studies can development of anticancer drugs from medicinal plants EH.

Keywords: Euphorbia hirta, HT29 cellline, phytoconstituents, Thin Layer Chromatography, MTT ((3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide), AO/EtBr dual staining.

INTRODUCTION

Cancer has become a serious unhealthiness worldwide. Cancer is associate in nursing abnormal growth and proliferation of cells. Cancer accounts for relating to 1 in each 7 deaths worldwide. In 2012, there square measure associate in nursing calculated 14.1 million cases of cancer diagnosed round the world and 8.2 million cancer deaths. [Deepika Sharma and Dr. C.B.S. Dangi, 2016]. The World Health

Organization (WHO) reportable that around 13% of all deaths among the world measure caused by cancer annually. Colorectal cancer (CRC) is that the third commonest cancer worldwide. CRC is one in every of the foremost reason behind death in worldwide and accounts for over 9% of all cancer incidence thus death from carcinoma is that the fourth highest among all cancer-related deaths. There square measure similar incidence rates for cancer of the colon in both genders, and a slight male predominance for rectal cancer. [Forouzesh, Flora, et al., 2018]. Throughout the year 2009, it's calculate that 49,920 individuals can die from colon and rectal cancer. Despite these statistics, mortality from carcinoma has attenuated slightly over the past 30 years, presumptively thanks to earlier designation through screening and better treatment modalities. [Engstrom, PaulF., et al., 2009][9]. Deaths from cancer worldwide unit projected to continue rising, with accolade calculate 12 million deaths in 2030 (WHO estimate) [Sidambaram, R. Raja, 2011] . Despite these statistics, mortality from carcinoma has diminished slightly over the past 30 years, presumptively attributable to earlier diagnosing through screening and higher treatment modalities. [Engstrom, PaulF., et al., 2009]. This treatment makes use of the compounds naturally present in plants considerably secondary metabolities that posses ability to inhibit or kill cancer cells [Deepika Sharma and Dr. C.B.S. Dangi, 2016] .

Plant natural product measures a historically very important part among the treatment and interference of sickness. Plants are a rich supply of active ingredients for health care product. The genus Euphorbia is that the foremost vital among the plant family Euphorbiaceae, comprising regarding 2000 known species and ranging from annuals to trees. All contain latex and have distinctive flower structures.[Ozbilgin, Serkan, et al., 2012] . Euphorbia hirta features a worldwide distribution, and its common names include asthma attack weed and milk weed. The phytopharmacological investigations showed that its bioactive parts possessed varied medical science properties like medication, anti-microbial, anti-diarrheal, sedative, analgesic, anti-pyretic, anti-oxidant, anti-asthmatic, anti-tumor, larvicidal, diuretic, anti-cancer curative agent, etc. In India, the plant parts measure traditionally used to treat against worm infestations in babies and for disease, gonorrhea, jaundice, acne, pimples, biological process disorders, cancer, polygenic disease for various tumors. consistent with many ethnomedicinal varieties of literature the plant has known for increasing milk flow in women, and since of it milk like latex, it's also used for various female disorders. [Ghosh, Pranabesh, et al, 2019]. several plants of family Euphorbiaceae were tested for his or her antineoplastic property, however most of them measure used in ancient medication as treatment for varied human diseases, like Gastro organ disorders, metabolism diseases, system, sex organ instrumentation, varied ocular ailments, skin and mucous membranes issues and tumour [Asha, Sivaji, et al, 2014]. Several plants of Euphorbiaceae were tested for his or her antitumour property, but most of them used in ancient medication as treatment for various human diseases. Antitumor activity against cancer and pathology, cancer of the blood in mice and cytotoxic activity against certain cancer cell lines were also determined. [Aleksandrov, Mihail, et al., 2019] . In developing countries, in step with estimates by the World Health Organization, regarding 80% of the population still depends on North American country medicines factory-made from plants for interference and treatment. Plants having secondary

metabolites . The Secondary metabolites in plants include several terms of molecules, also as steroids, alkaloids, phenolic, lignans, carbohydrates and glycosides, etc., [Tran, Ngan, et al., 2020] . To assess for preliminary antitumor activity in terms of cell viability, the MTT and MTS in vitro totoxicity assays are thought of two of the foremost economic, reliable and convenient ways. This can be supported their easy of use, accuracy and rapid indication of toxicity, additionally as their sensitivity and specificity [McCauley, Janice, et al, 2013]. The aim of this study was to analysis the in vitro antitumor activity of E. hirta and to evaluate its potential for clinical use as a natural antitumor agent.

MATERIALS AND METHODS

Collection of the plant materials

Fresh leaves of *E. hirta* specimens were harvested. The plant sample was collected in and around Chengalpattu district, Tamil Nadu, India. The plant were washed in tap water and air-dried in the shade for 10 days. The sample grained to a fine powder using an electric blender, and stored in clean, labelled, airtight bottles.

Preparation of Ethanolic extract

The leaves of *E.hirta* was cleaned and shade dried in open air for 8-10 days then pulverized to dry power using electric grinder.The powdered form of *E. hirta* (10g) was taken and add 100ml of ethanol and allow to rotary shaker for 24-48hr of slow shaking. After 2days the extract was filter with whatman no.1 filter paper and the filtrate was allow to evaporate into laminar air flow. After evaporation scrab the sample and collect the powered crude extract. The ethanolic crude extract put in air tight containers stored in a refrigerator for experimentation purpose.

Phytochemical screening

Qualitative chemical tests were carried out using extract from plant to identified phytochemicals, the plant extract was screened for the presence of biologically active compounds like sugars, amino acids, proteins, phenols, terpenoids, Steroids etc.

Test for terpenoids

In a test tube 1ml of chloroform, 0.5ml of extract then followed by the addition of 2ml conc. Sulphuric acid. Which forms a Reddish brown coloration of the interface indicates Terpenoids.

Test for saponins

To 0.5ml of extract then mixed with 5ml of Distilled Water in a test tube it was shaken briskly. The formation of stable foam which indicate the presence of saponins .

Test for steroids

To 0.5ml of extract then mixed with 0.5ml of acetic anhydride then followed by few drops of conc. Sulphuric acid. A green colouration indicated the presence of Steroids.

Test for alkaloids

To 0.5ml of extract was dissolved with 1ml of 1% HCL and heated gently, then Mayers reagent were added to the mixture. Turbidity forms thus the confirmation for the presence of Alkaloids.

Test for tannins

To 0.5ml of extract and 10% alcoholic ferric chloride was added; formation of greenish black colour shows the presence of Tannins.

Test for flavanoids

To 0.5ml of extract and lead acetate solution was added, shows the yellow colour precipitated to conform the Flavanoids.

Test for betacyanin

To 0.5ml of extract, 1ml of 2N NAOH was added and heated for 5 minutes at 1000C. Formation of yellow colour indicates the presence of Betacyanin.

Test for coumarins

To 0.5ml of extract and 1ml of 10% NAOH was added. Formation of yellow colour indicates presence of Coumarins.

Test for carbohydrates

To 0.5ml of extracts, 1ml of distilled water, then 2ml of Fehlings solution was added and boiled for 5 minutes and observe the results . The red colour shows the presence of Carbohydrates.

Test for phenol

To 0.5ml of extract, distilled water, then a drop of ferric chloride was added to a solution and observe the results. Appearance of purple colour shows the presence of Phenol.

Test for quinones

To 0.5ml of the extract, 1ml of conc. Sulphuric acid was added. Appearance of red colour indicates the presence of Quinones.

Test for acid

To 0.5ml of the extract treated with sodium bicarbonate solution. Appearance of effervescence indicates the presence of Acids.

Test for proteins

A few drops of the Million's reagent was added to the 1ml of extract, when us then heated gently. A reddish colour, occurs the formation of Proteins.

Test for reducing sugar

To 0.5 ml of extract, Fehling's reagent was added and heated gently formation of red colour occurs the presence of Reducing Sugar.

Test for glycosides

To add 2ml of chloroform and 0.5ml of the extract and then add 10% ammonium solution. The appearance of pink color indicates the presence of Glycosides. [Das, B. K., et al., 2014]

TLC (Thin Layer Chromatography)

Ethanol leaf extract of *Euphorbia hirta* were tested to determine the various phytochemical constituents such as alkaloid, steroid, terpenoids using TLC techniques. [Chitra, M., et al., 2011]

CYTOTOXICITY ASSESSMENT BY MTT TEST

The assay is disbursed out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial Succinate dehydrogenase and enzyme reductase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell range and inversely proportional to the degree of cell toxicity.

CELL CULTURE AND MAINTANANCE:

HT29 (Human body part adenocarcinoma) cell line was procured from NCCS, stock cell was refined in medium supplemented with 100% inactivated craniate Bovine body fluid (FBS), antibiotic drug (100 IU/ml), antibiotic (100 µg/ml) in Associate in Nursing humidified atmosphere of 5% carbon dioxide at 370c till convergent.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide MTT ASSAY:

To each well of the 96 well microtiter plate, 100µl of the weakened cell suspension (50,000cells/well) was supplemental. After 24 hr, when a fractional monolayer was molded, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different centralizations of investigate medication were supplemental on to the incomplete monolayer in microtiter plates. The plate was then brooded at 37^0C for 24hrs in five-hitter carbonic corrosive gas climate. when brooding the investigate arrangements inside the wells were disposed of and 100µl of MTT was supplemental to each well. The plate was hatched for 4h at 37^0C in five-hitter carbonic corrosive gas climate. The supernatant was eliminated and 100µl of DMSO was supplemental and furthermore the plate was tenderly upset to solubilize the designed formazan. The absorbance was estimated utilizing a microplate peruser at a frequency of 570 nm. The extent of reasonability was determined abuse the ensuing recipe:

Percentage of viability = Sample abs/Control abs x 100

ACRIDINE ORANGE-ETHIDIUM BROMIDE (AO/EtBr) FLUORESCENT STAINING

Acridine Orange–Ethidium Bromide Dual Staining The degree of apoptosis in the phones treated with MTT measure was examined infinitesimally utilizing an acridine orange/ethidium bromide (AO/EtBr) double staining strategy. Double staining was performed to recognize the chromatin buildup of dead apoptotic cells by staining them with a fluorescence stain. The tumor cell HT29 were independently filled in 24 well plates (1 × 105 cells for each well) as depicted above, and the cells were treated with an IC50 convergence of MTT test. The treated cells were washed with phosphate-cushioned saline, and the double stain (AO/EtBr) (1 mg/mL AO and 1 mg/mL EB in PBS) was then added, and the combination brooded for 5 min. The cells were again washed with PBS support, and the abundance stain was taken out and afterward pictured the cells. [Pannerselvam, Balashanmugam, et al., 2021]

3. RESULTS:

Phytochemical Screening:

The qualitative phytochemical constituents of ethanolic leaf extract of *E. hirta* –shown in Table 1. Analysis revealed the presence of terpenoids, saponins, steroids, alkaloids, tannins, flavonoids, betacyanin, comarins, except phenol, quinones, acid, protein, reducing sugar, and glycosides respectively.

Thin Layer Chromatography:

The quantitative analysis of thin layer chromatography for the leaf extract of E. hirta are shown in Table 2. Steroid, terpenoids and alkaloides where present in the leaf extract of E.hirta. That Rf value was calculated 0.90, 0.88, 0.95 respectively.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide MTT ASSAY:

Euphorbia hirta ethanolilc leaf extract exhibits targeted antitumor impact against HT29 cell line: Antitumor impact of ethanolic leaf extract of E.hirta estimated by MTT assay and morphological studies. The results of the MTT assay and morphological study shown in (Figure 2 & 2.1) revealed specific antitumor activity of the ethanolic leaf extract towards colon cancer cell line HT29. The IC50 value of the given investigate test E.hirta and moreover the normal 5-Fluorouracil (5-FU) was discovered to be 112.20 µg and a couple of 0.79 µg, severally. The results suggested that the toxicity of the extract is extremely specific to colon cancer cells and non-toxicity to traditional cells at a selected concentration. Graphical analysis for comparing the ethanolic extract of E.hirta with the Fluorocuracil (5FU) value in µg level. Once these analysis has shown in graphical representation in (Figure 3).

ACRIDINE ORANGE-ETHIDIUM BROMIDE (AO/EtBr) DUAL STAINING:

The extent of cell death at intervals of the cells treated with biogenic AgNPs was associate and degree analysed microscopically exploitation an acridine orange/ethidium bromide (AO/EtBr) dual staining technique. Dual staining was performed to detect the chromatin granule condensation of dead apoptotic cells by staining them with a visible light stain. The tumour cells HT29, were on an individually fully grown in 24 well plates (1 _ 105 cells per well) as described higher than, and conjointly the cells were treated with an IC50 concentration. The treated cells were washed with phosphate-buffered saline, and conjointly the dual stain (AO/EtBr) (1 mg/mL AO and 1 mg/mL EB in PBS) was then added, and also the mixture incubated for 5 min. The cells were once more washed with PBS buffer, and also the excess stain was removed excitation filter of 482 nm (figure 4). [Pannerselvam, Balashanmugam, et al., 2021]

DISCUSSION:

The constituents of several medicinal plants are used since ancient times to treat a variety of diseases. *Euphorbia* genus has a wide variety of terpenoids (mono, sesqui, and diterpenes to triterpenoids and steroids). Many of these compounds are investigated for their therapeutic effects or their toxicity activity , and some nof them are used as medicines . Also, the biological activities of them are antiproliferation, antiviral, antimicrobial, modulability of multidrug resistance, cytotoxic, antifeedant, antidiarrheal, molluscicidal activities and anticancer activities .[Forouzesh, Flora, et al., 2018] .

This examination amied to screen the anticancer and antioxident potential of methanol, petrol ether, chloroform, ethyl acetate, butanol of Euphorbia hirta Linn sample was analysed for qualitative phytochemical analysis was carried out to evaluate the bioactive compounds in each extract. The extraction method is the first step to separate phytochemicals from raw materials. According to the principle of extraction, solvent extraction has been used**.** [Tran, Ngan et.al. (2020)][4]. Similarly this study evaluated the chemical composition, antioxidant, anti-inflammatory and anticancer activities of a

Euphorbia hirta L. ethanol extract. [Sharma, Neelesh, et al., 2014]. Similarly the ethanolic leaf extract of Euphorbia

hirta was analysed in vitro anticancer activity from this study are to find the presence of secondary metabolites by preliminary phytochemical investigation was screened for the presence of biologically active compounds like sugars, amino acids, proteins, phenols, terpenoids. This study was analysed the cytotoxicity against Dalton Lymphoma Ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cell lines [Anitha, P., et al., 2014].

Similarly during this study was planned to evaluate TLC analysis of phytochemicals and in vitro inhibitor activity of the leaf extract of Euphoria hirta Linn. The phytochemical constituents were screened through Thin Layer Chromatography (TLC) studies and therefore the in vitro inhibitor activity of the alcohol and solvent acetone extract was additionally investigated. The E.hirta has flavanoids, steroids and phenols that were screened through TLC analysis. [Chitra, M., et al. 2011] . Similarly in this study was to analyse the phytochemical analysis of E,hirta and its cytotoxicity activity on Ma104. From the phytochemical analysis the results shows flavonoids, tannins, saponins, alkaloids and terpenes present in the curde acetone extract of E.hirta. Then the cytotoxicity activity in non-toxic concentration. [Ponnambalam, A., et al., 2019] . Similarly this study was to evaluvate the several polyphenols from Euphorbia hirta ethanolic extract to inhibit melanoma growth and metastatic potential.[Mishra, S. M. 2019] .

Similarly this study analysed the anticancer effects of the alcoholic extract of Euphorbia condylocarpa. The cytotoxicity of the methyl extracts of genus Euphorbia was performed on Adenocarcinoma Gastric Cell line (AGS) gastric cancer.[Mohammadi, Sepideh et.al., 2016] . Similarly the Cytotoxic Activity of the Root of Euphorbia Tehranica Ethanolic Extract Against Caco-2 Colorectal Cancer Cell Line was analysed [Forouzesh, Flora, et al. 2018] . Similarly this study was to analyse the two Euphorbia species (i.e.terracina and paralias) were investigated for his or her cytotoxic and antioxidant activities. For toxicity the methanol and chloroform fractions was examined towards human acute myeloid leukemia and human colon epithelial cancer cell lines. The secondary metabolites are phenolic classes, alkaloids, terpenes, saponins and antioxidant activities were assessed in plant extracts. [Soumaya Ben Jannet et al 2017].

Siminarly in this investigation shows the anticancer activity from the plant Euphorbia hirta from this study was analysed for the phytochemical analysis the results shows the presence of terpenoids, saponins, steroids, alkaloids, tannins, flavonoids, betacyanin, comarins. The phytochemical consituents were screened through TLC studies and the in Vitro anticancer activity of the ethanolic extract was also investigated. The phtocompounds of steroids, flavonoids and terpenoids which were screened through TLC analysis. Then the presence of phytochemical and cytotoxic development using HT29 cell line. Cytotoxic analysis were evaluated to utilizing (3-(4,5-Dimethylthiazol-2-yl)- 2,5-Diphenyltetrazolium Bromide) (MTT). It shows the IC50 esteems may determined its become a vaiability range is 112.20% from the ethanolic leaf concentrate of euphorbia hirta against HT29 cellline. Then analysed to investigate the double staining Acridine Orange-Ethidium Bromide (AO/EtBr) Fluorescent Staining respectively. It

is concluded that investigated ethanolic leaf extracts have the potential to act against the anticancer activity for the further process will be analysed for pharmaceutical industry.

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Table content

TABLE 1: Phytochemical screening of ethanolic leaf extract of E.hirta

S.NO.	PHYTOCHEMICAL TESTED	ETHANOLIC LEAF EXTRACT OF *E.hirta*
1.	Terpenoids	+
2.	Saponins	+
3.	Steroids	+
4.	Alkaloids	+
5.	Tannins	+
6.	Flavanoids	+
7.	Betacyanin	+
8.	Comarins	+
9.	Carbohydrates	-
10.	Phenol	-
11	Quinones	-
12.	Acid	-
13.	Protein	-
14.	Reducing Sugar	-
15.	Glycosides	-

+ indicates Positive

-indicates Negative

TABLE 2: QUANTATIVE ANALYSIS OF ETHANOLIC LEAF EXTRACT OF E.HIRTA

S.NO.	PHYTOCHEMICALS TESTED	ETHANOLIC LEAF EXTRACT OF *E. hirta*	Rf VALUE
1.	Steroids	+	0.90
2.	Alkaloids	+	0.88
3.	Terpenoids	+	0.95

+indicates positive

- indicates negative

Figure content

Figure 1: The plant of Euphorbia hirta [Asthuma Plant]

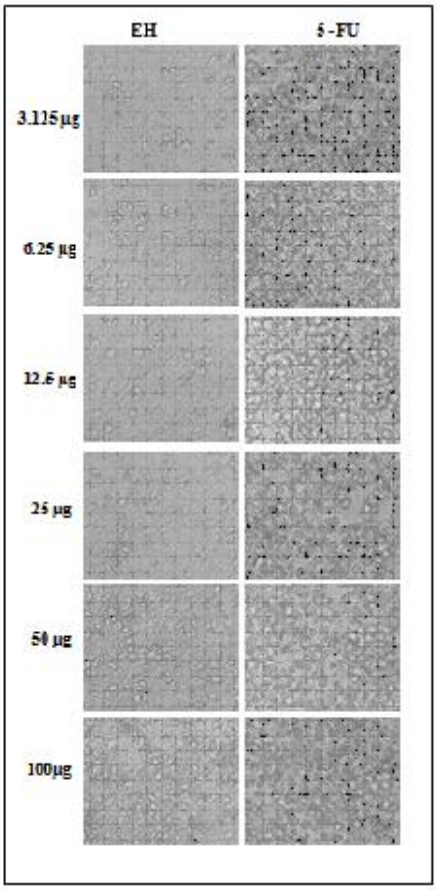

Figure 2 & Fig: 2.1: Its represents the phase contrast image shows specific and significant morphological change in HT29 cells. When cytotoxicity assay for Colon Cancer Cell line (HT29) the normal cell as treated with E.hirta ethanolic leaf extract.

MTT ASSAY					
SAMPLE (EH)	**3.125**	**6.25**	**12.2**	**25**	**100**
STANDARD (5-FU)	**3.125**	**6.25**	**12.2**	**25**	**100**
IC50 VALUE HT29 CELLLINE	**112.20**		**2.79**		

TABLE 3: This analysed results of ethanolic extract of E.hirta sample with the standard (5-FU) values in µg level

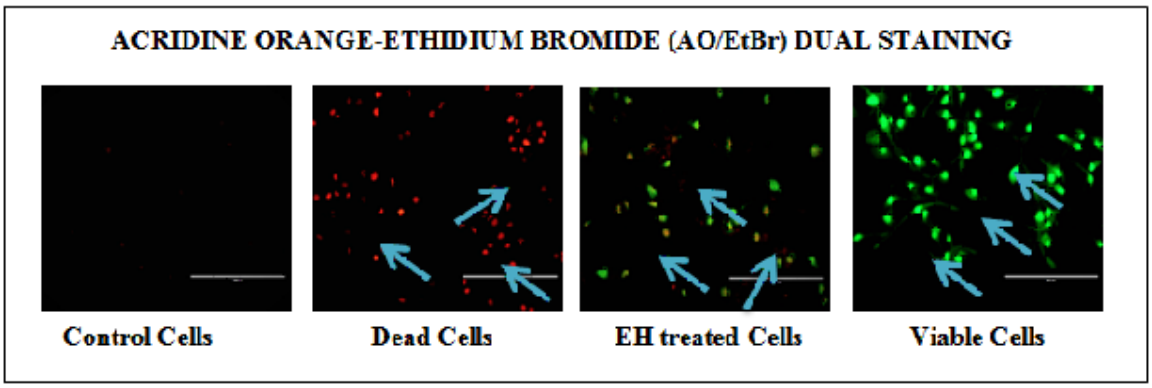

Figure 4: Acridine orange-ethidium bromide (AO–EB) staining of HT29 Cell line. When this images indicates (AO-EB) staining result which shows the images [a], [b], [c], [d] the image [a] indicates the control cells , [b] indicates the dead cells, [c] indicates the ethanolic extract of E.hirta treated sample, [d] indicates the viable cells.