IJMPR 2021, 5(5), 105-121

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

# ANTI-ANGIOGENESIS AND ANTI-CANCER ACTIVITY OF THE ETHANOLIC EXTRACT OF *SPATHODEA CAMPANULATA* ROOT BARK

***[1]Sandhya E., [2]Sankari M. and [3]Dr. Meena A.**

[1]M. Pharm (Pharmacology), [2]Associate Professor Department of Pharmacology, [3]Principal.
K K College of Pharmacy, Chennai, Tamil Nadu-600122.

**Received on: 27/08/2021**
**Revised on: 17/09/2021**
**Accepted on: 07/10/2021**

***Corresponding Author**
**Sandhya E.**
M. Pharm (Pharmacology), K K College of Pharmacy, Chennai, Tamil Nadu-600122.

## ABSTRACT

The study designed for identification of the anti-angiogenesis and anti-cancer activity of ethanolic extract of *Spathodea campanulata* root bark (EESC). The phytochemical chemical studies showed that the presence of phenolic and flavonoid constituents exerts antioxidant and free radical scavenging activity. The Pharmacognostical evaluation of EESC by fluorescence analysis with ethanol showed dark red in day light, black in short UV (254nm) and brown in long UV (366nm). The pharmacological assay includes *in-vitro* MTT assay and DNA fragmentation assay using MCF7 breast cell lines. The result revealed that the cell viability of MCF7 breast cell lines was 51.19% and IC50 value calculated for EESC was 62.5µg/ml which indicates that the presence of cytotoxic activity by MTT assay and DNA ladder formation indicated the cytotoxic effect of EESC caused by inhibition in the growth of breast cancer through apoptosis using DNA fragmentation method concludes the use of anti-cancer agent. *In-ovo* Chick Chorioallantoic membrane (CAM) assay includes 60 Fertile white leghorn eggs and on the sixth day of incubation 48 viable eggs are taken into experiment. The various concentration of EESC (10, 25, 50, 100, 150 and 200µg/ml) showed an anti-angiogenic activity based on the dose dependent manner. The result was compared with the standard (Prednisolone 5mg/ml) and control (0.9% NaCl) groups. A marked increase in the blood vessels was noted in normal saline (negative control) when compared to Prednisolone (Positive control). As the concentration of EESC increases the angiogenesis process was inhibited which revealed the anti-angiogenic activity of the EESC.

**KEYWORDS**: *Spathodea campanulata*, phytochemical, MCF7 breast cell lines, anti-cancer, anti-angiogenesis.

## INTRODUCTION

The human body has the ability to form new blood vessels from existing vessels to ensure proper vascularization during embryonic growth, reproductive cycles, and proper wound healing. Vascularization from pre-existing blood vessels is termed as angiogenesis. Angiogenesis is a normal and complex process controlled by certain biomolecules produced in the body. Angiogenesis plays a critical role in a various physiological and pathological processes such as embryonic development, wound healing, chronic inflammation, tumor growth and metastasis. Sustained, uncontrolled angiogenesis is associated with various pathological conditions including cancer, diabetic retinopathy and rheumatoid arthritis.[1-3]

Tumor angiogenesis is the consequence of an angiogenic imbalance in which pro-angiogenic factors e.g., vascular endothelial growth factor (VEGF), Fibroblast growth factor (FGF) angiopoietins etc. predominate over anti-angiogenic factors e.g., thrombospondins (TSP), angiostatins and endostatins. Tumor aniogenesis is defined as the proliferation of a network of blood vessels which supplies a tumor with supportive microenvironment rich with oxygen and nutrients to sustain optimal growth allowing the tumor to enlarge and the cancer cells to invade nearby tissue, to move throughout the body and to form new colonies of cancer cells called metastasis.[4-6]

Anti-angiogenesis is an important strategy for tumor therapy. Many studies have demonstrated that tumor angiogenesis can be inhibited by the flavones and other flavonoids. There is continuous search for the anti-angiogenic agents with an idea that these agents will prevent the growth and spread of cancer.

In comparison with chemical signals that induce blood formation there is another type of chemical signal known as an angiogenesis inhibitor (soluble VEGF, platelet factor, angiostatins etc.). These signals may systematically disrupt blood vessel formation or support removal of existing vessels. Inhibitors function by acting

on several proteins that have been identified as angiogenic activators.[7] It is very important to keep a balance between activators and inhibitors, and this balance regulates vascular homeostasis. Acquisition of the angiogenic phenotype is a rate-limiting step in tumor progression, wherein the tumor remains in a dormant state until it is able to stimulate blood vessel growth from nearby pre-existing capillaries in order to facilitate cancer cell progression and metastasis.[8]

Cancer burden in India has more than doubled over the last 26 years. The highest increase among all therapy areas, with breast cancer being the most common among Indian women, according to a recent report. As per the Indian Council of Medical Research (ICMR) data, India had 14 lakhs cancer patients in 2016 and this number was expected to increase. The government has laid down four priority cancer are Breast cancer, Cervical cancer, Oral cancer and Lung cancer which together constitute 41 per cent of cancer burden. Oral Cancer is among the top three cancers in India, number one among all cancers in men and number three among female cancers. Breast cancer is currently the most common cancer among Indian women, both in terms of incidence as well as mortality, with proportional prevalence in younger age-groups being higher than the global average. The age standardized rate is approximately 25.8 per one lakh women and is expected to rise to 35 per one lakh women in 2026, the report stated.[9] The *in-vitro* anti-cancer of plant *Spathodea campanulata* P. Beauv. was evaluated using MTT assay and DNA fragmentation assay using MCF7 breast cell lines done from Life Tech Research Lab, Chennai. The global cancer data from GLOBOCAN 2018 international agency for research on cancer around worldwide increased upto 18.1 million new cancer cases and 9.6 million cancer deaths.[10]

Various anti-cancer drugs were used for different kinds of cancers but the problems associated with conventional anti-cancer drugs such as serious side effects, chemo- and radio-resistance, disease relapse, and metastases, the researchers has looked for the development of alternative chemotherapeutic regimens, such as anti-angiogenic drugs. This anti-angiogenic strategy has been an important consideration for the development of cancer chemotherapeutics for the past three decades. To date, validation of more than 40 anti-angiogenic agents in clinical settings is underway and several classes of anti-angiogenic agents have been approved by the Food and Drug Administration as anti-cancer drugs are Axitinib, Bevacizumab, Cabozantinib, Everolimus, Lenalidomide, Pazopanib, Ramucirumab, Regorafenib etc.[8,11-13] At present, much attention has been focused on natural product-based therapeutics, especially phytochemicals, owing to numerous reports that revealed the interference of phytochemicals on cancer-related pathways. Some of the herbs used as anti-angiogenic agents are Polyphenols (Grapes, Tea, Curcuma longa), alkaloids **(**Strychnos nux-vomica, Colchicum autumnale, Catharanthus roses), terpenoids and tannins **(**Panax ginseng, Taxol, Triphala churna) and flavonoids **(**Artemisia annua, Scutellaria baicalensis, Apples, Onions, Ginkgo biloba, Broccoli).[14-16]

The plant *Spathodea campanulata* P. Beauv. belongs to a family Bignoniaceae has various synonyms are African tulip tree, Nandi flame, Fountain tree and Pichkari. This plant found majorly on Hawaii, Caribbean, south pacific islands, Atlantic and gaff coastal plain. In India its used as an ornamental plant. Its leaves used as an anti-diabetic and anti-convulsant agent whereas its seed used for HIV, CNS depressants. The plant *Spathodea campanulata* P. Beauv. bark has good antioxidant property and used for rheumatism. The flower of *Spathodea campanulata* are used for various conditions like malaria, HIV, diabetes mellitus, oedema, constipation. The root of *Spathodea campanulata* has anti-inflammatory and antioxidant property.[17-20] Here our study deals with the evaluation of anti-angiogenesis and anti-cancer activity of root bark of *Spathodea campanulata* P. Beauv. using *in-ovo* Chick Chorioallantoic membrane (CAM) assay and *in-vitro* MTT assay and DNA fragmentation assay. Spathodea campanulata was investigated its Pharmacognostical profile.

## MATERIALS AND METHODS

### Phyto chemical studies

### Plant part used

The root bark of *Spathodea campanulata* P. beauv was used for extraction which was collected in month of March 2019 at Chennai. It was examined and authenticated by Mr. P. Jayaraman, PhD Botany (Professor retd).

### Procedure for extraction of EESC

It is defined as separation of medicinally active parts of plant using selective solvent, ethanol through standard procedure called Hot continuous (soxhlet) extraction method. The principle behind this process is of continuous extraction method in which the solvent can be circulated through the extractor for several times. The vapours of solvent are taken to the condenser and the condensed liquid is returned to the extract for continuous extraction. The fresh root of *Spathodea campanulata* P. beauv collected from Gerugambakkam, Chennai. Then the root bark was peeled and dried in a shade. After that the dried root bark was crused well and coarsely powdered. The powder was used for the extraction. 50 grams of powder was packed into soxhlet apparatus and was subjected to extraction with 500ml of ethanol. The extraction was continued until the colour of the solvent in the siphon tube became colourless. Extract of ethanol were subjected to concentrated by using rotatory vacuum evaporator till a semisolid mass was obtained. It was dried and percentage yield was calculated.

### Phytochemical analysis[21]

The freshly prepared ethanol extract was subjected to phytochemical screening for the presence or absence of active constituents by following the methods.

**Test for alkaloids**
Crude extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was tested with various alkaloidal reagents such as,
Mayer's reagent – Cream precipitate
Dragendroff's reagent – Orange brown precipitate
Wagner's reagent– Reddish brown precipitate

**Detection of Glycoside**
**Borntrager's test**
The crude extract was boiled with 1ml of sulphuric acid in a test tube for 5 minutes. Filtered while hot, cooled and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume of dilute ammonia. Appearance of rose to red colour was produced in the ammonical layer indicated the presence of anthraquinone glycosides.

**Modified born Trager's test**
The crude extract was boiled with 2ml dilute sulphuric acid. This was treated with 2 ml of 5% aqueous ferric chloride solution (freshly prepared) for 5 minutes and shaken with equal volume of chloroform. The lower layer of solvent was separated and shaken with half of its volume with dilute ammonia. Appearance rose pink to red colour was produced in the ammonical layer.

**Legal's test**
The crude extract was treated with sodium nitroprusside in pyridine and methanolic alkali. Formation of a pink red colour indicated the presence of cardiac glycoside.

**Detection of Steroids and Triterpenoids**
**Libermann Burchard's test**
The crude extract was treated with few drops of acetic anhydride, boiled and cooled. Concentrated sulphuric acid was added from the sides of the test tube. Formation of brown ring at the junction of two layer and upper layer turns green which shown presence of steroids and formation of deep red colour indicated the presence of triterpenoids.

**Salkowski test**
Crude extract was mixed with 2ml of chloroform. Then 2ml of conc. Sulphuric acid was added carefully and shaken gently. Appearance of reddish brown colour ring indicated the presence of steroids and formation of yellow coloured lower layer indicated the presence of triterpenoids.

**Detection of Flavonoids**
**Shinoda's test**
Small quantity of crude extract was dissolved in alcohol. To this piece to magnesium followed by conc. Hydrochloric acid were added drop wise and heat. Appearance of magenta colour has shown the presence of flavonoids.

**Alkaline reagent test**
Small quantity of the crude extract was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicated the presence of flavonoids.

**Detection of Carbohydrates**
**Molisch's test**
To the extract solution, few drops of alcoholic alpha napthol and few drops of conc. Sulphuric acid was added through the slides of test tube. Appearance of purple to violet colour ring at the junction indicated the presence of carbohydrates.

**Fehling's test**
Crude extract was treated with equal volume of Fehling A and Fehling B reagents and mixed together and gently boiled. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugars.

**Detection of Tannins**
**Lead acetate test**
The test solution was mixed with basic lead acetate solution. Formation of white precipitate indicated the presence of tannins.

**Ferric chloride test**
A few drops of 5% aqueous ferric chloride solution were added to 2ml of an ethanol extract of the drug and appearance of bluish black colour indicated the presence of tannins.

**Detection of Proteins**
**Biurette test**
The crude extract was treated with 5 to 8 drops of 10% w/w copper sulphate solution. Appearance of violet colour indicated the presence of proteins.

**Detection of saponins**
A drop of sodium bicarbonate solution was added to the sample and the mixture was shaken vigorously and left for three minutes. Development of any honey comb like froth indicated the presence of saponins.

**Detection of gums and mucilage**
The small quantities of test substance were dissolved in 5 to 10 ml of acetic anhydride by means of heat, cool and 0.05 ml of conc. Sulphuric acid was added. Formation of bright purplish red colour indicated the presence of gums and mucilage.

**Detection of fixed oils and fats**
A small quantity of extracts was pressed between two filter papers. An oily stain on the filter paper indicated the presence of fixed oils and fats.

**Fluorescence analysis**
Many crude drug show fluorescence when the sample is expose to UV radiation. Evaluation of crude drug based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of the fluorescent

effect. Fluorescence lamps are fitted with suitable filter, which eliminate visible radiation from the lamp and transmits UV radiation of definite wavelength was used for the study several crude drugs show characteristic fluorescence which is very much useful for their evaluation. The drug powder was treated with acids such as 1N HCL and 50% $H_2SO_4$ and alkaline solutions such as aqueous sodium hydroxide, alcoholic sodium hydroxide and other solvent such as nitric acid, picric acid, acetic acid, ferric chloride and nitric acid with ammonia. They were subjected to fluorescence analysis in daylight and in the ultralight (UV)-light (254 nm and 365 nm).

**Pharmacological studies**

***In-vitro* studies**

**Cytotoxicity study (MTT assay)[22]**

It is a universally accepted *in vitro* method for screening the drugs having cytotoxic activity. It was described by Mosmamm (1983) & Monks (1991). This assay is used to determine the IC50 of drugs or extracts. The principle behind this is that the Tetrazolium salt, 3, -(4, 5-dimethyl thiazol-2-yl)-2, 5, diphenyl tetrazolium bromide is reduced into blue formazan product by the mitochondrial dehydrogenase enzyme of live or metabolically active cells. The intensity of blue or purple colored formazan produced is directly proportional to cell viability.

**Materials required**

**Plant extracts**

Ethanol extract of *Spathodea campanulata* (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8)

**Reagents**

Drugs used in this study includes: DPBS, TPVG, MEM, Trypan blue, MTT dye(5mg/ml), DMSO (0.1% v/v) and other materials were, MTT plate with plate with lid, Sterile pipette, tips, glove, mask, Aluminium foil, Incubator, Inverted microscope, Falcon tube, Hemocytometer and cover slip.

**Cell line and culture**

MCF 7 cell line was obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml $CO_2$ at 37 °C.

**Procedure (Mosmann, 1983)**

Cells ($1 \times 10^5$/well) were plated in 24-well plates and incubated in $37^0$C with 5% $CO_2$ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV-Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition ($IC_{50}$) was determined graphically. The % cell viability was calculated using the following formula:

**% Cell viability = A570 of treated cells / A570 of control cells × 100**.

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

**Apoptosis assay**

**DNA fragmentation analysis using gel electrophoresis (Wyllie, 1980)[23]**

This assay provides a qualitative method for assessing cell death by detecting DNA fragments using agarose gel electrophoresis. One of the classic features of apoptosis is the cleavage of the genomic DNA into oligonucleosomal fragments represented by multiples of 180-200 bp.

**Materials required**

The Reagents used in this study are: Agarose, TPVG 6, TTE solution, EDTA, NaCl (ice cold), Isopropanol (ice cold), Ethanol 70% (ice cold), Bromophenol blue dye. Other materials included were: $CO_2$ incubator, Centrifuge tubes, Gel electrophoresis equipment, Wide-bore, pipette tips and UV transilluminator.

**Procedure**

MCF 7 cells were plated in 6 well plate and kept in CO 2 incubator to attain confluency. 100µg/ml of Sample was added in to the well and incubated for 24 hrs. After this, cells were harvested using TPVG. Centrifuge cells at 200xg at $4^0$C for 10 min. Add to the pellet 0.5 ml of TTE Solution and Vortex Vigorously. This procedure allows the release of fragmented chromatin from nuclei, after cell lysis (due to the presence of Triton X- 100 in the TTE solution) and disruption of the nuclear structure (following Mg ++ chelation by EDTA in the TTE Solution). To separate fragmented DNA from intact chromatin, centrifuge tubes at 20,000xg for 10 min at $4^0$C. Carefully remove the supernatants and add 500µl of TTE solution into the pellet. Add 500µl of Ice-cold NaCl and vortex vigorously. The addition of the Salt should be able to remove histones from DNA. Add 700µl of ice-cold isopropanol and vortex vigorously. Allow precipitation to proceed overnight at $-20^0$C. After, precipitation, recover DNA by pelleting for 10 min at 20,000x g at $4^0$C. Rinse the pellets by adding 500-700µl of ice-cold 70% ethanol. Centrifuge tubes at 20,000x g for 10 min at $4^0$C. Dissolve DNA by adding to each tube 20-50 µl of TTE solution and place the tubes at $4^0$C. Mix the samples of DNA with loading buffer by adding 10x loading buffer to a final concentration of 1X. The addition of loading buffer to samples allows to load in wells more easily and to monitor the run of samples. Run the electrophoresis in standard TE buffer after setting the

voltage to the desired level. During electrophoresis it is possible to monitor the migration of samples by following the migration of bromophenol blue dye contained in the loading dye. Stop the electrophoresis when the dye reaches about 3 cm from the end of the gel. To visualize DNA, place the gel on a UV Transilluminator.

***In-ovo* studies**

**Chick Chorioallantoic membrane (cam) assay[1,6& 24]**

Anti-angiogenic activity of ethanolic extract of root bark of *S. campanulata* P. beauv was conducted on fertilized eggs by modified CAM assay method. Angiogenesis processes the formation of new blood vessels. But uncontrolled productions of blood vessels are one of the main criteria during cancer development. The inhibition of new blood vessels growth by anti-angiogenesis drugs to control cancer development. To control neovascularization, various assays is developed *in-vitro* and *in-vivo*. One of the important and ease assays chosen here was CAM assay. Fertile white leghorn eggs were obtained from a local hatchery. The following criteria should be taken into consideration are,

**Inclusion criteria-** Three days old fertilized, medium sized healthy country chick embryos, Crack free embryos, Experiment should be simple to perform and uniformly reproducible, Technique should be able to predict the potential properties of standard drug and it should consume minimal quantities of drugs.

**Exclusion criteria-** More than three days old fertilized chicken embryos, large size embryos, Diseased chick embryos, Embryos with crack and Double yolk embryos.

**Materials required**

The reagents used in this study includes: Saline (9% sodium chloride), Prednisolone (5mg/ml), Benzalkonium chloride and Ethanol extract of *Spathodea campanulata* and other materials includes: Egg incubator, Laminar air flow hood, Micropipette, Sterile disc, Petridish, 1ml vials, Scissor, Beaker, Forceps, Cotton swab and Parafilm.

**Procedure**

In this procedure, a total of 60 fertile country chicken eggs were obtained from a local hatchery. The eggs were weighed and cleaned with benzalkonium chloride. The eggs were incubated in a vertical position within the range of 35-37.5˚C (95-99.5˚F) in an egg incubator for 72 hours with 50-60% humidification. The egg tray was automatically tilted for 45˚ angle every 30 minutes mimicking the natural process. On the fourth day of incubation, By the process of candling, viable eggs with moving embryo were identified and the unfertilized eggs were removed. From 60 eggs, 3 eggs were found to be unfertile and discarded from the study. Remaining 57 eggs were taken for further process. The experiment was performed under aseptic condition on the laminar air flow hood. A fine driller was used to make a hole on the pointy end to remove nearly 2 ml of albumin from eggs out to provide a false air sac for ease of sample introduction inside the egg. After removal of albumin from eggs, sealed using paraffin wax and kept for incubation for 24 hours. On the sixth day of incubation, the 57 eggs were taken for candling to check out the viability. Out of 57, 9 eggs were found to be dead and the remaining 48 eggs were taken into experiment.

**Preparation of the extract on filter disk**

The control (Normal saline 0.9% NaCl 5ml/kg), standard (Prednisolone 5mg/ml) and different concentrations of ethanolic extract of root bark of *Spathodea campanulata* P. beauv (200, 150,100, 50, 25 and 10 mg/ml) was prepared and 10 µL of samples administered on sterile filter disk using micropipette. Then the filter disks are dried under laminar airflow.

**Placement of filter disk in-ova on eggs**

A window was made on the blunt side of egg and a cut of 1.5-2cm was made using driller and scissor. The sample dried filter disks are placed inside the egg on the vessels and sealed the eggs using parafilm. 48 eggs of 6 per group will be divided into the following 8 groups –

- Group 1: Normal control (0.9% NaCl 5ml/kg)
- Group 2: Standard (Prednisolone 5mg/ml)
- Group 3: EESC 10µg/ml
- Group 4: EESC 25 µg/ml
- Group 5: EESC 50 µg/ml
- Group 6: EESC 100 µg/ml
- Group 7: EESC 150 µg/ml
- Group 8: EESC 200 µg/ml

The eggs were placed for incubation for next 24 hrs. On the eighth day of incubation:

The window was opened and changes made in the CAM (anti-angiogenesis activity) were photographed and scored. For clear visualization the CAM was separated and placed on petridish and photographed. The images obtained were analyzed for the changes that the drug has brought to the angiogenesis process in the CAM of the developing chick embryo.

**Statistical analysis[6& 24]**

The results were expressed as Mean±SEM. Statistically significance was determined using one-way ANOVA followed by Dunnett's multiple comparison test. P values <0.01 were considered significant.

**RESULTS**

The Percentage yield of ethanolic extract of *Spathodea campanulata* p. Beauv root bark (Table 1) revealed by using Hot continuous extraction method. The root bark was semisolid in nature and brown in colour and % yield of EESC root bark was found to be 3.01 % w/w.

**Table 1: percentage yield of ethanolic extract of root bark of *Spathodea campanulata* P. beauv.**

| S.NO | Extract | Method of extraction | Physical Nature | Colour | YIELD (%W/W) |
|---|---|---|---|---|---|
| 1. | Ethanol | Continuous hot percolation method using soxhlet apparatus | Semi solid | Brown colour | 3.01 |

**Phytochemical analysis**

Phyto chemical analysis was performed with ethanolic extract of *Spathodea campanulata* P. beauv, listed (Table 2) and it shows following constituents: Steroids, Flavonoids, Triterpenes, Proteins, Alkaloids, Carbohydrates and Phenolic compounds.

**Table 2: Preliminary phytochemical analysis of the ethanolic extract of *Spathodea campanulata* root bark.**

| S.no | Chemical constituents | Powdered drug | Ethanol extract |
|---|---|---|---|
| 1. | Steroids | + | + |
| 2. | Glycosides | - | - |
| 3. | Saponins | - | - |
| 4. | Flavonoids | + | + |
| 5. | Tannins | - | - |
| 6. | Triterpenes | + | + |
| 7. | Proteins | + | + |
| 8. | Alkaloid | + | + |
| 9. | Carbohydrates | + | + |
| 10 | Phenolic compounds | + | + |

*Note: + and - indicates the presences and absence.*

**Fluorescence Analysis**

The experiments conducted in *Spathodea campanulata* root bark for analysis of fluorescent characters showed the following results (Table 3 and 4): The root bark powder of the plant sample was extracted in water, NaOH, HCl, acetic acid, alc. NaOH, Picric acid, $H_2SO_4$, $HNO_3$, Iodine, $FeCl_3$, KOH, alc. KOH, ammonia and ethanol. The fluorescence analysis of these root bark powder extract was observed under visible light (Day light) and also under UV of short wavelength (254 nm) and long wavelength (366 nm) and recorded.

**Table 3: Fluorescence characteristic of powdered root bark of *Spathodea campanulata beauv.***

| S.NO | TREATMENT | DAY LIGHT | SHORT UV (254nm) | LONG UV (366nm) |
|---|---|---|---|---|
| 1. | Powder | Brown | Brown | Orange |
| 2. | Powder + water | Brown | Pale green | Reddish brown |
| 3. | Powder + NaOH | Brown | dark green | Blue |
| 4. | Powder + Hcl | Orange | Green | Blue |
| 5. | Powder + Acetic acid | Brown | Brown | Orange |
| 6. | Powder + Alc. NaOH | Brown | Green | Orange |
| 7. | Powder + Picric acid | Yellow | Green | Orange |
| 8. | Powder + Sulphuric acid | Brown | Green | Black |
| 9. | Powder + Nitric acid | Orange | Yellow | Orange |
| 10. | Powder + Iodine | Brown | Brown | Reddish brown |
| 11. | Powder + Ferric chloride | Orange | Brown | Reddish brown |
| 12. | Powder + KOH | Brown | Yellow | Orange |
| 13. | Powder + Alc. KOH | Brown | Brownish green | Orange |
| 14. | Powder + Ammonia | Brown | Brown | Reddish brown |
| 15. | Powder + Ethanol | Brown | Green | Orange |

**Table 4: Fluorescence Analysis of root bark extract of *Spathodea Campanulata* Beauv.**

| S.NO | Treatment | Day Light | SHORT UV (254nm) | LONG UV (366nm) |
|---|---|---|---|---|
| **1.** | **Ethanol** | Dark red | Black | Brown |

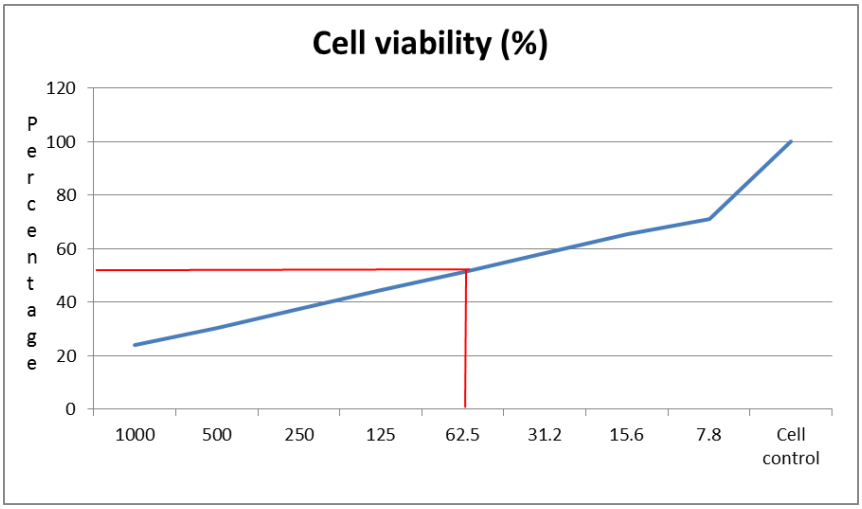
**Cytotoxicity test using MTT assay**

MTT assay with ethanolic extract of *Spathodea campanulata* P. beauv was performed in various concentrations. The cell viability and percentage of inhibition of cells (IC50) was determined. Cell viability of 51.19% was observed in concentration of 62.5 µg/ml which was taken as 50% of inhibition of cells (IC50). The cell viability of ethanolic extract of root bark of

Spathodea campanulata against MCF7 cell line was showed (Table 5) and the graph was plotted as concentration (µg/ml) (on x-axis) vs percentage of cell viability (on y-axis) showed (Bar diagram 1). The cytotoxic effect of various concentration (1000, 62.5 and 7.8µg/ml) of EESC against MCF7 cell line was observed microscopically and showed (Figure 1).

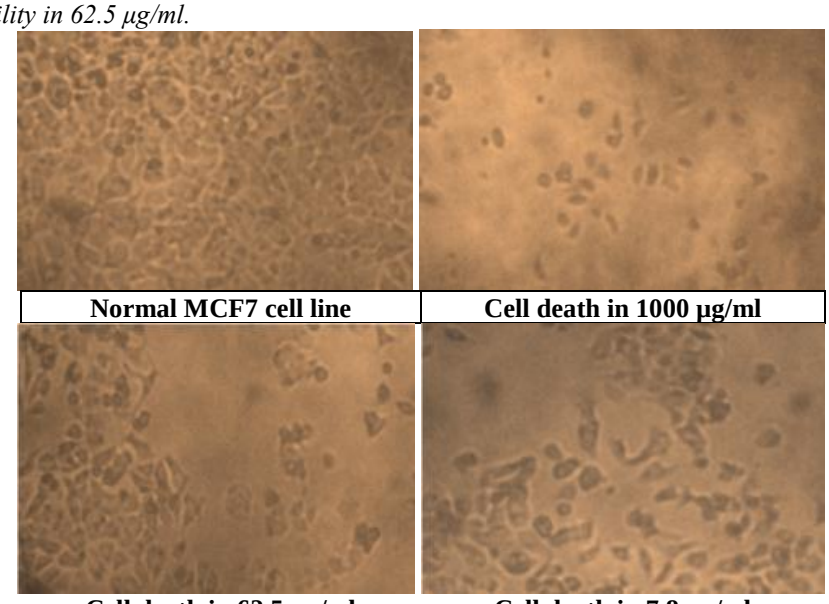
**Table 5: Anticancer effect of Sample *on MCF7* cell line.**

| S.no | Concentration (µg/ml) | Dilutions | Absorbance (O.D) | | | | Cell viability (%) |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | Avg. | |
| 1 | 1000 | Neat | 0.130 | 0.133 | 0.131 | 0.131 | 24.12 |
| 2 | 500 | 1:1 | 0.167 | 0.165 | 0.168 | 0.166 | 30.57 |
| 3 | 250 | 1:2 | 0.204 | 0.201 | 0.206 | 0.203 | 37.38 |
| 4 | 125 | 1:4 | 0.243 | 0.244 | 0.244 | 0.243 | 44.75 |
| 5 | 62.5 | 1:8 | 0.279 | 0.279 | 0.278 | 0.278 | 51.19 |
| 6 | 31.2 | 1:161` | 0.318 | 0.316 | 0.319 | 0.317 | 58.37 |
| 7 | 15.6 | 1:32 | 0.357 | 0.355 | 0.358 | 0.356 | 65.56 |
| 8 | 7.8 | 1:64 | 0.386 | 0.387 | 0.386 | 0.386 | 71.08 |
| 9 | Cell control | - | 0.543 | | | | 100 |




**Bar diagram 1: Cell viability in ethanolic extract of *Spathodea campanulata* P. beauv on MCF7 cell line.**

*It showed concentration vs percentage of cell viability. EESC (ethanolic extract of Spathodea campanulata) shows 51.19 % of cell viability in 62.5 µg/ml.*



**Normal MCF7 cell line** **Cell death in 1000 µg/ml**

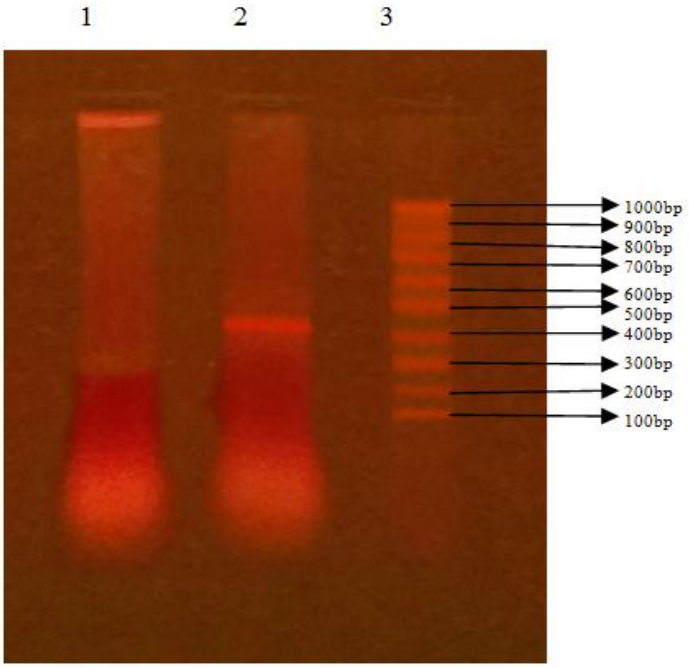
**Cell death in 62.5 µg/ml** **Cell death in 7.8 µg/ml**

**Figure 1: Anticancer effect of Sample on *MCF 7 cell* line.**

Figure 1: shows cell death of MCF7 cell line using different concentration of ethanolic extract of Spathodea campanulata. In this 62.5µg/ml concentration shows 51.19% of cell death, so 62.5µg/ml taken as IC50 concentration for further study.

**Apoptotic activity using DNA fragmentation assay**

The DNA fragmentation assay was performed in an agarose gel electrophoresis where the sample was exposed to MCF7 breast cancer cell lines at IC50 concentration. The apoptotic activity of the ethanolic extract of root bark of *Spathodea campanulata* was determined by ladder formation. As shown in figure 2, fragmented DNA was observed in sample treated cancer cell lines (lane 2) but no ladder formation in control treated lane (lane 1). The fragmentation of DNA by ethanolic extract on cell lines indicates the characteristics of apoptotic cells.




**Lane 1: Control, Lane 2: Sample and Lane 3: Marker. Figure 2: Showed the quick detection of DNA ladder apoptotic activity that can be visualized as a ladder pattern due to DNA cleavage by the activation of a nuclear endonuclease by standard agarose gel electrophoresis.**

**Figure 2: Formation of the DNA ladder in gel electrophoresis by induction of apoptosis in MCF7 cell line.**

**Anti-angiogenic activity using chick Chorioallantoic membrane (cam) assay**

Dose dependent changes with the angiogenesis process of the Chick CAM assay model were noted and compared with the Prednisolone (positive control) and normal saline (negative control). The anti-angiogenic activity was observed in 48 hours of observation with the process initiated at the dose of 10 µg/ml of EESC and the inhibition was increased upon increasing the concentration of EESC (25, 50, 100, 150 and 200µg/ml. The viability of the eggs till end of the experiment was noted and there was no death due to EESC was noted (Table 6). The changes made were photographed as showed (Figure 3-10):

I. During placement of disk with control, standard and various concentration of EESC.
II. Activity after 48 hrs.
III. CAM In petridish.

**Table 6: Effect of ethanolic extract of *Spathodea campanulata* (EESC) on angiogenesis in chick Chorioallantoic membrane.**

| (n=6) | Angiogenesis | Antiangiogenesis |
|---|---|---|
| Group I (Control-10 µL of NaCl) | 6 | 0 |
| Group II (Standard-10 µL of Prednisolone) | 0 | 6 |
| Group III (EESC-10 µg/ml) | 0 | 6 |
| Group IV (EESC-25 µg/ml) | 0 | 6 |
| Group V (EESC-50 µg/ml) | 0 | 6 |
| Group VI (EESC-100 µg/ml) | 0 | 6 |
| Group VII (EESC-150 µg/ml) | 0 | 6 |
| Group VIII (EESC-200 µg/ml) | 0 | 6 |

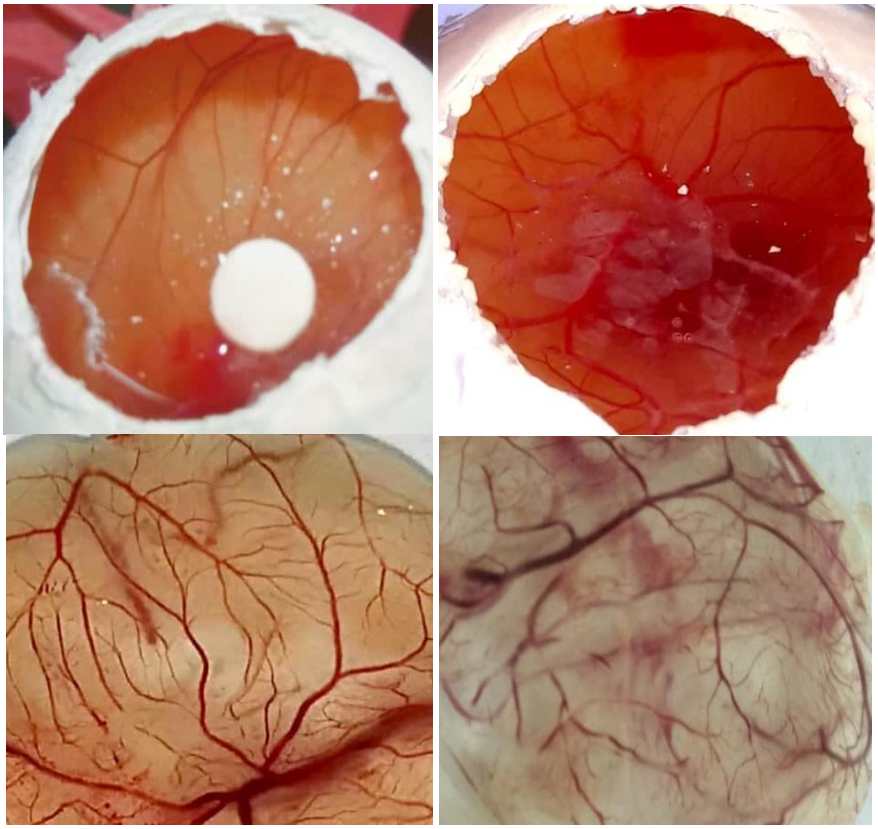


**Figure 3: Control 10 µL of Normal saline.**

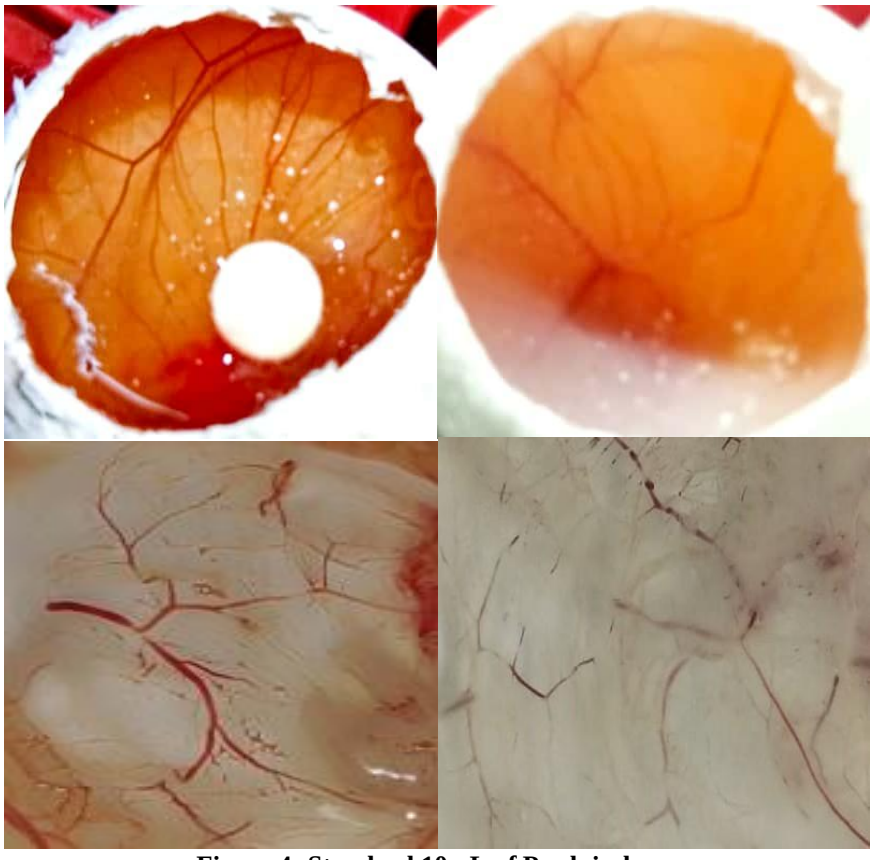


**Figure 4: Standard 10 µL of Prednisolone.**

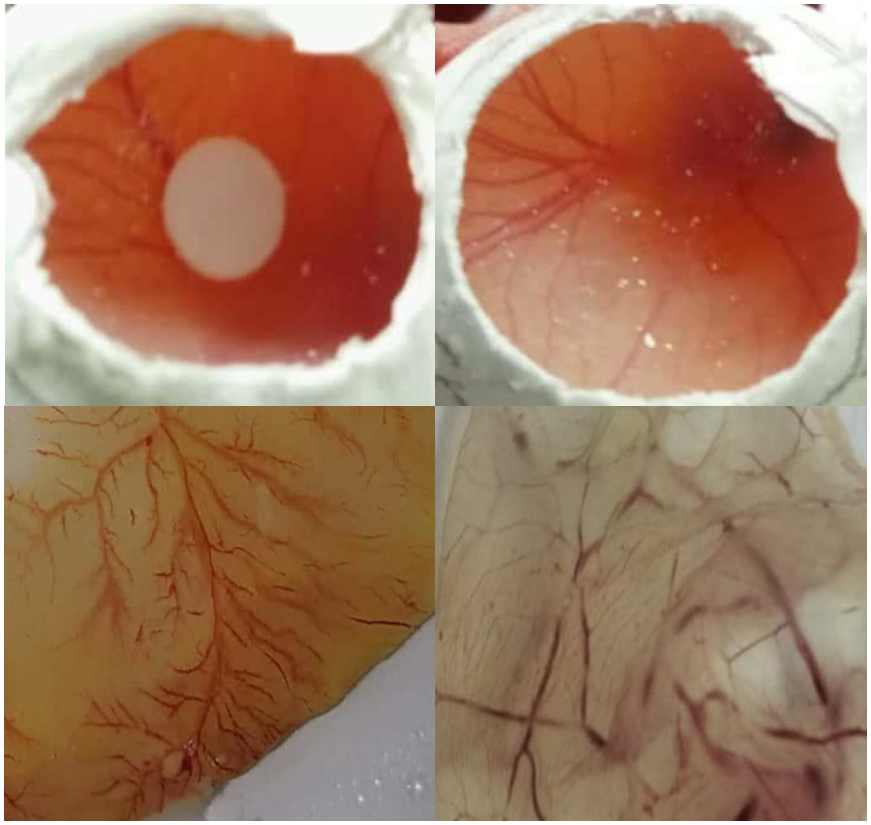


**Figure 5: 10 µL of 10 µg/ml concentration of EESC.**

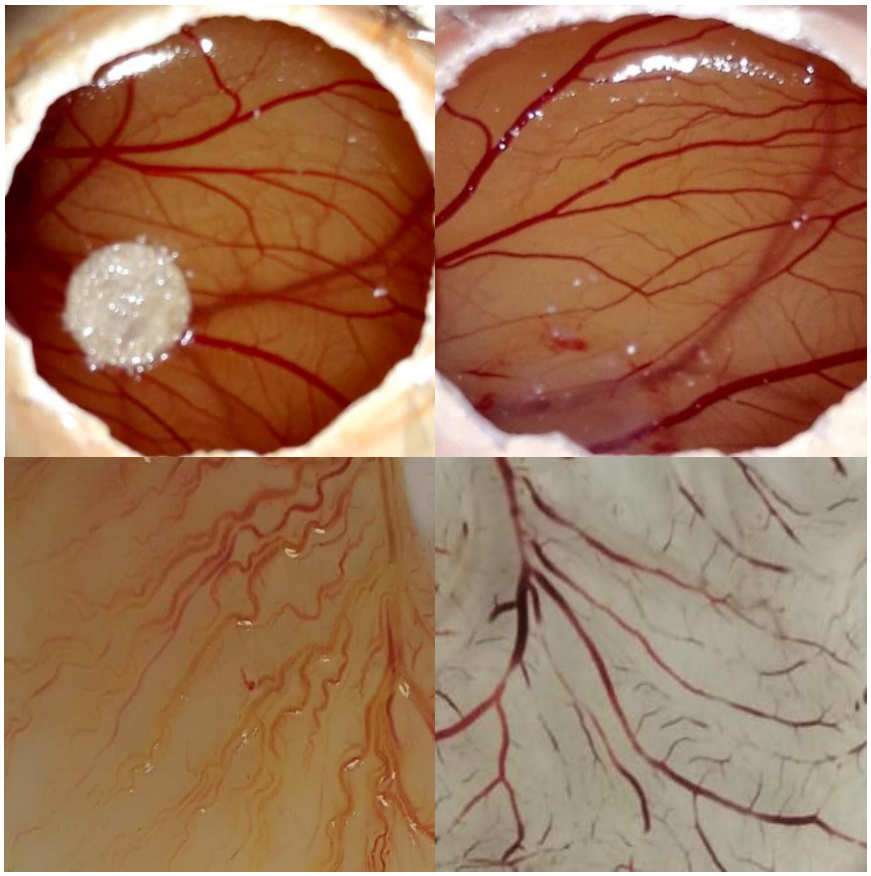


**Figure 6: 10 µL of 25 µg/ml concentration of EESC.**

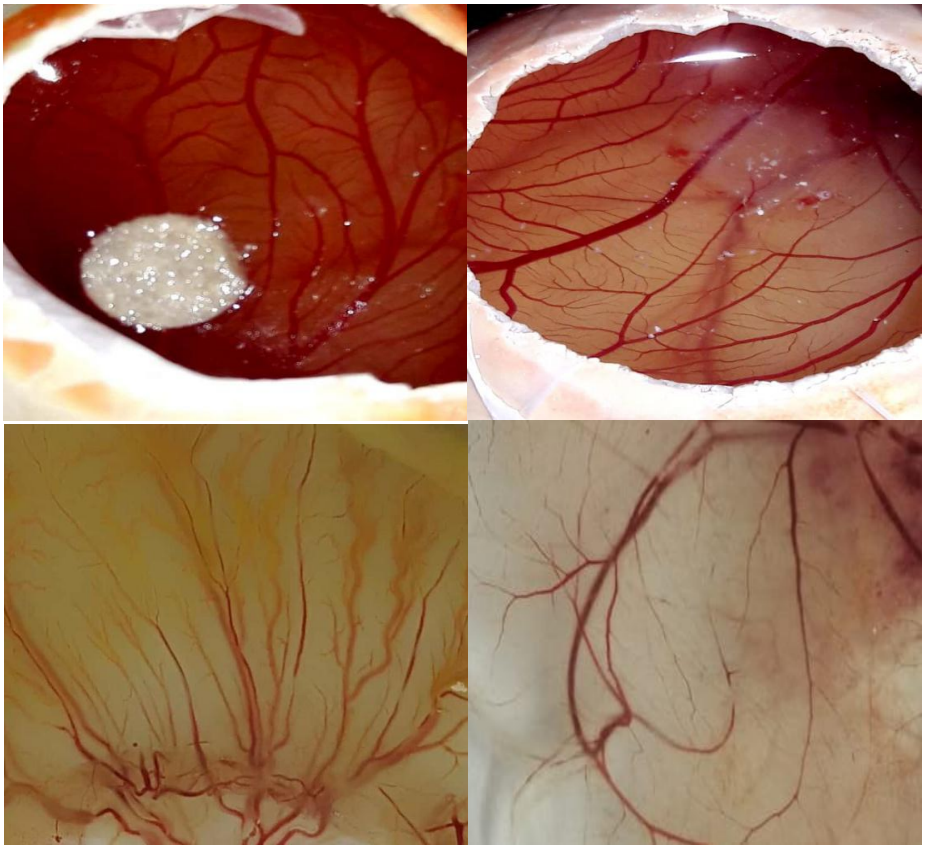


**Figure 7: 10µL of 50µg/ml concentration of EESC.**

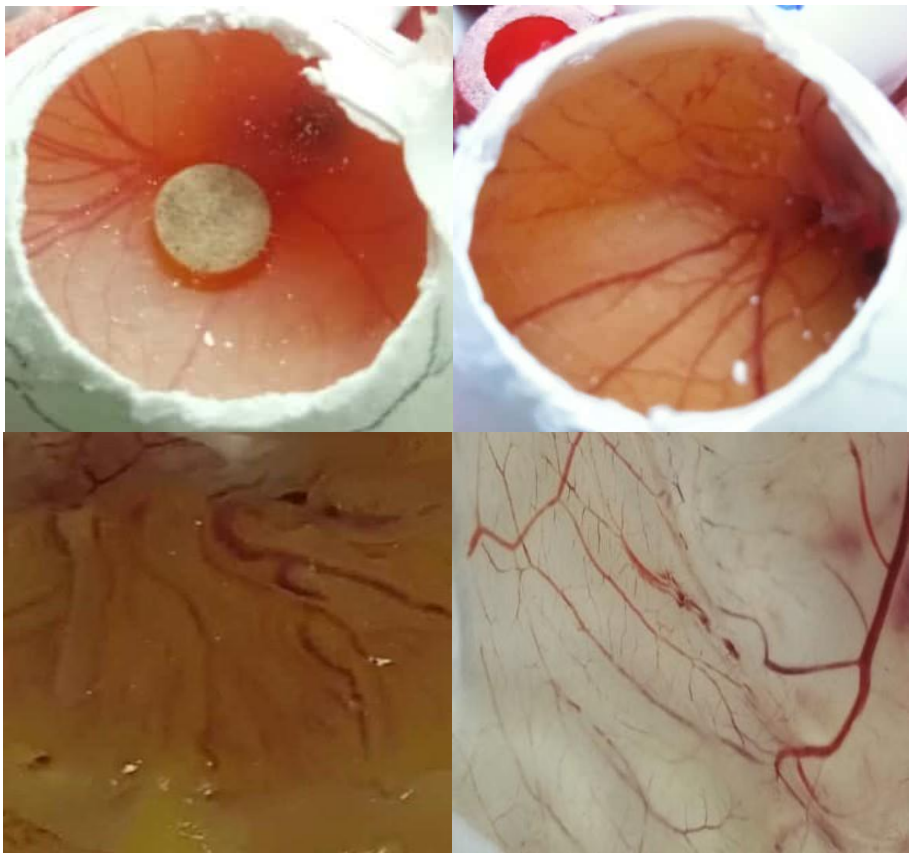


**Figure 8: 10µL of 100µg/ml concentration of EESC.**

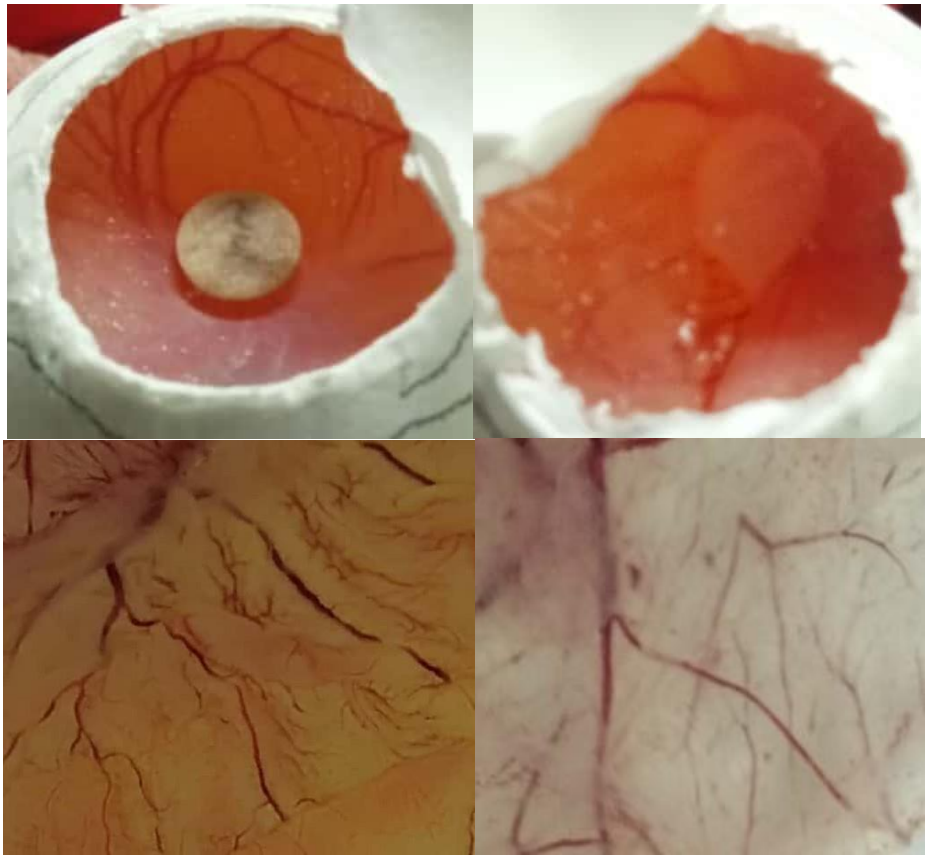


**Figure 9: 10µL of 150µg/ml concentration of EESC.**

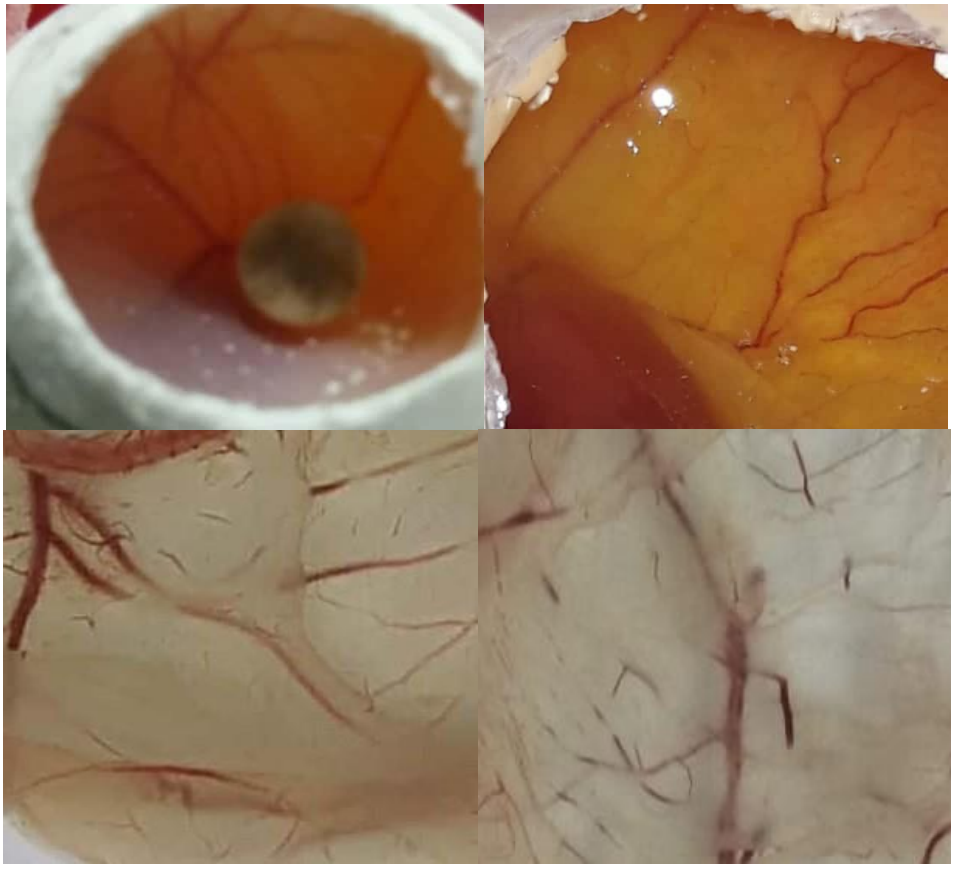


**Figure 10: 10µL of 200µg/ml concentration of EESC.**

**Table 7: The Average number of blood vessels before and after treatment of ethanolic extract of *Spathodea campanulata* on CAM model.**

| Group | 1 | 2 | 3 | 4 | 5 | 6 | Avg. no. of vessel(s) | Group | 1 | 2 | 3 | 4 | 5 | 6 | Avg. no. of vessel(s) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Normal saline** | 8 | 9 | 12 | 11 | 9 | 11 | 10 | **Normal saline** | 13 | 14 | 18 | 16 | 14 | 15 | 15 |
| **Prednisolone** | 12 | 11 | 9 | 11 | 10 | 9 | 10.33 | **Prednisolone** | 3 | 4 | 2 | 4 | 4 | 3 | 3.33 |
| **EESC 10 µg** | 10 | 9 | 11 | 12 | 9 | 11 | 10.33 | **EESC 10 µg** | 9 | 8 | 10 | 11 | 8 | 10 | 9.66 |
| **EESC 25 µg** | 12 | 11 | 9 | 10 | 12 | 9 | 10.5 | **EESC 25 µg** | 9 | 9 | 8 | 8 | 10 | 7 | 8.16 |
| **EESC 50 µg** | 9 | 9 | 10 | 10 | 11 | 12 | 10.16 | **EESC 50 µg** | 6 | 5 | 7 | 7 | 8 | 8 | 6.83 |
| **EESC 100 µg** | 10 | 11 | 9 | 10 | 9 | 10 | 9.83 | **EESC 100 µg** | 6 | 7 | 4 | 5 | 5 | 6 | 5.5 |
| **EESC 150 µg** | 11 | 12 | 10 | 11 | 11 | 10 | 10.83 | **EESC 150 µg** | 4 | 5 | 4 | 5 | 6 | 4 | 4.66 |
| **EESC 200 µg** | 10 | 9 | 11 | 10 | 11 | 12 | 10.5 | **EESC 200 µg** | 3 | 3 | 2 | 4 | 4 | 3 | 3.16 |

**Table 8: Anti-angiogenic effect of ethanolic extract of root bark of Spathodea campanulata on CAM model.**

| GROUP | DOSAGE | PERCENTAGE OF VESSEL INHIBITION (%) | | | | | | PERCENTAGE OF INHIBITION (MEAN± SEM) |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| **NORMAL SALINE** | 0.9 % | -62.5 | -55.55 | -50 | -45.45 | -55.55 | -66.66 | -59.95 ±3.181* |
| **PREDNISOLONE** | 5mg/ml | 75 | 63.63 | 77.77 | 63.63 | 60 | 66.66 | 67.94 ±2.881 |
| **EESC** | 10 µg/ml | 10 | 11.11 | 9.09 | 8.33 | 11.11 | 9.09 | 9.67 ±0.494* |
| **EESC** | 25 µg/ml | 25 | 18.18 | 11.11 | 20 | 16.66 | 22.22 | 18.83 ±1.956** |
| **EESC** | 50 µg/ml | 33.33 | 44.44 | 30 | 30 | 27.27 | 33.33 | 32.83 ±2.414** |
| **EESC** | 100 µg/ml | 40 | 36.36 | 55.55 | 50 | 44.44 | 40 | 44.33 ±3.029*** |
| **EESC** | 150 µg/ml | 63.66 | 58.33 | 60 | 54.54 | 45.45 | 60 | 57 ±2.683*** |
| **EESC** | 200 µg/ml | 70 | 66.66 | 81.81 | 60 | 63.63 | 75 | 62.7 ±3.232*** |

*Values are expressed as mean ± SEM, (n=6). One-way analysis of variance (ANOVA) followed by Dunnette's multiple comparisons test. * P<0.05,*
***P<0.01, ***P<0.001 when compared with positive control group.*

The 8th day old embryos after treatment, number of blood vessels and their reduction were examined. The analysis of blood vessel was based on the evaluation of angiogenesis by measuring the area of inhibition surrounding the applied disc and noted (Table 7)**.** The percentage of vessel inhibition (%) was calculated and compared with positive control for significance was showed (Table 8). The bar diagram was plotted against concentration (µg/ml) on x-axis vs % of inhibition on y-axis showed (bar diagram 2).

**Bar diagram 2: Anti-angiogenesis effect of EESC of root bark of *Spathodea campanulata* on CAM model**

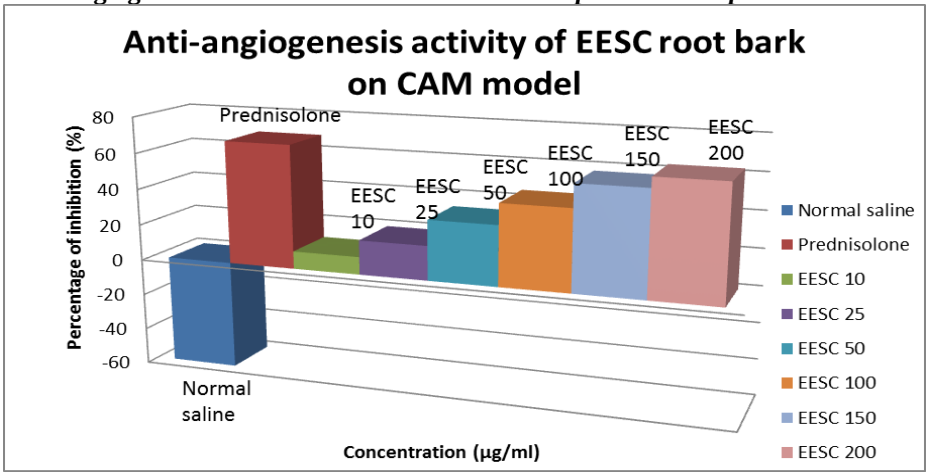



**Diagram showed Concentration (µg/ml) on x-axis vs percentage (%) inhibition on y-axis**

## DISCUSSION

Organized blood vessel formation is essential for development and physiological function of organs. Blood vessel formation is full of complicated and sequential processes initiated by vasculogenesis in which endothelial progenitor cells differentiate, proliferate, and subsequently assemble into primitive tubular networks. Vasculogenesis is followed by angiogenesis, wherein vascular networks remodel into more complex networks through dilatation, sprouting, and bridging. Another form of blood vessel growth after birth is arteriogenesis which defined as structural enlargement and remodeling by growth of preexisting arteriolar connections.[25]

Angiogenesis is a critical player in many pathologic processes, especially in neoplasm. Uncontrolled angiogenesis is thought to be indispensable in tumor growth and metastasis. Many pathological conditions such as ischemic tissue injury is also benefited by revascularization. On the other side, excessive angiogenesis may result in different diseases including cancer, atherosclerosis, rheumatoid arthritis, Crohn's disease, diabetes, psoriasis, endometriosis and adiposity. These diseases may benefit from the therapeutic inhibition of angiogenesis. Angiogenesis inhibitors, also called anti-angiogenesis, are drugs that block angiogenesis. Blocking nutrients and oxygen from a tumor "starves" it. Many of the body's normal functions depend on angiogenesis. Anti-angiogenesis drugs are useful though it contains adverse effects. Therefore, selection of angiogenic inhibitors is very important. Various researches on herbal plants are still ongoing to improve anti angiogenic activity with very lesser side effects.[2,7]

Cancer incidence in India to double every 20 years, a study reported on the "THE HINDU" Business Line as updated on 2019, August 05. Uttar Pradesh, Bihar, Jharkhand and Odisha, which are currently passing through huge epidemiological changes, will bear the biggest cancer burden in the next 10-20 years, a study has shown.[26] Chemotherapy drugs work by stopping cancer cells dividing and growing. Different drugs affect the cancer cells in different ways. Usually, cancer drugs work by damaging the RNA or DNA that tells the cell how to copy itself in division. If the cancer cells are unable to divide, they die. The faster that cancer cells divide, the more likely it is that chemotherapy will kill the cells, causing the tumor to shrink. However, these drugs have a side effect which affects mainly the fast-dividing cells of the body, such as blood cells and the cells lining the mouth, stomach and intestines.[27] Alternative to these drugs, many researchers focusing on the herbal drugs that shows maximal anti-cancer effect with minimal side effects. The mechanism of inhibition of tumor progression by natural phytochemicals range from inhibition of genotoxic effects, increased anti-inflammatory and ant oxidant effect, inhibition of cell proliferation, protection of intracellular communications to modulate apoptosis and signal transduction pathways.

Various parts (stem, bark, leaves and roots) of *Spathodea campanulata* has been reported to show a various biological activity in traditional medicine and it has been used for malaria, diabetes mellitus, oedema, dysentery, constipation, GI disorders, ulcers, skin diseases, wounds, fever, urethral inflammation, liver complaints and as a poison antidote.[17,18]

### Phytochemical analysis

The preliminary phytochemical analysis of the ethanolic extract of *Spathodea campanulata* (EESC) showed the presence of steroids, flavonoids, triterpenes, proteins, alkaloids, carbohydrates and phenolic compounds.

Previous studies which can supports for our present study that the presence of phenolic and flavonoid contents showed a good inhibition of protein denaturation and antioxidant property in the rhizomes of *Nephrodium cicutarium* and leaves of *Spathodea campanulata*.[28] Another study reported that presence of flavonoids and phenols showed antioxidant activity of leaf extracts of *Gliricidia sepium* and *Spathodea campanulata* with good free radical scavenging activity.[29] According to the previous study, ethanolic extract of leaf of *Spathodea campanulata* showed higher antioxidant level and higher level of total phenolic content which in turn used to prevent or treat the oxidative stress damaged induced cancer.[30]

### Fluorescence Analysis

The fluorescence analysis of S. campanulata root bark powder showed brown colour under UV light of short wavelength (254nm) when treated with acetic acid, Iodine, $FeCl_3$, ammonia and when the powder was used as such as well as in the root bark extract in semisolid form under UV light of longer wavelength (366nm). Also, brown colour was observed under visible light when the root bark powder was treated with water, NaOH, acetic acid, alc. NaOH, $H_2SO_4$, Iodine, KOH, alc. KOH, ammonia and ethanol, as well as, when used in the powder form as such. Reddish brown colour was observed under UV light of long wavelength (366nm) when the root bark powder was treated with water, Iodine, $FeCl_3$ and ammonia. Brownish green colour was observed when root bark powder was treated with alc. KOH and black colour was observed when root bark extract was treated with ethanol under short UV light (245nm) and with $H_2SO_4$ under long UV (366nm) light. Dark red was observed when root bark extract was treated with ethanol under visible light. The powdered root bark showed orange colour under long UV light (366nm) when treated with acetic acid, alc. KOH, Picric acid, $HNO_3$, alc. NaOH, KOH and ethanol and as well as, when powder used as such. Also, orange colour observed in day light when root bark powder treated with HCl, Ferric chloride and $HNO_3$. The yellow colour was showed under short UV light (254nm) when root bark powder treated with nitric acid and KOH and also under day light when treated with picric acid. Green colour was observed under short UV (256nm) when root bark

powder treated with picric acid, alc. NaOH, $H_2SO_4$, HCl and ethanol. The root bark powder when treated under short UV light (254nm) with water showed pale green colour and with NaOH showed dark green colour. Blue colour was observed under long UV (366nm) light when root bark powder treated with NaOH and HCl.

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant. Some showed fluorescence in visible day light and some showed under UV light. The UV light produces fluorescence in many natural products which doesn't shows fluorescent under visible light. Therefore, it plays important role in qualitative analysis of Pharmacognostical evaluation and in determination of quality and purity of the drug.

**MTT assay**

MTT assay is a universally accepted *in-vitro* method for screening the drugs having cytotoxic activity. *In-vitro* cytotoxic activity against MCF7 cell line at different concentrations of ethanolic extracts of *Spathodea campanulata* (EESC) was evaluated. In our study, we showed that EESC inhibited the growth of MCF7 breast cancer cell lines and had strong cytotoxicity in a concentration-dependent manner. Cytotoxic effect against the MCF7 cell line is considered as a prognostic anti-cancer activity indicator. The cell viability of MCF7 breast cell lines was 51.19% and IC50 value calculated for EESC was 62.5µg/ml which indicates the presence of cytotoxic activity. Several authors reported that phenolic acids, flavonoids, steroids, terpenoids, glucosides are known to be bioactive principles undergoes either of mechanism that inhibits the cell cycle progression as a chemo preventive mechanism and induces cell arrest by down-regulation of cyclin/Cdk complex kinase activity, inhibition of E2F release in MCF-7 breast carcinoma cells which resulted into anti-proliferation activity.[31-33] The phytochemicals such as vitamins, saponins, flavonoids, terpenoids, polyphenols, alkaloids, tannins and minerals present in *Fragaria vesca* and *Rubus idaeus* block various hormonal actions and metabolic pathways that are associated with the development of cancer and showed antioxidant and cytotoxic activity.[34]

**DNA fragmentation assay**

Apoptosis is a programmed cell death characterized by cleavage of chromosomal DNA into oligonucleosomal fragments, plays an essential role in the anticancer properties of many anticancer molecules by preventing or controlling abnormal development.[35] Apoptosis was initially described by its morphological characteristics, including cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation.[36]

In our study, the result showed that degradation of chromosomal DNA into small inter nucleosomal fragment as shown in figure 17, when MCF7 breast cancer cell lines treated with ethanolic extract of *Spathodea campanulata* (EESC) which was regarded as a biochemical hallmark of cells undergoing apoptosis, thus fragmented DNA confirmed antiproliferative effect of the extract. Therefore, DNA ladder formation indicated that the cytotoxic effect of EESC caused inhibition in the growth of breast cancer through apoptosis. Previous study reported that the phenolics with antioxidant properties and flavonoids have been found to be beneficial in preventing or treating the oxidative damage induced cancers and also regulate the gene expression during cell proliferation and apoptosis process. The presence of flavonoids has been reported to modulate the key mechanism and receptors involved in signal transduction pathway of cell proliferation, differentiation, apoptosis, angiogenesis, inflammation, metastasis and reversal of multidrug resistance.[37-39]

**Chick Chorioallantoic Membrane (CAM) Assay**

The cancer biologists, developmental biologists and ophthalmologists have described the chick Chorioallantoic membrane (CAM) as a model system for studying development, cancer behavior, properties of biomaterials, angiogenesis and photodynamic therapy. This assay is the most widely used assay for screening of both the angiogenesis and anti-angiogenesis substances. The CAM assay is relatively simple and inexpensive and thus suitable for large scale screening.[40] The CAM is composed of a multilayer epithelium; the ectoderm at the air interface, mesoderm (or stroma) and endoderm at the interface with the allantoic sac. Furthermore, the CAM contains extracellular matrix proteins (ECM) such as fibronectin, laminin, collagen type I and integrin $\alpha v\beta_3$. The presence of these extracellular matrix proteins mimics the physiological cancer cell environment.[41]

In our study, the ethanolic extract of *Spathodea campanulata* P. beauv (EESC) root showed the antiangiogenic property on the dose dependent manner. As the concentration increases the angiogenesis process was inhibited which revealed the anti-angiogenic activity of the EESC. The various concentration of EESC (10, 25, 50, 100, 150 and 200µg/ml) showed an anti-angiogenic activity based on the concentration showed in ascending order and the result was compared with the standard (Prednisolone 5mg/ml) and control (0.9% NaCl) groups. A marked increase in the blood vessels was noted in normal saline (negative control) when compared to Prednisolone (Positive control). The percentage inhibition for normal saline was -59.95 ± 3.181 (*$p<0.05$) and not significant with the Prednisolone which expressed as 67.94± 2.881(*$p<0.001$). The % inhibition was calculated for various concentration of EESC and p value was compared with positive control. The 10µg/ml concentration of EESC showed less inhibition and was found to be 9.67 ± 0.494 (*$p<0.05$) less significant when compared to prednisolone. The concentration of 25 and 50µg/ml of EESC was observed as moderate inhibition of 18.83 ±1.956 (**$p<0.01$) and 32.83 ±2.414 (**$p<0.01$) which showed significant result when compared to positive control. The concentration of 100, 150 and 200µg/ml of EESC was found to be 44.33 ±3.029 (***$p<0.001$), 57 ±2.683 (***$p<0.001$) and 62.7

±3.232 (***p<0.001) sowed high significant when compared to prednisolone. The previous study reported that, CAM assay model has emerged as one of the important animal models to understand the anticancer property of herbal extracts.[6,42] Our study was based on the indirect anticancer property of the plant was through the method of antiangiogenesis. With the drug in a quantity close to 10µg/ml bringing angiogenesis in the chick embryo to a halt without becoming lethal to the developing chick embryo itself was interesting and promising. Previous studies have reported, by blocking the development of new blood vessels, one hopes to cut off the tumor's supply of oxygen and nutrients and therefore, its growth and spread to other parts of the body. This may be due to the induction of apoptosis by phytochemical present in these plants.[4]

## CONCLUSION

The result of the investigation concludes that the presence of phytochemical constituents in ethanolic extract of *Spathodea campanulata* showed good antioxidant property. By MTT assay, the IC50 value of ethanolic extract of *Spathodea campanulata* was concluded that EESC possessed cytotoxicity effect. From DNA fragmentation assay, the ethanolic extract of *Spathodea campanulata* possessed apoptotic activity. By Chick Chorioallantoic membrane (CAM) assay, the ethanolic extract of *Spathodea campanulata* exerted the anti-angiogenic property. Our current investigation supports the ethanolic extract of *Spathodea campanulata* as an anti-angiogenesis and anticancer agent in the traditional medicine system.

## ACKNOWLEDGEMENT

We thank the Management and Department of Pharmacology, K K College of Pharmacy for excellent support, encouragement and valuable advice throughout this work. We would like to express my sincere thanks to the Life Tech Research Lab, Chennai and Microbiology Lab, Vellore for their support.

## REFERENCES

1. Jaywant Jadhav *et al*. Antiangiogenic properties of *Boerhaavia diffusa* extracts in chick Chorioallantoic Membrane (CAM) assay. *Int. J. Drug Dev. & Res.*, 2011; 4: 307-317.
2. Folkman J *et al*. Angiogenesis in cancer vascular rheumatoid and other diseases. *Nature Med.,* 1995; 1: 27-31.
3. Mohd Rias Mustafa *et al*. *In vitro* and *invivo* anti-angiogenic activities of Panduratin A. PLoS ONE, 2012; 7: 38103.
4. Kota K *et al*. Study of antiangiogenic activity of aqueous extract of *Nigella sativa* seeds in chick chorioallantoic membrane (CAM) model. *International Journal of Advances in Medicine*, 2018; 5: 895-900.
5. Liu C *et al.* Inhibition of tumor angiogenesis by TTF1 from extract of herbal medicine. *World J Gastroenterol*, 2011; 17: 4875-4882.
6. Magdalin Joy A. L. *et al*. Antiangiogenic activity of *Strychnos nux vomica* leaf extract on chick chorioallantoic membrane mode. *J. Chems. Pharm., Res.,* 2016; 8: 549-552.
7. Shaker A. Mousa *et al*. The Role of Angiogenesis in Cancer Treatment. *Biomedicines*, 2017; 5: 34.
8. Angel Medina M. *et al.* Angiogenesis and signal transduction in endothelial cells. *Cell Mol Life Sci.*, 2004; 61: 2224–2243.
9. https://www.indiatoday.in/education-today/gk-current-affairs/story/cancer-rate-india-stats-cure-treatment-1386739-2018-11-12.
10. www.googleimages/356-india-fact-sheets-1.jpg
11. Samant RS. *et al.* Recent advances in anti-angiogenic therapy of cancer. *Oncotarget*, 2011; 2: 1–13.
12. Wakelee H.A. *et al.* Monoclonal antibodies targeting vascular endothelial growth factor: Current status and future challenges in cancer therapy. *Bio Drugs*, 2009; 23: 289–304.
13. Angiogenesis Inhibitors – National Cancer Institute – Fact Sheet. Reviewed 0n, 2017.
14. Bae H. *et al.* Antiangiogenic phytochemicals and medicinal herbs. *Phytother Res.*, 2011; 25: 1–10.
15. Sujit Basu *et al.* Plants and their active compounds: natural molecules to target angiogenesis. *Angiogenesis, HHS Public Access*, 2016; 19: 287-295.
16. Wong R.K., *et al.* Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer-part 1. *Current Oncology, Review article*, 2006; 13: 14-26.
17. Anita, *et al.* Plant Profile, "Phytochemistry and Pharmacology of *Spathodea Campanulata. International journal of pharmacy and pharmaceutical sciences*, 2018; 10: 1-6.
18. Burkill HM, The useful plant of west tropical Africa. 2 edition, United Kingdom, 1985.
19. Yogananarasimhan S. N. *Medicinal plant of India interline publishing pvt ltd.* 1, Karnataka, 442.
20. Ram.p. Rastogi, B.N. Mehrotra. Compendium of Indian medicinal plants, 1970-79; 5: 800-801.
21. Drug screening methods. 2nd edition, Jaypee Brothers medical publishers; S. k. Gupta.
22. J. L. Kaspers. Cell Sensitivity Assays: The MTT Assay. Cancer cell culture, 2011: 237-245.
23. Mitsuhiro Yokoyama *et al*. Harmonic Interplay of Angiogenic Growth Factors in the Development of Coronary Blood Vessels. *Circ Res.,* 2001; 88: 1099-1101.
24. Bae H. *et al.* Antiangiogenic phytochemicals and medicinal herbs. *Phytother Res.*, 2011; 25: 1–10.
25. www.google.in/cancer.net-the-hindu.
26. www.wikipedia.in/caner-and-its-therapeutics.
27. Maushumi Kulkarni *et al*. Total flavonoid, total phenolic content, in-vitro anti-inflammatory and antioxidant activity of extracts of two medicinal

plants. *Journal of Pharmacy Research,* 2014; 8: 584-588.
28. F.C. Akharaiy *et al*. Antibacterial, Phytochemical and Antioxidant Activities of the leaf extracts of *Gliricidia sepium* and *Spathodea campanulata*. *World Appl. Sci. J.,* 2012; 16: 523-530.
29. Vellingiri Vadivel *et al*. Evaluation of total phenolic content and antioxidant activity of different solvent extracts of leaf material of *Spathodea campanulata* P. Beauv. And investigation of their proliferation inhibition potential against EAC cell line. *Journal of Applied Pharmaceutical Science*, 2016; 6: 121-127.
30. Rathinam Prema *et al*. Invitro cytotoxicity study on combined plants extracts (Cissus quadrangularis and Aegle marmelos). *European Journal of Experimental Biology,* 2012; 2: 882-888.
31. Shankul Kumar *et al*. Antioxidant and in vitro cytotoxic activity of extracts of aerial parts of Cocculus hirsutus (L) using cell line cultures (breast cell line). *The Journal of Phytopharmacology,* 2014; 3: 395-399.
32. Shun Wong *et al*. Pharmacogenomics and the Yin/Yang actions of ginseng: anti-tumor, Angio modulating and steroid-like activities of ginsenosides. *Chinese Medicine,* 2007; 2: 1-21.
33. Shaikh *et al*. Evaluation of Anticancer, Antioxidant, and Possible Anti‐inflammatory Properties of Selected Medicinal Plants Used in Indian Traditional Medication. *Journal of Traditional and Complementary Medicine*, 2014; 4: 253-257.
34. Susan Elmore. Apoptosis: A Review of Programmed Cell Death. HHS public access, 2007; 35(4): 495-516.
35. Cell death: apoptosis. Cellular and molecular biology, 2016.
36. Potchanapond Graidist *et al*. Cytotoxic Activity of Piper cubeba Extract in Breast Cancer Cell Lines. *Nutrients,* 2015; 7: 2707-2718.
37. Chaitanya G Joshi *et al*. Antiproliferative activity of crude extract and different fractions of Butea monosperma against lung cancer cell lines. *Indian J Pharm Sci.,* 2018; 80: 875-882.
38. Deep Jyoti Bhuyan *et al*. Phenolic Compounds Potential Health Benefits and Toxicity. Utilization of Bioactive Compounds from Agricultural and Food Waste, 2017; 2.
39. Vinoth Prabhu V *et al*. A Review on Angiogenesis Assays. *International Journal of Pharmaceutical Sciences and Drug Research,* 2010; 2: 159-164.
40. Martin K. Oehler *et al*. Chick Chorioallantoic Membrane (CAM) Assay as an In Vivo Model to Study the Effect of Newly Identified Molecules on Ovarian Cancer Invasion and Metastasis. *Int. J. Mol. Sci.,* 2012; 13: 9959-9970.
41. Jin-zhong Wu *et al*. Anticancer and anti-angiogenic activities of extract from *Actinidia eriantha* Benth root. *Journal of Ethnopharmacology,* 2017; 203: 1–10.
42. Muhammad Farhan Bashir *et al*. Effect of ginger extract on angiogenesis using CAM assay. *Bangladesh J Pharmacol,* 2017; 12: 348-353.