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EVALUTION OF ANTICANCER ACTIVITY OF METHANOLIC EXTRACTOF EUPHORBIA MILII LEAVES

Name of Author: Miss. Priyanka Jamade M.Sc
Department of Pharmaceutical Chemistry,
Karnataka State Akkamahadevi Women University, Vijayapura

ABSTRACT : Euphorbia milii is a herb used as a folk medicine from ancient times. Historically the plant has been utilized as an antiinflammatory, antioxidant ,antisparmodic as well as parasite and wart treatment. Then solvent extraction is carried out using hot methanol. The methanolic extract showed positive result for glycosides, steroids, flavanoid, alkaloids and phenols the anticancer activity was tested using different concentrations, these chloroform fractions demonstrated potent anti-DPPH radical activity. This study used a finely tuned combination of phytopharmacological and cutting-edge computational tools to examine the anticancer potential of Euphorbia milii (E. milii). The outcomes of antioxidant assays revealed a consistent pattern, with the antioxidant reduction activity of the methanol extract often outperforming that of the water extract by a factor of 2:1. Significant toxicity results were seen in both tests of toxicity. The proposed genera Euphorbia triaculeata extract demonstrated genotoxic effects against the PC 3 and MCF-7 DNA cell lines, but not on the HEPG2 cell lines, by increasing the mean value of the DNA damage in the comet assay.

Keywords: DPPH, MTT, Tetrazolium, Phytochemical, Radical scavenging

INTRODUCTION:

The term "medicinal plant" refers to a variety of plants used in plant medicine (herbology and herbal medicine). The word "herb" comes from the Latin word "herba" and the Old French word "herbe." Today, the term "herb" refers to any part of a plant, including the fruit, central bark, flower, leaf, stigma, and root, as well as non-woody plants. Plants have been employed for medicinal purposes since the prehistoric era. Herbs have been used as medicine for almost 4,000 years, according to evidence from the unani hakims, Indian vaids, and European and Mediterranean cultures. Various indigenous societies, including those in Rome, Egypt, Iran, and Africa, used herbs in healing rituals, while others created traditional medical systems like uanani, ayurveda, and Chinese medicine where herbal remedies were used.

A large variety of plants with medicinal and aromatic properties are found mostly in India's forests, where they are gathered primarily as raw materials for medicines and perfumery products. India has a reputation as one of the first civilizations with a wealth of medicinal herbs. Indigenous medicine, ayurveda, unani, and siddha are the main indigenous medical systems. The most developed and commonly practised of these systems in India are ayurveda and unani medicine[1].

According to a recent WHO (global health agency) estimate, over 21,000 plant varieties have the potential to be used as medicinal plants, and 80% of people worldwide rely on herbal remedies for some part of their primary healthcare needs. According to research, more than three-quarters of the world's population relies primarily on botanical extracts and plants for their medical needs. At one point, more than 30% of all plant species were employed for medicinal purposes[2]. According to estimates, plant-based medications make for up to 25% of all drugs consumed in industrialised nations like the United States while accounting for up to 80% of all drugs consumed in rapidly emerging nations like India and China, countries such as to provide two third of the plants used in morden system of medicine and the health care system of rural population depend on indigeneous system of medicine.

Euphorbia milii is a herb used as a folk medicine from ancient times. Historically the plant has been utilized as an antiinflammatory, antioxidant ,antisparmodic as well as parasite and wart treatment. In the present work we collected leaves in the KSAWU Vijaypur campus in the month of November 2022 shade deied and creshed in to raw powder.

THE PRESENT PLANT :

It is an branded evergreen shrub reaching to a weight of 60-90 cm it is sensitive to temperature below 35^0 F in the winter. Although euphorbia milii Des moul is tolerant of poer soils, especially rocks –sandy soils and even of drought, regulator application of moderate hydration may result in improved bloom with less leaf drop. It performs best in location with adequate air movement and indoor plants require intense light and thrive in a coarse soil- based potling mix and which propagate from culturing of the tip it is also known as crown of throwns due to presence of plants of thorns on stem.

Classification[3]:
Kingdom :plantae
Division :Magnoliophyta
Class: Magnoliopsida
Order: Malipighiahiales
Family : Euphorbiaceae
Genus : Euphorbia
Species : Milii
Origin: Madagascar
Flowering : yes
Fragrant : Slightly
Growing case : No extra care requires
Temperature : 30-40^0c
Vernacular names : crown of thrown, Christ plant , Christ thorn

MATERIAL AND METHOD:

Extraction is the term used for the separation of medicinally active portion of plant tissue from the inactive or inert components by solvents in standard extraction procedures in this method the finally grind plant material of euphorbia milli placed in a humble made of strong filter paper which is placed in a chamber of soxhlet apparatus. The extracting solvent (Methnol-350ul) is taken boiling flask which is heated and solvent vapours condense in condenser. The condensed extract drips into the thimble containing the crude sample and extracts it by contact when the level of liquid in chamber rises to the top of the siphan tube the liquid contents of chamber siphon into flask this process is continuous and is carried out until a drop of sovent from the siphan tube does not leave residue when evaporated[4].

SOLVENT RECOVERY BY DISTILLATION UNDER REDUCED PRESSURE: Excess of methanol used during extraction process is recovered by vacuum distillation to distil compounds that have a point of boiling or any compounds that undergo decay upon heating at atmospheric pressure. Since the point of boiling of the create is lower at an external pressure, the compound will not need to be heated to a high temperature in order to boil.thevaccum is provided by a mechanical pump it is used because substance boil at lower temperature. This save heating costs and also keeps sensitive substance from decomposing under the high temperature that would the high temperature that would be required at atmospheric pressure . Plant powders were steeped in methanol for 12 hours while being occasionally shaken. The filtrate was collected after filtering this mixture. The filtrate was obtained after the residue was steeped in an additional 300 mL of methanol for an hour. The leftover material was then filtered after being resoaked in 300 mL of methanol. Using a rotary evaporator, the filtrates were then mixed and condensed to a volume of approximately 100 mL. Next, a volumetric flask was used to transfer the concentrated extract, which was then diluted with methanol[5].

DRGING: After distillation the crude extract is taken into the petridish the methanol remain evaporates leaving a dry solid which has been kept for at least two days later extract was subjecting to phytochemical screening and testing anticancer activity using sensor detecting detoxifying radicals originating from DPPH.

DPPH Radical Scavenging activity

The a solution of an approach which was dissolved in a mixture of 95% methanol at a dosage level of 1 mg/mL, was made using the stock solution. Utilising 95% a solution of 10 mL of a 1 mg/mL botanical extract was created.A colorimeter was used to measure the absorbance at 510 nm. The following equation was used to calculate the extract's capacity to scavenge free radicals[6]:

DPPH Scavenging effect (%) = X_0-X_1/X_0 x100

Where X0 denotes the intensity of the sample and that of the control, respectively. The control was created and tested exactly identical way as the sample with the extract omitted.

PHYTOCHEMICAL SCREENING :

According to conventional phytochemical procedures, it is an initial assessment of secondary substances such alkaloids, saponins, lignans, flavonoids, phenols, steroids, and glycosides.

Confirmatory presence of Alkaloid

The sample and methanol ratio was taken in the ratio of 10:1in the alkaloid test. Following filtration, 1 mL of the filtrate was added along with two or three drops of Wagner's reagent. Alkaloids were present when brown or reddish-brown precipitates were formed(6).

Confirmatory presence of Flavonoid

Shinoda's technique was employed to determine the presence of flavonoids. 200 mg of the plant material were extracted for the test using 10 mL of chloroform. After filtering, 2 mL of the filtrate was mixed with concentrated hydrochloric acid, HCl, and magnesium ribbon. The presence of flavonoids is indicated by the emergence of pink-red tint[7].

Test for saponins: A foaming test was performed to screen for saponins. In order to do this, 0.50 mL of the filtrate was added to 5 mL of distilled water in a test tube, which was then shaken vigorously for 30 seconds. The presence of saponins was established by persistent foaming[7].

Steroid screening test: The Liebermann-Burchard's test was employed to identify steroids. By soaking 200 mg of plant samples in 10 mL of chloroform, the analyte was removed from the sample. It was filtered, and 2 mL of the filtrate was combined with 1 mL of concentrated sulfuric acid, H_2SO_4, and 2 mL of acetic anhydride. The development of a blue-green ring is proof that steroids are present[6].

Test for glycosides:

The Keller-Killiani test was used to determine whether glycosides were present. Pipetting 2 mL of the crude extract and adding it to 1 mL of $FeCl_3$ and 1 mL of concentrated H_2SO_4 was done for this. The presence of glycosides is indicated by the emergence of green-blue color[7].
. Table No. 1 summarises the outcome of the screening for phytochemical constituents.

ANTICANCER ACTIVITY

Euphorbia spicies' standard assay[8].
To assess the extract's genotoxicity, a similar MTT assay approach is being examined.
The methanolic suspension of Euphorbia growing triaculeata was compared to the well-known chemotherapy drugs 5-FU and DOX because of its curative properties against the female breast carcinoma tumour cell line MCF-7, cancer of the prostate cell path PC-3, the cancer of the liver organism line HEPG2, the conventional breast implants epithelial cell accordance MCF-10A, and the frontal skin cells cell line Hs68.

MTT stands for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
In living cells, tetrazolium salt (MTT) preferentially degrades into formazan blue, and the amount of formazan that is produced is inversely correlated with the quantity of living cells present. Exponentially growing cells have been placed in triplicate in 96-well sterilised plates at a density of 1 104 cells per well. During 24 hours, the cells were cultured in a humid atmosphere with 5% CO_2 and subjected to increasing quantities of the test extract. The cells received treatment using MTT (0.5 mg/ml) for an additional four hours at 37°C after being exposed to the plant extract for 48 and 72 hours. The blue MTT formazan precipitate was incubated for a further 2 hours after becoming dispersed in detergent (50% final amount of N, N-dimethylformamide and 10% sodium dodecyl sulphate). On a multiwell ELISA plate reader, absorbance was measured at 570 nm. In the medium control, the mean absorbance was left blank and subtracted. Cells without samples served as the negative control, while the anticancer medications doxorubicin and 5-fluorouracil were employed as the positive controls. The association between surviving component and the concentration of extract was plotted in order to obtain the curve of survival of the three lineages of cancer cells using the set amount of extract.

PRINCIPLE :

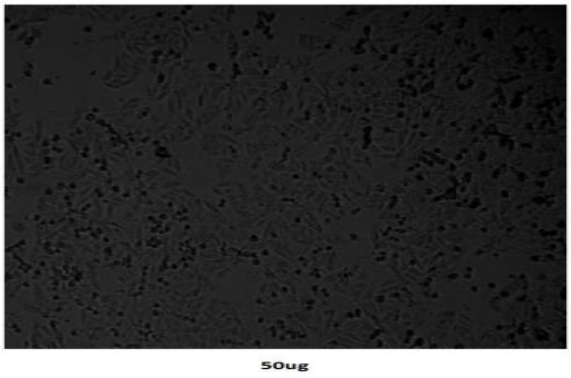
The yellow tetrazolium MTT (3-(4,5 dincetlylthiazoly -2) -2,5 dipheneyl tetrazolium bromide) is reduced by its metabolic activity in cells, in part by the reaction of declydrogenase enzymes in order to produce reducing equivalent such as NADH and NADPH, also the resulting introduced purple formazan can be solubilized and quantified by spectrophotometeric methods the assay measures tetra

PROCEDURE:

1. Trypsinization and aspiration of the cells into a 15 ml centrifuge tube. Centrifugation at 300 x g produced a pellet of cells. Utilising DMEM media, the cell density was adjusted so that 200 microliters of suspension contained about 10,000 cells.
2. 200 microliters of the cell slurry were applied to each of the 96 wells in the microtitre plate, and the entire setup was then incubated at 37 °C and 5% CO_2 for 24 hours.
3. The used medium was aspirated after 24 hours. 200 microliters of various test concentrations of test medicines (50,100,150,200, and 250 microliters/ml from stock) were applied to the appropriate well.The plate was then incubated for 24 hours at 37°C and 5% CO_2.

4. The drug-containing media was aspirated when the plates were taken out of the incubator. The plate was then incubated at 37°C and 5% CO2 for 3 hours after 100 microliters of media containing 10% MTT reagent were added to each well to reach a final concentration of 0.5 mg/ml.
5. The crystals were entirely extracted from the culture media without being damaged. The produced formazan was then solubilized using 100 microliter of solubilization (DMSO), which was added, and the plate was gently shaken in a gyratory shaker.olubilise the formed formazan.

6. A microplate reader was used to measure the absorbance at 570 and 630 nm wavelengths.After subtracting the background and the blank, the percentage of growth inhibition was computed, and the dose-response curve for the cell line was used to generate the concentration of test medication required to inhibit cell growth by 50% (IC50).



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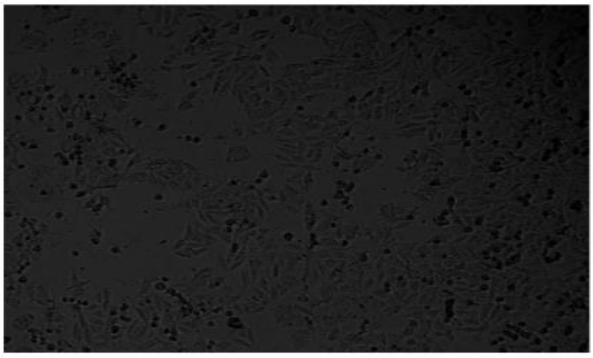

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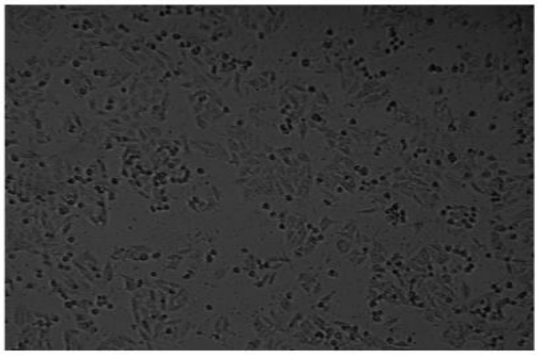

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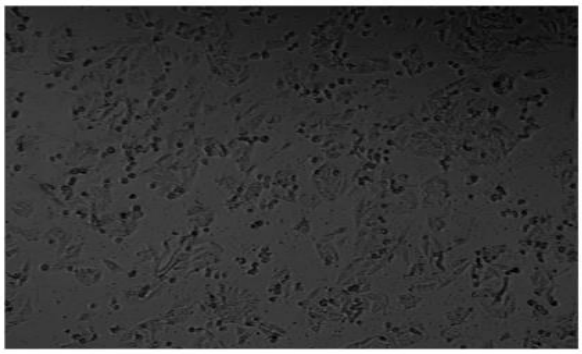

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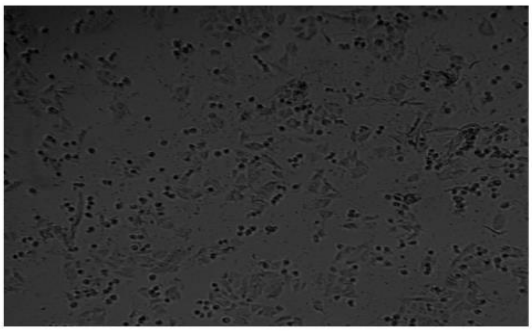

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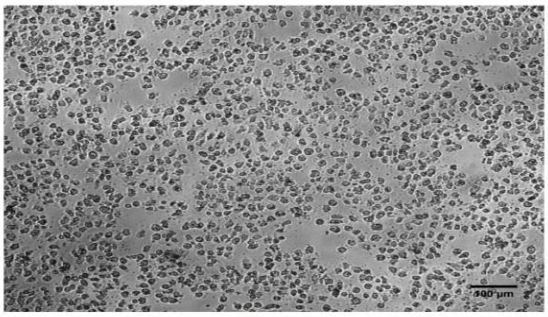
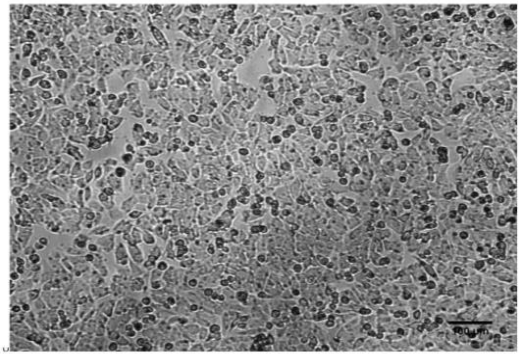


Standard



Untreate

Calculation:

The following formula is used to calculate the inhibition

Percentage inhibition= O.D of treated cells/O.D of positive x 100

Result:

Sample	A375 cell line IC50 (in ug/ml) 24 hr
Methanolic extract	199.45ug/ml

Result =199.45ug/ml

RESULT AND INTERPRETATIONAL ANALYSIS

The test compounds' IC50 values for a 24-hour treatment on the A375 cell line were determined to be:
The values given above are simply predicted values for IC50 values outside of the actual experimental levels used; further testing at higher/lower concentration ranges may be necessary for confirmation.

Phytochemical composition[9].

According to the findings in Table, Euphorbia milli was affirmative for the phytochemicals alkaloids, carbohydrates, flavonoids, saponins, and tannins, but inconclusive for the presence of anthraquinones, glycosides, and steroids. It was discovered that the extracts' antioxidant and radical-scavenging abilities depended on dose, with methanolic extracts having the highest concentration of active components (MeOH> two-fold vs. H2O extracts). It was discovered that the extracts prevented pancreatic cancer cells from proliferating, indicating that they may one day be developed into therapeutic treatments. (MTT) to assess the evaluated extracts' antiproliferative effects on cancer cell lines. The assay relies on the mitochondrial enzyme succinate dehydrogenase to convert the tetrazolium salt (MTT) into formazan blue. Therefore, the MTT assay may be helpful for assessing antiproliferative effects of substances. The MTT assay was used to evaluate cytotoxicity based over the metabolic degradation of MTT.

HeLa cell line (59.61%) than the Em-MeOH extract (52.59%).

Discussion In the current work, the MTT test was used to thoroughly explore the chemopreventive potential of E. milii aerial parts. Cancer cells decreased MTT to produce formazan. The shift in color provided a qualitative indication of the decline.

Using a programme called CalcySynTM (USA), the IC 50 values have been determined. After the MTT experiment utilising the MeOH extracted and its 6 parts in the mediums in an increasing sequence of polarity was finished, an IC50 value for the HeLa cell line was calculated. It was discovered that the Em-C fraction had a better ability to inhibit the

Antimicrobial, anti-inflammatory, and antioxidant capabilities are only a few of the medical advantages that the secondary metabolite provides[10].

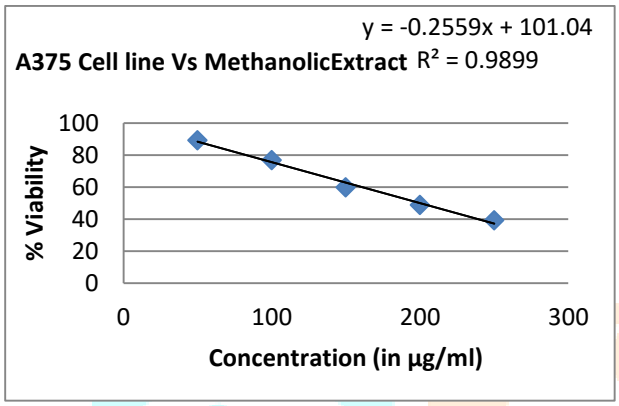
DNA damage, oxidative stress, and chronic inflammation are a few of the pathogenic pathways that link cancer and other chronic diseases. According to Aly and Mahmoud (2013), these diseases can be treated by developing resistance to mutagens and carcinogens as well as by using chemopreventive medicines to halt the disease's course. Surgery and chemotherapy are common treatments for certain disorders, albeit they have not always been completely successful. The majority of anti-tumor medications now utilized in chemotherapy are harmful to immune cells as well as normal cells. Therefore, it's crucial to keep treatment doses as low as possible while also attempting to reduce any negative side effects associated with these medications.

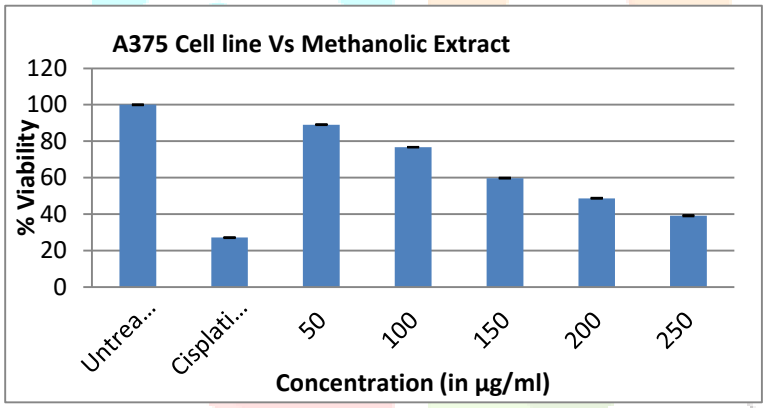
Comparing the methanolic extract of Euphorbia to the reference anticancer medications, our findings revealed that it demonstrated a moderate to significant growth inhibition between 0 and 50 g/ml concentrations. According to our findings, the cytotoxic effect gets stronger as extract concentration rises because succinate dehydrogenase, a mitochondrial enzyme, is active and cleaves the tetrazolium ring, turning MTT into an insoluble purple formazan.Incubation period was related to the genotoxic activity of the Euphorbia triaculeata methanol extract[11].

Table No-I

Phenols	+
Flavanoids	+
Steroids	+
Glycosides	-
Saponins	-
Alkaloida	-

Figures showing concentration and growth





S N	A375 cell line	**Test concentration in (ug/ml)**							
01		BLANK	UNTREATED	CISPLATIN 15UG/ML	50	100	150	200	250
02	Reading 1	0.027	1.515	0.428	1.353	1.169	0.915	0.74	0.616
03	Reading2	0.027	1.525	0.436	1.361	1.176	0.922	0.75	0.607
04	Mean OD	0,027	1.52	0.432	1.357	1.172	0.918	0.75	0.611
05	Mean OD mean blank		1.493	0.405	1.33	1.145	0.891	0.72	0584
06	Standard deviation		0.0070	0.0056	0.005	0.004	0.004	0.06	0.006
07	Standard error		0.005	0.004	0.004	0.003	0.003	0.04	0.004
08	%standard error		0.3348	0.267	0.262	0.234	0.234	0.30	0.301
09	%viability		100	27.126	18.082	76.72	59.71	48.6	39.14
	IC50 =199.45ug/ml								

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