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Research Article

Evaluation Of Antimitotic Activity of Phytochemicals Present In Extracts Of *Costus Igneus* Leaves

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ABSTRACT

Costus Igneus known as Insulin plant is one such traditional plant which is getting global acceptance and is now widely used as an ayurvedic medicinal herb.The present study investigates the Antimitotic activity of the plant leaves. The leaves of plant were collected, and the aqueous and ethanolic extracts were prepared using soxhlet apparatus. These extracts were tested for phytochemicals and showed the presence of alkaloids and flavonoids, anthraquinones, taninns, phenolic compounds, terpenoids, saponins and proteins and absences of carbohydrates. The Antimitotic activity of ethanolic extract and aqueous extract of costus igneus leaves were evaluated using Seed germination method. The costus igneus leaves extracts is capable of inhibiting the germination of green gram seeds as result indicates 55.3% and 63.5% inhibition by ethanolic extract at 5mg/ml and 10mg/ml respectively whereas aqueous extract showed 69.4% and 70.5% inhibition at 5mg/ml and 10mg/ml when compared with the percentage inhibition of standard paclitaxel which showed 96.6% inhibition indicates that the ethanolic and aqueous extract of costus igneus is having antimitotic activity. Hence this indicates that plant leaves having alkaloids and flavonoids and absence of carbohydrates show antimitotic activity. The aqueous extract of costus igneus leaves showed more potent antimitotic activity when compare to the ethanolic extract.Further studies can be carried out to determine the most effective chemical compound responsible for anti mitotic activity in this plant to treat different stages of cancer.

INTRODUCTION

CELL CYCLE - A cell cycle is a series of events that a cell passes through from the time of cell division until it reproduces its replica.

-It is the growth and division of single cell into daughter cells and duplication.

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-In prokaryotic cells, the cell cycle occurs via a process termed as binary fission. In eukaryotic cells, the cell cycle can be divided in two periods:-

a) Interface
b) Mitosis

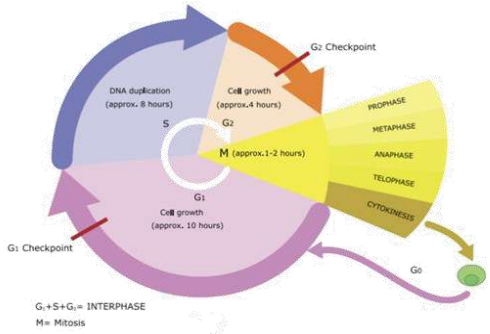


Figure 1: Cell cycle

INTER PHASE
G1 (pre-synthetic phase)
S (DNA synthesis)
G2 (pre-mitotic phase)
CELL DIVISION

a) Interphase- During this phase the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA.
b) Mitosis (M)-phase- During which the cell splits itself into two distinct cells. The duration of the cell cycle varies from hours to years. A typical human cell has duration of 24h. (**Error! Reference source not found.**)

INTERPHASE

Go PHASE:

It is the resting phase.

In these cells cyclin D is in decreased concentration. Rb protein is in hypo-phosphorylated (active form). Hence, holds the cell cycle at check point I by inhibiting the expression of several transcription proteins (E2F) that codes cyclins A and E necessary for cycle progression. Growth factor stimulation takes the Go cells to G1 phase.

G1 PHASE:

G stands for gap. It is the first phase within the interphase. It is also called the growth phase. This phase is the gap period between a mitotic phase and the S phase of the cycle. Cell is preparing for S phase .This period starts immediately after division. The daughter cells grow and size during this phase.

S PHASE:

Cyclin E/cdk and cyclin A/cdk regulate the processes in phase S. By phosphorylating and activating proteins and enzymes that are involved in DNA synthesis.S stands for synthesis. During this phase DNA synthesis occurs. The DNA molecule duplicates. All the chromosomes have been replicated. This period lasts for 35 to 40% of interphase.

G2 PHASE:

Pre-mitotic phase.

The G_2 phase is the gap period between S-phase and mitotic (M) phase of a cell cycle. It is the second growth phase. It is a period of rapid cell growth and protein synthesis which the cell ready itself for mitosis. The nucleus increases in volume. Metabolic activities essential for cell division, occur during this phase, mRNA, tRNA and RNA synthesis also occur. (**Error! Reference source not found.**)

M PHASE:

G_2 cells are divided into two daughter cells which may enter the cycle again at G1 phase or come out of the cycle to Go phase. Mitosis is the distribution of the two sets of chromosomes into two separate and equal nuclei.This is the division phase. During this phase the cell divides. This phase has a short duration. A typical human cell cycle has duration of 24hours, of these the M phase has duration of 45 to 60min. This phase has two sub-phases called karyokinesis and cytokinesis (**Error! Reference source not found.**)

- Karyokinesis refers to the cell division of nucleus into two daughter nuclei. It has 4 sub-stages, namely prophase, metaphase, anaphase and telophase.
- Cytokinesis refers to the cell division of the cytoplasm resulting in two daughter cells.

1) Prophase- mitotic spindle formation
2) Metaphase- metaphase plate
3) Anaphase- mitotic apparatus
4) Telophase- cytokinesis (**Error! Reference source not found.**)

Prophase:

1. Chromatid: Each of the original chromosomes is called chromatid
2. Centromere: - Two chromatids are joined together.
3. Mitotic apparatus: Two centrioles separated, by mitotic spindle, nuclear Envelope disappears.

Metaphase: -

The chromatids align on the centre of the spindle attached by their Centromere. **(Error! Reference source not found.)**

Anaphase-:

The Centromeres separate and one of each pair of sister chromatids at the end of spindle which forms mitotic spindle contract.

Telophase: -

The mitotic spindle disappears, the chromosomes uncoil and the nuclear envelope reforms.

Cytokinesis

The cytoplasm intracellular organelles and plasma membrane split forming two identical daughter cells. **(Error! Reference source not found.)**

PHYTOCHEMICALS: Medicinal plants have bioactive compounds known as phytochemicals that plays an important role in curing and healing of various diseases in human. processes.

The word 'phyto' is derived from the Greek word phyto which means plant. These phytochemicals have antifungal, antibacterial, antioxidant, anti-inflammation activities.

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds etc. **(Error! Reference source not found.)** Plants produce these chemicals to protect itself, but recent research demonstrates that they can protect humans against diseases. Most of these active plant's phytochemicals can be classified into sub-groups according to their chemical structure, which includes the following:-**(Error! Reference source not found.)**

Phytochemicals	Medicinal Uses
Alkaloids	Analgesic, anti-inflammatory, Anti-helminthic
Carbohydrates	Antithrombic anticoagulant
Anthraquinone glycosides	Anticancer, laxatives,Antimicrobial
Cardiac glycosides	Treatment of cardio vascular diseases.
Saponins	Expectorant, Carminative
Sterols	Lipid lowering property
Phenols	Antioxidants, Anti-inflammatory
Flavonoids	Antioxidants.
Tannins	Astringent, Antiseptic

Table: 1 Phytochemicals and its Medicinal Uses

CHECK POINTS

The control of cell division: The control of cell division depends on specific cyclins (G- cyclin and M-cyclin) and cyclin-dependent protein kinases (Cdks). The Cdks are switched on and off by cyclins, which are synthesized or broken-down during cell cycle. The joining of specific cyclin and cdk molecule trigger various events that control cell division.Tumor Supressor Genes Defective genes that regulate the cell cycle or apoptosis are associated with many diseases. The loss or inactivation of tumour suppressor genes, whose normal function is to inhibit cell proliferation, causes some types of cancer Two proteins produced by tumour suppressor genes are the Rb protein which normally stops cells from passing through the G, checkpoint, and the p protein, which responds to DNA damage by arresting the cell cycle at the G CLINICA checkpoint or triggering cell death by apoptosis.

For this reason, the p53 gene is nicknamed "the guardian angel of the genome."**(Error! Reference source not found.)**

PHYTOCHEMICALS AS ANTICANCER DRUGS

The first clinically formulated and used phytochemicals were isolated from the plant Catharanthus roesus and named as Vinblastine and Vincristine.

They were used in the treatment of breast, lung and blood cancers along with other anticancer drugs in combination. Paclitaxel isolated from the bark of the tree Taxus brevifolia, is one of the most widely

used and effective phytochemical compounds against ovarian, cervical, breast and pancreatic cancer. The synthetic derivtives of camptothecin are topotecan and irinotecan isolated from camptotheca acuminata used for treating colorectal cancer and ovarian cancer. The given table indicates the different tests to be performed for identification of phytochemicals. (**Error! Reference source not found.**)

Phytochemials	Tests	Reactants	Inference
Alkaloids	Dragendorff's test	Extract+Solution of potassium bismuth iodide	A red precipitate is formed
	Wagner's test	Extract+Iodine in potassium iodide.	A brown precipitate is formed
	Mayers test	Extract+Potassium mercuric iodide	A yellow precipitate is formed
	Hager's test	Extract+Saturated picric acid solution	A yellow precipitate is formed
Saponins	Froth test	Extract+Water	Formation of 1 cm layer of foam
Phenols	Ferric chloride test	Extract+Ferric chloride solution.	Formation of bluish black colour
Tannins	Gelatin test	Extract+1%gelatin solution, Nacl	Formation of white precipitate
Flavonoids	Alkaline reagent test	Extract+NaOH solution	Formation of intense yellow colour which becomes colourless on addition of dilute acid
	Shinoda test	Extract+Mg turnings, conc Hcl	Formation of pink colour
Glycosides	Modified borntagers test	Extract+Ferric chloride, benzene, ammonia solution	Formation of rose pink colour in the ammonical layer
Cardiac glycosides	Legal test	Extract+Sodium nitroprusside in pyridine, NaOH	Formation of pink to blood red colour
Terpenoids	Liberman burchard test	Extract+Acetic anhydride,conc H2so4	Formation of deep red colour
Steroids	Salkowski test	Extract+conc Sulphuric acid	Formation of red colour at the lower layer

Table 2: Different tests to be performed for identification of phytochemicals

Drugs inhibiting mitotic phase

Vinca alkaloids: Vincristine Vinblastine Vinleuroside

Taxol derivatives: Paclitaxel Docetaxel

colchicine

Vinca alkaloids:

It is dimeric indole alkaloid obtained from Catharanthus Roseus, family Apocynaceae. Indole containing moiety known as Cathranthine Indoline containing moiety known as Vindoline. (**Error! Reference source not found.**)

E.g., Vincristine, Vinblastine.

Vinleuroside.

Mechanism of action: It causes mitotic arrest by promoting the dissolution of microtubule in cell.

Use: Acute leukemia, Hodkin's Disease, lymphocyte lymphoma, Breast carcinoma.

Heterocyclic Amine as an anti-cancer agent (**Error! Reference source not found.**)

Isolated from Chinese tree Camptotheca acuminate.

E.g., Camptothecin, Hydroxy Camptothecin Use: Colorectal and Ovarian cancer.

Lactone (Alkaloids) as an anti-cancer agent

Podophylotoxin and Deoxypodophylotoxin are obtained from Himalaya shrub Podophyllam Emodi and P.Peltatum.

Mechainism of action: It inhibits mitosis by destroying the structural organization of mitotic apparatus.

Taxol derivatives

E.g., Paclitaxel and Docetaxel (**Error! Reference source not found.**)

It is obtained from western yew tree Taxus Bravifolia.

M/A: It binds with ß-Tubulin subunit of microtubule and appears to antagonize the disassembly of the key cytoskeletal protein and arrest in mitosis follows.

Colchicine:

Main use: Terminating acute attack of Gout M/A: It inhibits mitosis at metaphase by disorienting the organization of spindle.

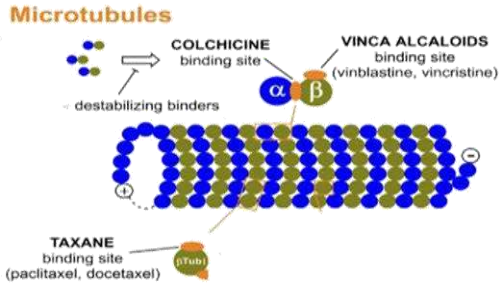


Fig 3: Mechanism of action of drugs inhibiting Mitotic phase

PLANT PROFILE

COSTUS IGNEUS [INSULIN PLANT]

TAXONOMIC CLASSIFICATION

Kingdom: Plantae

Order: Zingiberales

Genus: Chamaecostus

Family: Costaceae

Scientific name: Chamaecostus cuspidate

Species: C.cuspidatus

MORPHOLOGY

It is a perennial upright spreading plant reaching about 2feet tall with the tallest stems falling over and lying on the ground.

Leaves are simple, alternate, entire oblong, evergreen 4-8 inches in length with parallel venation. The large smooth dark green leaves of the tropical ever green have light purple under sides and are spirally arranged around stems forming attractive from underground root stocks. Flower 1.5 inch diameter, orange flower cone like head at the tips of branches[(Error! Reference source not found.)]

2. Commonly called fiery costus or spiral flag, is a species of herbaceous plant belonging to the family costaceae. The native of this plant is Eastern Brazil and America. The herb is widely used in Ayurveda and Herbal medicine. It is primarily used in the treatment of diabetes because it has anti-diabetic properties.
3. The leaves of this plant have corosolic acid that helps to generate insulin to treat diabetes.
4. It strengthens the beta cells for buildup of insulin in the human body that's why it is called insulin plant in India.
5. It is a perennial plant that reaches upto 2(ft) with spirally arranged leaves and the flowers of the insulin plant are very attractive [(Error! Reference source not found.)]
6. Apart from the antidiabetic property some reasearches have proved thar thid herb is boosted with various pharmacological activities such as diuretics, hypolipidimic, anti-microbial, Antioxidant and anticancerous.
7. In addition the research also reveals the presence of various phytochemicals such as triterpenoids, carbohydrates, alkaloids, proteins, saponins, tannins and flavonoids [(Error! Reference source not found.)]

Costus belongs to the family Costaceae, commonly known as insulin plant in India because its leaves help to build up insulin in the human body. Since oral hypoglycemic agents possess various side effects, there is a growing demand for herbal remedies for the treatment of diabetes mellitus.

Many plant preparations are used in folklore and traditional system of medicine to manage diabetes mellitus. Investigation on new oral hypoglycemic compounds from medicinal plants will set a milestone for the development of pharmaceutical

entities or as a dietary adjunct to existing therapies in the future. Insulin plant is one such traditional plant which is getting global acceptance nowadays and is now widely used as an ayurvedic medicinal herb. Consumption of the leaves are believed to lower blood glucose levels, and diabetics who consumed the leaves of this plant said to have a fall in their blood glucose levels. Insulin plant is native to Southeast Asia, especially on the Greater Sunda Islands in Indonesia. It is relatively a new entrant to India and is being grown as an ornamental plant in Kerala. In the Ayurvedic system of medicine, diabetes is traditionally treated by chewing the plant leaves for a period of one month to get a controlled blood glucose level. Costus igneus N.E. Br. is a perennial, upright, tropical evergreen plant belongs to the family Costaceae. Possesses evergreen leaves which are simple, alternate, entire and oblong, having 4-8 inches length with parallel venation.

The large, smooth, dark greens leaves possess light purple undersides and are spirally arranged around stems, forming attractive, arching clumps arising from underground rootstocks. It reaches a height of about 60cm with the tallest stems falling over and lying on the ground. Beautiful orange flowers are produced in the warm months having a 2.5-12.5cm diameter, appears on cone-like heads at the tips of branches2. Propagation of insulin plant is by stem cutting1.

Common names: Fiery Costus, Spiral flag, Insulin plant, Step ladder **(Error! Reference source not found.)**

TAXANOMIC POSITION

Botanical Name	Costus igneus
Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Viridaeplantae
Phylum	Tracheophyta
Subphylum	Euphylophitina
Infraphylum	Radiotopses
Class	Liliopsida
Subclass	Commelinidae
SuperOrder	Zingiberane
Order	Zingiberales
Family	Costaceae
SubFamily	Asteroideae
Tribe	Coriopsidae
Genus	Costus
Specific epithet	Igneus

MATERIALS AND METHODS

Costus igneus leaves were collected from the RBVRR womens college of pharmacy, Hyderabad. Chemicals such as wagner's reagent, chloroform, 2% H2SO4,concentrated sulphuric acid,10%lead acetate, Benedicts reagent,0.1% ferric chloride, Fehlings solution, dilute NaOH, 2% HCL,10% ammonia,10% HCL, distilled water, Ethyl alcohol are provided by the management of the college.

PREPARATION OF EXTRACT

Fresh leaves of Costus igneus were collected and washed under running tap water followed by distilled water. The leaves cut into pieces, air dried and powdered. 25 gram of powdered sample were taken and extracted with 300ml of ethanol in Soxhlet apparatus for 12 hours of time. The crude extract was filtered and the solvents were further condensed using rotary evaporator. The crude extract was stored at room temperature in airtight container for further analysis. A portion of the extract was used for evaluation.

SOXHLET APPARATUS

The crude substance is placed in a thimble-shaped filter paper which is then kept in a glass cylinder. This cylinder is provided with a siphon tube and an inlet tube. A water condenser is attached to the cylinder at the top. This entire assembly is fitted into the neck of a round bottom flask containing the solvent. **(Error! Reference source not found.)**

Fig 4: Soxhlet extraction for ethanolic extract

Soxhlet extraction procedure

The flask is heated in a water bath or sand bath. The solvent vapors reach the cylinder through the inlet tube and conduce on passing upward into the condenser. The condensed solvent comes in contact with the crude organic substance and dissolves it. As soon as the solution reaches the top end of the siphon tube. In this way, a continuous supply of solvent vapors is maintained in the cylinder, and the dissolved organic compound flows back into the flask. Finally, the heating is stopped and the solution in the flask is distilled to recover the solvent, While the organic compound is left behind **(Error! Reference source not found.)**

Fig 5: Soxhlet extraction for Aqueous extract

The extracts were filtered and concentrated using a rotary evaporator at70 °C and then used for further analysis.

CHEMICAL TESTS

a) Phytochemical screening: Chemical test is carried out on the prepared extract using standard procedure to identify the constituents.

b) Procedure for alkaloids: 2ml of extract is taken and added 2ml of wagner's reagent a brownish precipitate indicates the presence of alkaloids.

c) Cardiac glycosides: 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer.Deep reddish brown colour at the inter face of steroid ring indicates the presence of cardiac glycosides.

d) Flavonoids: 2ml of extract is treated with 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.

e) Saponins: 2ml of extract is dissolved with 2ml of benedicts reagent. Blue black ppt indicates the presence of saponins.

f) Tanins: 2ml of extract with 0.1% of ferric chloride. Brownish green indicates the presence of tannins.

g) Terpenoids: (salkowski test) 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour indicates the presence of terpenoids.

h) Anthraquinones: 1ml of extract is boiled with 10%HCL for few minutes in a water bath. It is filtered and allowed to cool. Equal volumeof CHCL3 is added to the filterate few drops of 10% Ammonia is added to the mixture and heat. Formation of rose pink colour indicates the presence of reducing sugars.

i) Reducing sugars: The extract was shaken with distilled water and filtered. The filterate was boiled with Fehlings solution A and B for few minutes an orange red ppt indicates the presence of reducing sugars.

j) Glycosides: The extract was hydrolysed with HCL solution and neutralized with NAOH solution. A few drops of Fehlings solution A and B are added, red ppt indicates the presence of glycosides.

k) Phlobatanins: The extract is dissolved in distilled water and filtered. The filterate is

boiled with 2% HCL solution. Red precipitate shows the presence of phlobatanins.

l) Carbohydrates: The extract was mixed with 2 drops of Alpha-Naphthol and 1ml of conc h2so4. it indicates absence of carbohydrates

m) Steroids: 1 ml of extract was mixed with 2ml of chloroform and concentrated H2SO4 was added it indicates absence of steroids.

ANTIMITOTIC ACTIVITY

Seed Germination Assay

Seed germination assay was evaluated by using green gram seeds [Vigana Radiata]

PROCEDURE FOR EVALUATING ANTIMITOTIC ACTIVITY OF PREPARED ETHANOLIC EXTRACT OF COSTUS IGNEUS LEAVES:

Experimental design:

Green gram seeds were divided in to 4 groups and each group containing 10 green gram seeds.

Procedure:

Green gram seeds were collected from the local market and each seed weighed individually.5mg/ml, 10 mg/ml concentrations of seed coat with ethanolic extracts were prepared. Paclitaxel was used as standard drug. Distilled water was used as a control. Equal weights of seeds were added in the sample vials containing different concentrations. The test tubes were left at room temperature for 24hrs for inhibitions of water. After 24hrs and 72hrs drug treatment dried on dry tissue paper and weighed. The time of sprouting was extended to 72hrs and photographs were taken. [39]

The percentage inhibition is calculated

Percentage of inhibition=

The percentage weight inhibition of the seeds in P, ET1, ET2 was calculated.

Percentage inhibition in standard = [(Weight of seeds in C-Weight of seeds in M)/Weight of seeds in C] ×100

Where, C= control and P=standard

Percentage inhibition in ET1(5mg/ml) = [(Weight of seeds in C-Weight of seeds in ET1)/Weight of seeds in C] ×100

Where, C= control and ET1=Test(5mg/ml)

Percentage inhibition in ET2(10mg/ml) = [(Weight of seeds in C-Weight of seeds in ET2)/Weight of seeds in C]×100

Where, C= control and ET2=Test(10mg/ml) [40]

PROCEDURE FOR EVALUATING ANTIMITOTIC ACTIVITY OF PREPARED AQUEOUS EXTRACT OF COSTUS IGNEUS LEAVES:

Experimental design:

Green gram seeds were divided in to 4 groups and each group containing 10 green gram seeds.

Procedure:

Green gram seeds were collected from the local market and each seed weighed individually.5mg/ml, 10 mg/ml concentrations of seed coat extracts were prepared. Methotrexate was used as standard drug. Distilled water was used as a control. Equal weights of seeds were added in the sample vials containing different concentrations. The test tubes were left at room temperature for 24hrs for inhibitions of water.

After 24hrs and 72hrs drug treatment dried on dry tissue paper and weighed. The time of sprouting was extended to 72hrs and photographs were taken. [39]

The percentage inhibition is calculated

The percentage weight inhibition of the seeds in P, AT1, AT2 was calculated.

Percentage inhibition in standard =

[(Weight of seeds in C-Weight of seeds in P)/Weight of seeds in C]×100

Where,C= control and P=standard

Percentage inhibition in AT1(5mg/ml)=

[(Weight of seeds in C-Weight of seeds in AT1)/Weight of seeds in C] ×100

Where,C= control and AT1=Test(5mg/ml)

Percentage inhibition in AT2(10mg/ml)=

[(Weight of seeds in C-Weight of seeds in AT2)/Weight of seeds in C]×100
Where,C= control and AT2=Test(10mg/ml) [40]

RESULTS

Table showing results of Phyto chemical Analysis Costus igneus.

S. N	Constituents	Phytochemical test	Ethanol	Aqueous
1	Tanins	Fecl3 Test	+	+
2	Anthraquinones	Bromine Test	+	+
3	Flavanoids	Shinoda Test	+	+
4	Alkaloids	Dragendroffs Test	+	+
5	Terpenoids	Liebermann burchard Test	+	+
6	Saponins	Foam Test	+	+
7	Cardiac glycosides	Raymonds Test	+	+
8	Glycosides	Borntragers Test	+	+
9	Phlobatanins	Alkaline Test	+	+
10	Steroids	Salkowskis Test	-	-
11	Phenols	Litmus Test	+	+
12	Aminoacids	Sakaguchi Test	+	+
13	Proteins	Buret Test	+	+
14	Carbohydrates	Molischs Test	-	-

Table 3: Results of Phytochemical Analysis of Costus igneus

EVALUATION OF ANTIMITOTIC ACTIVITY OF ETHANOLIC EXTRACT

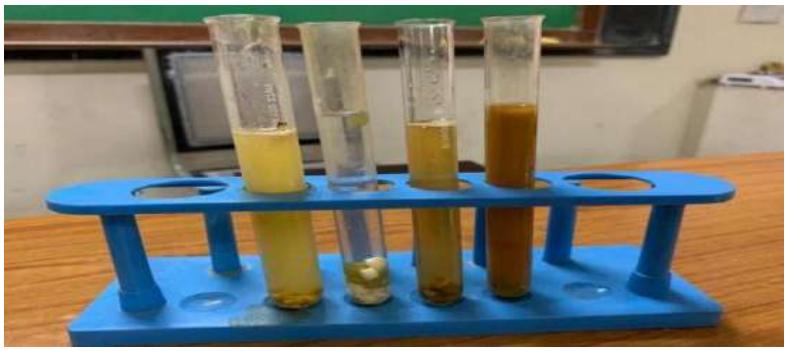


Fig 6: Antimitotic activity of ethanolic extract

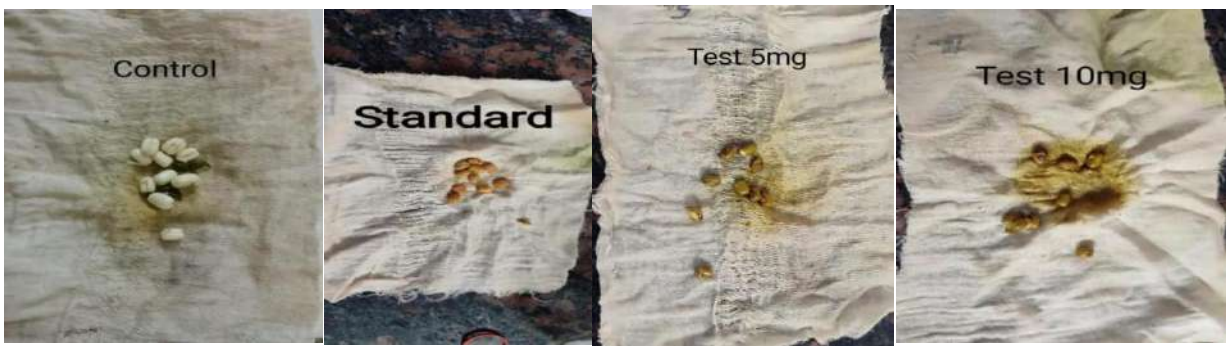
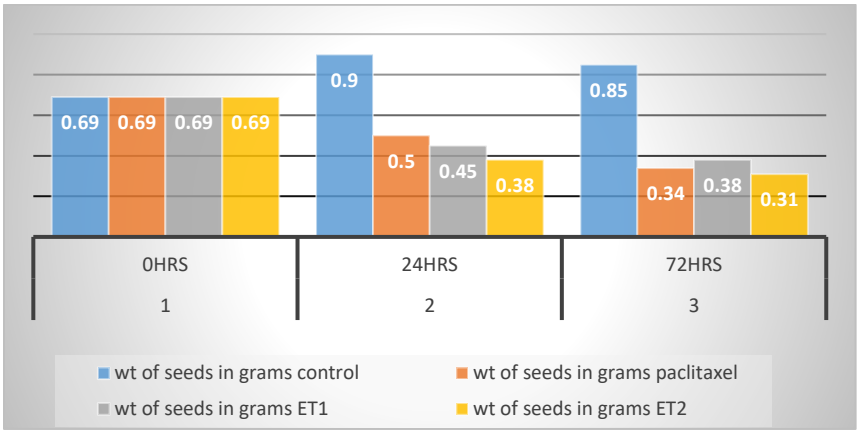


Figure 7: Antimitotic activity of Ethanolic extract

RESULTS OF ANTIMITOTIC ACTIVITY BY SEED GERMINATION METHOD

Groups	Time	Wt Of Seeds In Grams			
		CONTROL	PACLITAXEL	ET1	ET2
1	0hrs	0.69	0.69	0.69	0.69
2	24hrs	0.9	0.50	0.45	0.38
3	72hrs	0.85	0.34	0.38	0.31
Mean		0.81	0.62	0.50	0.46
Standard deviation		0.10	0.19	0.16	0.20
percentage inhibition			96.60%	55.3%	63.5%

Table 4: Results of antimitotic activity by seed germination method of Ethanolic Extract



EVALUATION OF ANTIMITOTIC ACTIVITY OF AQUEOUS EXTRACT

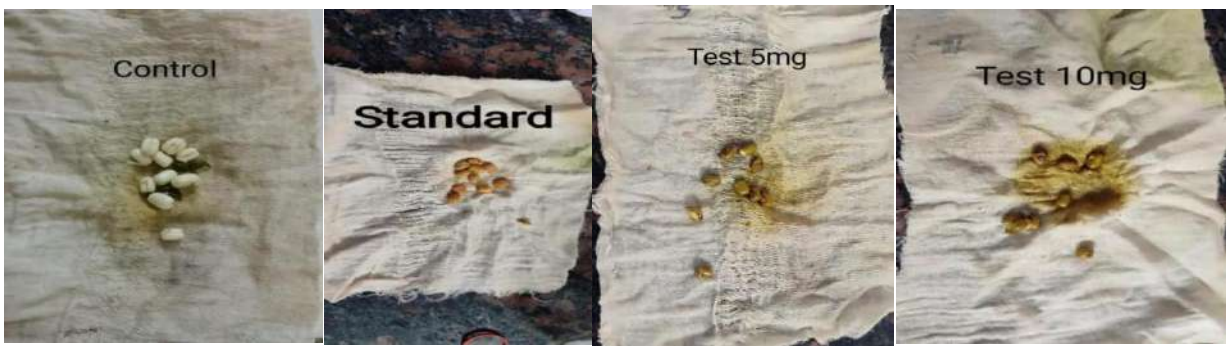


Fig 8: Antimitotic activity of aqueous extract

Seed Germination Assay

Seed germination assay was evaluated by using green gram seeds (vigana radiata).

Table 5: Results of Seed Germination method of Aqueous extract

Groups	Time	Wt of seeds in grams			
		CONTROL	PACLITAXEL	A T1	A T2
1	0hrs	0.69	0.69	0.69	0.69
2	24hrs	0.9	0.50	0.50	0.48
3	72hrs	0.85	0.34	0.26	0.25
Mean		0.81	0.62	0..61	0.59
Standard deviation		0.109	0.19	0.32	0.31
percentage inhibition			96.60%	69.4%	70.5%

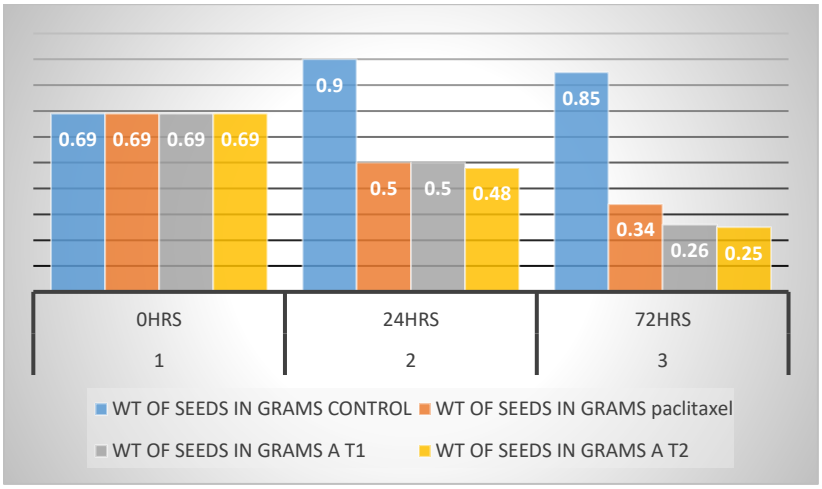


Fig 9: Antimitotic activity of Aqueous extract

DISCUSSION

The Antimitotic activity at different concentrations of costeus igneus leaves extract were evidenced by evaluating macroscopic parameters that is reduction in germination of seeds which were indicative of sprout growth inhibition.

Maximum number of non dividing cell were observed, which results, cell arrest in mitosis and eventually by apoptosis. [10]

The result from the study showed that the extract of costeus igneus leaves had antimitotic activity that were compared to the activity of paclitaxel.

It is widely reported by many researches that any plant with alkaloids and flavonoids will have potential anti cancer properties and antimitotic activity. [4] The phytochemical screening was performed which indicates the presence of flavonoids, alkaloids, anthraquinones, taninns, phenolic compounds, terpenoids, saponins and proteins.

In the present study the presences of alkaloids and flavonoids but absence of carbohydrates also indicates the antimitotic activity as cancer cell doesnot grow due to insufficient availability of glucose.

In the present study the antimitotic activity of ethanolic extract and aqueous extract of costus igneus leaves were evaluated using germination seed method. [7] The costus igneus leaves extracts is capable of inhibiting the germination of green gram seeds as result indicates 55.3% and 63.5% inhibition by ethanolic extract at 5mg/ml and 10mg/ml respectively whereas aqueous extract showed 69.4% and 70.5% inhibition at 5mg/ml and 10mg/ml. The percentage growth inhibition at different concentrations of extract clearly indicates the efficiency in the inhibition of growth of cancer cells by affecting microtubules polymerization.

The aqueous extract of costus igneus leaves showed better antimitotic activity when compare to the ethanolic extract.

CONCLUSION

The Anti mitotic activity of Aqueous and ethanol extract of costus igneus was studied by using Seed Germination Method. The phytochemical constituents like alkaloids, Glycosides, terpenoids, tannins, proteins, were found to be present and absences of Carbohydrate by performing Phytochemical screening studies. Thus the aqueous and ethanolic extract of costus igneus has arrested the germination of seeds. These findings suggest that the antimitotic property of the plant may be due to the key role of Phytochemical constituents like Alkaloids and flavanoids

compounds present in the extract and absence of carbohydrates confirms antimitotic activity.

In seed Germination method the aqueous and ethanolic extract of costus igneus significantly increased the percentage of inhibition after 24hrs and 72hrs. The present study results proved that the aqueous extract of costus igneus is having better antimitotic activity than ethanolic extract against the seed germination method. The inhibition of mitosis by test compound is beneficial for their possible application for life-threatening diseases such as Cancer.Thus,by this study we suggest that the cytotoxic action of the test compound can involve disturbance of mitotic processes in the fast dividing cancer cells which will be benefical for cancer treatment.

Further studies can be carried out to know the mechanisms and most effective chemical compounds responsible for anti mitotic activity in this plant.

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