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(RESEARCH ARTICLE)

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Investigation of antimitotic activity in ethanolic leaf extract of herbals: *Euphorbia milii*

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Abstract

Humans are using plants as medicine around from 6000 years and most of the drugs in modern science are plant derived or plant extracts. As per the science we have investigated antimitotic activity in a plant extract. The antimitotic activity of ethanolic extract of *Euphorbia milii* leaves was studied by using seed germination assay. The antimitotic drugs which are formerly developed has the phytochemicals such as alkaloids, flavonoids and saponins. As the phytochemical study of *Euphorbia milii* proved that it has phytochemicals such as carbohydrates, alkaloids, tannins, saponins and phenolic compounds. The ethanolic extract of *Euphorbia milii* leaves has shown a significant amount of antimitotic activity in the seed germination assay. When compared to the methotrexate (0.1 mg/ml), the 40 mg/ml ($p<0.0001$) of the ethanolic extract of *Euphorbia milii* leaves at different doses (10 mg/ml, 20 mg/ml, and 40 mg/ml) shown an exceptional effect or significance. Thus, ethanolic leaf extract of *Euphorbia milii* shows antimitotic activity and can be used in the treatment of cancer. Further evaluations should be done in the future.

Keywords: Ethanolic Leaf Extract; Seed Germination Assay; *Euphorbia Milii;* Anti-Mitotic Activity; Anti-Cancer Activity; Methotrexate; Soxhlation; Phytochemical Screening

1. Introduction

Humans have been using natural products—plants, animals, microbes, and marine organisms—in medicines to treat illnesses since ancient times. Records show that people have been using plants as medicine for at least 60,000 years [1]. In the modern medicine most of the API's or the source of the API are the natural products. In the postgenomic era most of the drugs are natural products [2]. Along with the medicines there is a growth of diseases as well such as cancers, infections etc. For these cancer and infectious diseases 60-70% of medicines are from the sources of natural drugs [3]. 3000 species of plants were used over the time for the treatment of the cancer. [8]

What is cancer? Cell growth that is aberrant is called cancer. Any organ or bodily structure can develop cancer, which is made up of microscopic cells that are unable to stop growing [4]. Over nine million people died from cancer in the past year, making it the second most common cause of death globally [5]. India register more than 11lakhs cases every year while the worldwide it exceeds 14 million [4]. As there is no successful treatment for cancer, there are some treatments like radiotherapy, surgery, and chemotherapy and some advanced technologies like gene therapy, stem cell therapy, natural antioxidants, targeted therapy, photodynamic therapy, nanoparticles, and precision medicine are available to diagnose and treat cancer [6]. Since several different factors may lead to malignancy; For pharmaceutical experts, identifying cancer cells without damaging healthy cells has proven to be a difficult undertaking [7]. For the efficacy in the treatment of the cancer it is convincing to use plant based traditional medicines. [8]

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1.1. Antimitotic activity

Mitotic or mitosis is a process by which cell's nucleus split into two which further divide into two individual cells. Mitosis is the process by which eukaryotic cells acquire chromosome balance. The main mitotic events include

- **Prophase**- this marks the start of the prophase, during which the duplicate chromosomes condense until metaphase. As two sets of centrioles form, the spindle starts to move in the direction of the opposing poles.
- **Prometaphase**- it starts with making the nuclei membrane into small vesicles, these microtubules continuously assemble and disassemble to grow out from the centromere to chromosome kinetochores.
- **Metaphase**- Here the microtubules pull the chromosomes with equal force and it allows the movement of each chromosome to the cell's midplane to form the "metaphase plate".
- **Anaphase-** Each chromosome splits into two identical pieces during anaphase. These pieces then go to the opposing ends of the cell.
- **Telophase**Telophase marks the end of mitosis; to return to an interphase state, the chromosomes decondense and the nuclei and nuclei membrane reform. [9,10]

To inhibit the process of mitosis is known as Antimitotic activity. There are certain drugs which shows antimitotic activity these are also called as anticancer drugs. These anticancer drugs inhibit the DNA synthesis and prevent the cell division. The mechanism of anticancer drugs is they compete with the folic acid and restrict the synthesis of tetrahydrofolic acid (THF) which is required for the DNA synthesis further it effects the cell division [11]. We have been performing the evaluation of antimitotic activity form the herbal extracts of *Euphorbia milii*; there are other herbal extracts which have shown the antimitotic activity such as; *Rotula aquatica,* Muccino pruriens, Asteracantha longifolia and Sphaeranthus indicus. [11,12]

For the evaluation of the antimitotic activity there are some in-vitro methods; seed germination assay and allium cepa root tip assay.in seed germination we evaluate the activity by considering the mitotic index of sprouting seeds [13]. For the evaluation of the *Euphorbia milii* we are performing the seed germination assay. *Euphorbia milii* commonly known as Christ thorn is a decorative plant which as well has its own medicinal uses. It is traditionally used to treat skin irritations, body pains, skin ulcers, snake and scorpion bites [14]. There are several pharmacological actions to *E. milii* include molluscicidal, antifungal, anticancer, anti-inflammatory, anti-helmenthic, and antioxidant qualities. [15,16,17,18,19and20]

1.2. Plant profile

Botanical description

Euphorbia milli or crown of thorns is a shrub native to Madagascar with the name "songosongo". It has woody succulent spiny shrub which grows 1.5 -2 meter tall (5-7 ft.). The spines grow slender and straight upto 3cm long. There are fleshy green leaves are oblong, acute shaped and grow upto 3-3.5cm long and 1.5cm breadth. These flowers are small and cup shaped suspended in the two petal like brats and has different colors such as red, pink and white upto 12mm. Euphorbia grows in warmer climates so grows well in spring and summer but blooms the flowers all year around. [21and22]

Taxonomical classification

- Domain: Eukaryota
- Kingdom: Plantae
- Class: Dicotyledons
- Order: Malpighiales
- Family: Euphorbiaceae
- Genus: Euphorbia
- Species: *Euphorbia milii*

Figure 1 *Euphorbia milii*

Phytochemistry

The phytochemical profile of the *E. milii*- There are vast number of phytochemical constituents in the extracts of *E. milii* - Glycosides, alkaloids, steroids, flavonoids, phenols, carbohydrates, amino acids, tannins and saponins.

Different parts of *Euphorbia milii* has phytochemicals

- Leaf: Glycosides, alkaloids, steroids, flavonoids, phenols, and saponins.
- Stem: Steroids/phytosterols, terpenoids, anthocyanin and betacyanin.
- Root and aerial parts: Tannins, steroids, amino acids, cardiac glycosides, phenolic compounds, carbohydrates, anthraquinones, and alkaloids. [14]

Toxicity

The white coloured sap in the shrub is poisonous as it causes irritation to the skin and eyes when gets contacted. If the sap is ingested it causes severe stomach ache, vomiting and also causes irritation to the throat and mouth. It is harmfull for the domestic animals but slightly harmful for humans. The poisonous ingredient in the euphorbia is found to be "phorbol esters". [21and22]

2. Methods and materials

2.1. Collection and processing of plant materials

Euphorbia milii leaves are available locally and collected from the plants at a bridge of Afzalgunj, Hyderabad, Telangana on October 8th 2024. The leaves were dried in shade for 15 days after completely drying the leaves they were grinded into a coarse powder.

The chemicals which are required for the experiment such as; methotrexate, ethanol, methanol, hexane and ethyl acetate are purchased.

Figure 2 Collection and processing of plant

2.2. Extraction

The extraction process was carried out utilizing the soxhlation method. This technique typically involves loading a thimble composed of thick filter paper into the main chamber of the Soxhlet extractor with a solid substance that contains a portion of the entire plant's dried powder form material. Using ethanol, 30g of plant material is treated to soxhlet extraction until ten to fifteen solvent cycles are completed. Following extraction, the extracted component is obtained by removing the solvent, usually using a rotary evaporator. Additionally, their respective yield percentages are computed. [13]

$$\text{Percentage yield \%(w/w)} = \frac{\text{weight of extract}}{\text{weight of drug taken}} \text{X } 100$$

Figure 3 Soxhlet extraction

Figure 4 Ethanolic leaf extracts of solvents

2.3. Extraction values:

In order to investigate the distribution of different elements in the extract of Euphorbia milii leaves, the extractive values were recorded in several solvents. In a glass-stoppered conical flask, 5.0 g of coarsely ground, air-dried material was precisely weighed. It was macerated with 100 mL of the solvent for 6 hours, shaking constantly, and then left to stand for 18 hours. To ensure that no solvent was lost, the mixture was filtered quickly. A tared thin porcelain dish was filled with 25 milliliters of the filtrate, which was then dried by evaporation on a water bath. Following six hours of drying at 105°C and thirty minutes of cooling in a desiccator, the residue was promptly weighed and the percentage w/w of extractive was computed in relation to the air-dried medication. [13]

$$\text{Extractive value } \% = \frac{W2 - W1}{\text{weight of the drug taken}} X\ 100$$

- Where,
- W_1=weight of empty dish
- W_2=weight of dish + residue

Figure 5 Solvent extracts

2.4. Phytochemical screening

Table 1 Phytochemical screening [23and24]

Test for alkaloids:		
Test	**Procedure**	**positive results**
Dragendroff's test	1-2 ml of Dragendroff's reagent with a few milliliters of filtrate	A precipitate of reddish-brown
Mayer's test	A few milliliters of filtrate with one or two drops of Mayer's reagent	A creamy yellow/white precipitate
Wagner's test	A few milliliters of filtrate plus one or two drops of Wagner's reagent	A precipitate of reddish-brown
Hagner's test	1-2 ml of Hager's reagent with a few milliliters of filtrate	A creamy white precipitate
Test for amino acids:		
Ninhydrin test	2 ml of filtrate with two drops of solution of Ninhydrin	Purple coloured solution
TEST FOR REDUCING SUGARS:		
Benedicts reagent test	Boil for two minutes with 0.5 ml of filtrate and 0.5 ml of Benedict's reagent.	Green/yellow/red colour
Test for proteins:		
Biuret test	1 drop of 2% copper sulphate sol, 1 milliliter of filtrate, and 1 milliliter of 95% ethanol + KOH pellets	pink coloured solution
Test for carbohydrates:		
Molish test	1 ml conc. H_2SO_4 + 2 ml filtrate + 2 drops of alcoholic α-napthol	A violet ring
Benedicts test	Boil for two minutes with 0.5 ml of filtrate and 0.5 ml of Benedict's reagent.	
Fehling's test	1 ml of Fehling's solutions A and B, 1 mL of filtrate, and 1 mL of boiling water	A red precipitate
Test for flavonoids:		
Shinoda test	Extract is dissolved in 5ml alcohol +Fragments of magnesium ribbon + few drops of conc. HCl	A pink crimson coloured solution
Alkaline reagent test	Plant extract + 10% ammonium hydroxide solution.	A yellow fluorescence
Test for saponins:		
Foam formation test	0.3g of extract + 6ml of distilled water (vigorously shake)	Foam formation
Hemolysis	Filtrate + 1ml of 1.8% NaCl solution + 2-3 drops of blood. (observe under microscope)	Hemolysis formation
Test for phenolic compounds		
Ferric chloride test	Add a few drops of 5% ferric chloride sol to the extracted aqueous solution.	The colour is bark green or blue black.

Gelatin test	5 mL of distilled water, 1% gelatin solution, and 10% NaCl are used to dissolve the plant extract.	A white precipitate
Test for steroids and triterpinoids		
Lieberman-Buchard test	Filtrate plus a few drops of boiled and cooled acetic anhydride plus concentrated H2SO4	Two green layers on top and a brown ring at the intersection (steroids) Deep red colour at the bottom
Salkowski test	a filter with a few drops of concentrated H2SO4	layer of golden yellow at the bottom
Test for tannins:		
Gelatin test	Plant extract in distilled water + 1%gelatin solution + 10% NaCl	A precipitate that is white
10% sodium hydroxide test	0.4 mL plant extract, 4 mL 10% NaOH, and a good shake	Emulsion formation

2.5. Seed germination assay method

This assay was evaluated by the *Phaseolus radiatus* commonly known as green gram. Here these green gram seeds are weighed individually and separated into 6 groups and vials are kept ready for the procedure.

Procedure

Green grams seeds are collected and weighed individually. A concentration of 10 mg/ml, 20 mg/ml and 40 mg/ml should be used to prepare the extract solution. Additionally, make water the control and methotrexate the standard solution. Now place similarly weighed green gram seeds in the vials with the samples containing different concentrations. In order to inhibit water, these vials are left at room temperature for a whole day, during which time they are monitored and photographed. Again, placed for another 24 hrs and observed and lastly for 72 hrs they were left at room temperature and observed the sprouting along with photographs. [13and25]

After 24 – 72 hrs of drug treatment green gram seeds were dried with a tissue and weighed.

$$\text{Percentage of inhibition (\%)} = \frac{\text{wt D} - \text{wt E}}{\text{wt D} - \text{wt M}} \text{ x } 100$$

Where

- wt D = seed weight with distilled water
- wt E = seed weight with extract sample
- wt M = seed weight with methotrexate

Figure 6 Seed germination assay

3. Results and discussion

3.1. Phytochemical analysis

Table 2 Results of phytochemical screening of extracts of Euphorbia milii leaves

Phytoconstituent	Test	Hexane	Ethylacetate	Methanol	Ethanol
Alkaloid	Dragondroff	✓	✓	×	×
	Mayer's	✓	×	×	×
	Wagner's	×	×	✓	✓
	Hager's	×	×	×	×
Amino acid	Ninhydrin	×	×	×	×
Carbohydrates	Molisch's	×	×	✓	×
Flavonoids	Alkaline reagent	✓	×	✓	✓
Saponins	Forth formation	×	×	✓	✓
Phenolic compound	Ferric chloride	×	×	×	×
	Biuret	×	×	×	×
Phenolic compounds	Shinoda	✓	✓	✓	✓
	Keller kiliani	✓	×	×	×
	Picric acid	✓	×	×	✓

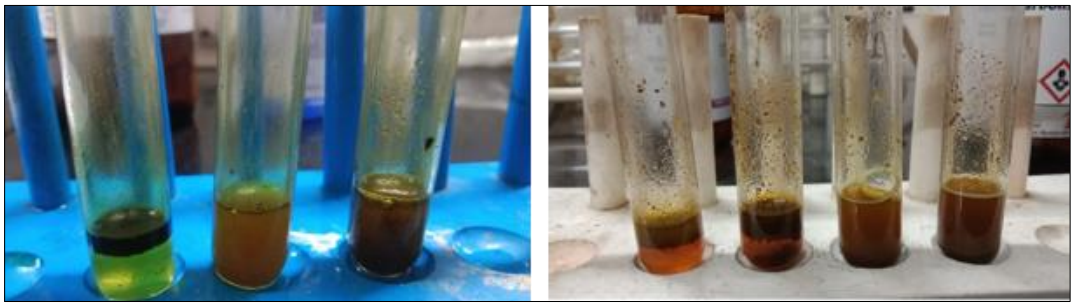

Figure 7 Phytochemical screening

Figure 8 Seeds after the treatment

3.2. Seed germination assay

The presence of carbohydrates, flavonoids, saponins, alkaloids, and phenolic chemicals was the outcome of the phytochemical screening in the different extracts of the *Euphorbia milii* leaves such as ethylacetate extract, hexane extract, methanol and ethanol extracts. Compared to the other extracts, the ethanol extract contains a higher concentration of alkaloids, flavonoids, and phenolic chemicals. Majority of the anticancer drugs consists rich amount of alkaloids as the ethanolic extract. These alkaloids help in development of chemotherapeutics as well so the ethanolic leaf extract of *Euphorbia milii* may show the antimitotic activity

Table 3 Values of seed germination assay

	DOSE (mg/ml)	Weights(g) Mean ± SEM after treatment		
		24 Hrs	48 Hrs	72 Hrs
Control	0	0.139 ±0.0009***	0.180 ± 0.013***	0.247 ± 0.0315***
Standard	0.1	0.182 ± 0.006***	0.171 ± 0.007***	0.167 ± 0.014***
MLE	10	0.123 ± 0.004***	0.113 ± 0.008***	0.089± 0.002***
MLE	20	0.120 ± 0.006***	0.107 ± 0.003***	0.086 ± 0.005***
MLE	40	0.110 ± 0.003***	0.104 ± 0.005***	0.064 ± 0.002***

The table represents the results of seed germination assay, the * indicates p significance value. where, **** = p<0.0001, *** = p<0.001

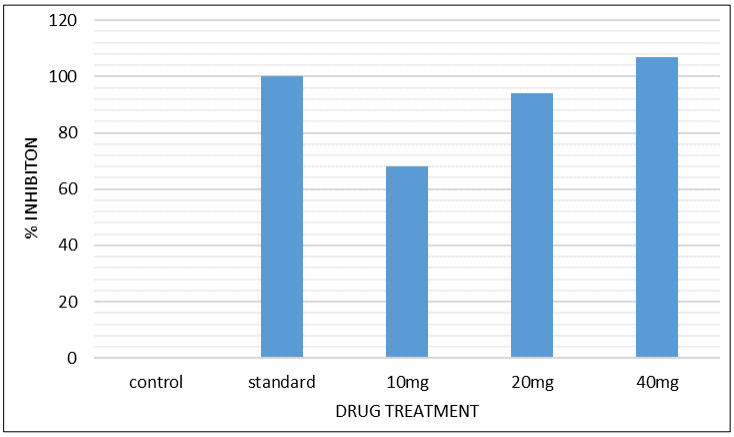

Figure 9 % Inhibition of assay at 72 hrs

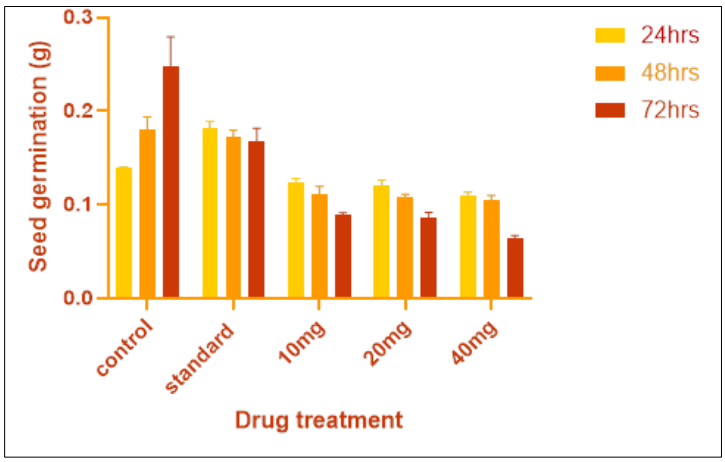

Figure 10 Seed germination vs drug treatment

4. Conclusion

According to our phytochemical screening, the ethanolic leaf extract has a greater quantity of phytochemicals than other solvents. Significant amounts of phytochemicals, including saponins, tannins, flavonoids, alkaloids, and phenolic compounds, are present in the ethanolic leaf extract of Euphorbia milii. Ethanolic leaf extract of *Euphorbia milii* has shown significant inhibition of seed germination resulting in the presence of antimitotic activity. Ethanolic extracts of concentrations 10mg/ml, 20 mg/ml and 40mg/ml shown significance ($p<0.0001$) towards the standard (0.1 mg/ml) ($p< 0.0001$). Among these concentrations 40 mg/ml shown more activity than the other concentrations and found significant. As we conclude that the ethanolic leaf extract of *Euphorbia milii* shows a significant amount of antimitotic activity and can be used in the treatment of cancer. Further evaluations on ethanolic extract of *Euphorbia milii* leaves should be done.

Compliance with ethical standards

Disclosure of conflict of interest

No potential competing interest.

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