RESEARCH PAPER

Exploring the anticancer potentials of canavalia virosa (roxb) wight &arn ethanol extract against in vitro and in vivo animal model

C. Bhuvaneswari[1*] , R. Sivasubramanian[2], G. Sridharan[3]

[1*]Department of Nutrition and Dietetics, Dr. Kalaignar Government Arts College, Affiliated to Bharathidasan University, Kulithalai, Tamil Nadu – 639 120, India. Email Id: hariharanbhuvana5@gmail.com
[2]Department of Biochemistry, Srimad Andavan Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu - 620 005, India. Tel: + 91 9994378860, Email Id: biosiva2000@gmail.com
[3]Department of Biochemistry, Srimad Andavan Arts and Science College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu - 620 005, India. Tel: + 91 9994378860,

***Corresponding Author;** C. Bhuvaneswari
Email Id: hariharanbhuvana5@gmail.com

Abstract

Aims: Cancer is a heterogeneous group of diseases that is characterized by unrestrained growth. It is a diverse set of disorders characterized by unrestrained cellular proliferation, local invasion, and distant metastasis, and is one of the most important problems in modern medicine.

Methodology: Medicinal flora represents an abundant source of bioactive metabolites, and many successful antineoplastic agents have been obtained from plant-derived phytochemical substances. The Fabaceae family haslisted several sizable anticancer constituents. Accordingly, Kattuthampattan, which botanically is Canavalia virosa (Roxb.), was selected for the present study.Wight &Arn, evaluation of its antioxidant & antitumor activity against Ehrlich Ascites Carcinoma (EAC).

Results: Preliminary research for phytochemicals of the ethanolic extract of C. virosa (EECV) revealed the presence of tannins, alkaloids, flavonoids, phenols, and saponins; quantitative determination showed the presence of alkaloids in high and significant amounts. The in vitro antioxidant potential was assessed using DPPH, ABTS, and reducing power measures, all of which demonstrated good free radical scavenging activity. Cytotoxic and antiproliferative activities against EAC cells were determined using trypan blue exclusion, MTT, LDH, and DNA fragmentation test. EECV showed dose-related cytotoxicity and caused apoptosis in the tumour cells. In vivo antitumor efficacy using an experimental model of cancer in Swiss albino mice with EAC was studied. Treatment with EECV (150 and 300 mg/kg of body weight) for 14 days caused a significant decrease in ascitic tumour volume, viable tumour cell count, nucleic acid and glycoprotein concentrations, and reversed the altered hematological and antioxidant profiles. Furthermore, EECV significantly increased the survival of tumour-bearing mice, effects commensurate with those of a standard chemotherapeutic agent, 5-fluorouracil.

Conclusion: These data collectively show evidence that the ethanolic extract of Canavalia virosa harbours potent antioxidant, cytotoxic, and antitumour activities, thus the potential of this medicinal plant as a natural anticancer therapeutic.

Keywords: Cancer, Canavalia Virosa (Roxb.), EAC, Anti-Tumor, EECV, In-Vitro, In-Vivo

How to cite this article: Bhuvaneswari C, Sivasubramanian R, Sridharan G. Exploring the anticancer potentials of canavalia virosa (roxb) wight & arn ethanol extract against in vitro and in vivo animal model. Int J Drug Deliv Technol. 2026;16(62s): 400-415. DOI: 10.25258/ijddt.16.62s.47

Introduction

Millions of cells make up the human body, and each cell divides, grows, and dies normally. Sometimes the mechanism malfunctions, causing unregulated cell growth that eventually results in cancer. When cancer cells unite, they become tumors, which are additional masses of tissue. Cancer is a prevalent illness that spreads via the body's bloodstream. Certain tumors, such as benign tumors, develop uncontrollably but do not spread across the body. Their growth is regulated by normal cells, which self-destruct when unhealthy [1]. For thousands of years, people have utilized natural goods, particularly plants, to treat a variety of illnesses. Egypt, India, China, and Greece have all used terrestrial plants as remedies for a very long time. These plants are employed to develop contemporary drugs. The first recorded descriptions of plant drugs date back to 2600 BC and were written by the Sumerians and Akkadians [2]. Ayurveda is an ancient Indian medicinal practice that uses a variety of plant sources to prevent or inhibit different kinds of tumors and treat them in different ways. The National Cancer Institute got 35,000 plant samples across 20 nations, analyzed around 114,000 extracts demonstrating anti-cancer effectiveness. There are more than 3000 plant species known to have antitumor effects. Cancer is a common disease in humans, ongoing development of novel anticancer drugs derived from natural products is currently of great scientific and commercial interest.

Natural products are considered significant sources of potential chemotherapeutic agents, as well as various anticancer drugs with natural origins. According to [3], more than half of the drugs being evaluated for anticancer effects within clinical settings were either sourced from or associated with natural sources.Plant-based anticancer drugs that are now in use include vincristine, camptothecin, taxol, and podophyllotoxin. Research on employing medicinal plants as sources for drug discovery is active in the domains of cancer and infectious diseases. 60% anticancer, along with 75% anti-infectious drugs of natural origin, are approved by the FDA. Whether they are pure chemicals or plant extracts, several in vitro as well as in vivo methods have been developed to evaluate the efficacy of natural cancer drugs.
Therefore, the objective of the current investigation was to choose a common plant with a traditional origin. The ability of selected plant extracts to stop the development of Ehrlich ascites carcinoma cell lines was then examined.

Highlights
- Ethanol extract of Canavalia virosa shows strong anti-cancer activity on Ehrlich ascites Carcinoma.
- High alkaloid content contributes to major cytotoxicity and antiproliferative activity.
- In the latter, "the extract induces apoptosis and inhibits proliferation of tumor cells in vitro."
- Especially in vivo treatment reduces tumor burden and prolongs survival in EAC-bearing mice.
- Antitumor action occurs by mediating antioxidant defense and normalization of metabolism.

2. MATERIALS AND METHODS

2.1Identification and Authentication

The current study used Canavalia virosa (Roxb.)Wight&Arn as its plant source. The selected plant's leaves had been collected from the vicinity of Trichy, recognized utilizing the Flora of Presidency, Madras [4], verified using specimens from the Department of Botany at St. Joseph's College, Trichy's RAPINAT Herbarium.

2.2Determination of Total Ash[5],

In a silica dish, 2g of a carefully weighed plant drug was ignited at utmost 450°C until it was carbon-free and weighed. The air-dried drugs were used to compute the percentage of ash.

2.3Determination of Acid Insoluble Ash

25ml diluted HCl had been used for boiling the total ash for 5min. To collect the insoluble elements, ash-free filter paper that has been washed using hot water, and burned to constant weight is used. The percentage of ash that is insoluble in acid has been determined using air-dried drugs.

2.4Determination of Water Soluble Ash

Remaining ash from the total amount of ash had been heated for 5min after 25mldistilled water had been added. Insoluble material had been collected using ash-free filter paper,hot water washing was done, andthen burned at 450 degrees Celsius for 15min. The difference between the weight of the ash and the weight of insoluble elements indicates the weight of water-soluble ash. The percentage of water-insoluble ash in the drugs that had been air-dried had been estimated.

2.5Determination of Extractive Values

Hexane, chloroform, and ethyl acetate were among the solvents used in increasing polarity to extract ten grams of the air-dried plant-based drugs from an extraction thimble using a Soxhlet's extraction apparatus (for six hours). After filtering the extract into a tarred evaporating dish, the solvent was evaporated in a water bath. Residue reached a constant weight after drying at 105 degrees Celsius. Employing air-dried drugs as a standard, the extractive values of various solvents had been estimated.

2.6Determination of Alcohol Soluble Extractive

A closed flask containing 5g of air-dried drugs along with 100ml alcohol had been macerated for 24 hours. After being shaken a lot for the first six hours, the flask was allowed to stand for the next eighteen hours. Immediately filtered with caution to prevent solvent loss. 25 ml of filtrate were dry evaporated in a tar-covered, flat-bottomed china dish, mixture had been dried at 105 degrees Celsius until weight remained constant. Calculated drug extract’s percentage that was soluble in alcohol after being air-dried.

2.7Determination of Water-Soluble Extractive

5g air-dried drugs, and 100ml water were placed in a closed flask, shaken frequently for the first 6 hours before being permitted to stand for 18 hours. Place 25 ml of filtrate in a flat-bottomed, tarred china dish, immediately filter, and evaporate until dry, then dry at 105 degrees Celsius until the weight stays constant. With the drug air-dried, the percentage of water-soluble extractives had been determined.

2.8Fluorescence Analysis[6],

After being treated with the drug's acidic and alkaline solutions, as well as in daylight and ultraviolet light, the drug's fluorescence was measured using a range of solvent extracts. Powder had been treated with neutral solvents such as benzene, hexane, ethyl acetate, chloroform, alcohol, and acetone; alkaline solutions including alcoholic and aqueous 1N NaOH; and acids including 50% sulphuric acid and 1N hydrochloric acid.

2.9Preliminary phytochemical screenings of various extracts and drug powder

The standard textual technique was used to perform preliminary phytochemical testing on a variety of extracts and drug powders [7].

2.10Quantitative estimation of the secondary metabolites and vitamin C

2.10.1 Estimation of phenol[8].

Ten times the volume of 80% ethanol was used to weigh and thoroughly grind one gram of the substance. For 20 minutes, the homogenate had been centrifuged at 10,000rpm. After collecting the supernatant, five times as much 80% ethanol was used to extract the residue again. After another centrifugation, the sample's supernatant has been collected. After that, it evaporated until it was dry. The resulting residue was dissolved in 5mldistilled water.0.5ml sample had been pipetted into a test tube, 3 distilled water had been added to raise the volume. After 3min, 0.5ml Folin's reagent was added, then 2ml of a 20% sodium bicarbonate solution. Following a minute in a boiling water bath, the components had been well mixed, the test tubes had been cooled, colour produced had been determined at 650nm.

2.10.2 Estimation of total Flavonoids

Ethyl acetate was used to extract plant extracts from water. A boiling water bath was used to concentrate the extracts until they reached a concentration of 1g/ml after they had been filtered and dried over anhydrous sodium sulphate. Using ethyl acetate to further dilute them produced the 0.01g/ml solution used in the investigations. A 25ml volumetric flask was filled with around 10ml of solution, 1ml of 2% aluminum chloride, and methanol-acetic acid. After the solution was left for half an hour, absorbance at 390nm was measured, and a blank was kept. A concentration range of 1-10µg/ml had been employed to generate a quercetin calibration curve.

2.10.3 Estimation of total Alkaloids[9].

After the alcoholic extract from the plant sample had been treated employing 0.1N HCl, chloroform was used in a separating funnel to separate the resultant aqueous acidified layer. Following the removal of the chloroform layer, the aqueous layer was basified using ammonium hydroxide to an alkaline pH, separated with chloroform in a separating funnel. After discarding the aqueous layer,along with evaporating the chloroform layer, the resulting material was handled as a total alkaloid and verified by using Dragendorff's reagent.

2.10.4 Determination of Ascorbic acid (vitamin C)[10].

After adding 1.0ml TCA and 0.5ml water to 0.5ml homogenate, the mixture was well combined and centrifuged. After adding 0.2 mL DTC reagent to 1.0 mL supernatant, the mixture was incubated for 3 hours at 37 degrees Celsius. Solutions had been kept at room temperature for a further half hour after 1.5ml sulfuric acid had been added until completely mixed.A UV spectrophotometer was used to determine the color produced at 520 nm.

2.11 In-vitro Antioxidant assay

2.11.1 DPPH Radical Scavenging Assay[11].

DPPH was used to assess the ethanolic extract of Canavalia virosa (Roxb) Wight and Arn's ability to scavenge free radicals. A solution of DPPH (200µM) was made in 95% methanol. Five test tubes were filled with 50, 100, 150, 200, and 250 µg/ml of the stock plant extract solution. Spectrophotometer had been employed to estimate absorbance at 517nm following a 10-min incubation period with the test drug in 0.5ml of freshly made DPPH solution. The reference was standard ascorbic acid.

2.11.2 Reducing the Power of EECV

One milliliter of extract was mixed with 1.5ml potassium ferricyanide and 2.5mlphosphate buffer at several doses (100–500 µg/ml). The mixture had been incubated for 20 minutes at 50 degrees Celsius. The mixture was mixed with 2.5ml TCA in aliquots and centrifuged for 10min at 3000rpm.Absorbance at 700 nm was measured after adding 0.5ml freshly prepared ferric chloride solution to the upper layer of the solution (2.5ml) that had been combined with an equivalent volume of distilled water. The reaction mixture's higher absorbance indicates an increase in reducing power.

2.11.3 ABTS+ Radicals Scavenging Activity

A series of test tubes was filled with conventional ascorbic acid solutions and plant extract at varying concentrations (100-500µg).After adding 0.3ml ABTS solution, the volume increased to 2.5ml using phosphate buffer. 0.3 ml ABTS solution as well as 2.2ml phosphate buffer remainedadditional to the control. The solutions were measured proximately at 734 nm.

2.12 ANTICANCER SCREENING

2.12.1 Experimental animals

The Tamilnadu Veterinary and Animal Sciences University in Chennai provided a total of 25-35g of healthy adult male as well as female Swiss Albino mice. The animals were allowed five days to acclimate to the lab setting before the trial. Standard polypropylene cages were utilized to house the animals. Each cage included six animals to provide them with enough room and avoid unnecessary sickness, leading to mortality. The animals were kept in a typical atmosphere with a 12-hr light/dark cycle at 23±2°C, along with 65±5% absolute wetness. The rodents were given an ordinary rat food pellet via Sai Durga Foods and Feeds, Bangalore, India, along with a

continuous supply of water. After receiving committee permission, every study was conducted in accordance with the CPCSEA's ethical criteria (Approval No: SAC/IAEC/BC/2015/M.phil-004).

2.12.2 Maintenance of Cells[12].
The Ehrlich Ascites Carcinoma cells had been supplied by Amla Cancer Research Centre in Thrissur, kept at a rate of 1x106 cells/mouse by weekly intraperitoneal inoculation.

2.12.3 Preparation of ethanol extract
For 48 hours, 250g of dehydrated plant material had been immersed in ethanol. After the solution was filtered, evaporation was used to dry the filtrate. For the investigation, the residue was dissolved in isotonic saline.

2.13 In-Vitro Cytotoxicity Assay
2.13.1 Trypan blue method[13].
Ehrlich Ascites Carcinoma cell lines were used to examine short-term in-vitro cytotoxicity of Canavalia virosa (Roxb.) Wight & Arn's ethanol extract, which was incubated for three hours at 37°C. An insulin syringe was used to remove the tumor cells from the tumor-bearing mice's peritoneal cavity and place them in a test tube filled with isotonic saline. A hemocytometer was employed to determine the cell count, which was subsequently adjusted to 1 × 10^6 cells/ml after the cells had been washed in normal saline. Different extract concentrations (100-1000µg/ml) had been added to each tube for the cytotoxicity test, and the final volume had been adjusted to 1ml using normal saline. There was no plant extract in the control tubes containing saline and tumor cells. For three hours, each tube was incubated at 370 degrees Celsius. Following incubation, 0.2% trypan blue dye and isotonic saline were added to 0.1ml test sample in a watch glass. Employing a hemocytometer, the number of viable (unspotted) along with non-viable (damaged) cells was measured.

$$\% \text{ Dead cells} = \frac{\text{Entire cells counted} - \text{total viable cells}}{\text{Entire cells counted}} \times 100$$

2.13.2 MTT ASSAY[14].
The cells were exposed to higher concentrations of EECV and cultured for 24 hours at 37 degrees Celsius in a CO2 incubator containing 5% CO2. A new growing medium containing 20µl of 3(-4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide(MTT,Sigma), along with MTT reagent were employed. Incubated at 37 degrees Celsius for 4 hours.Following incubation,cells were provided with 200µl of 0.1% 0.1N acidic isopropyl alcohol for dissolving Formazan crystals after the growth media was removed, and purple precipitate was observed under an inverted microscope. Covered plates had been stored in the dark for the entire night at 18-24 degrees Celsius. Every two hours, samples were taken, and the reading at 570 nm was recorded. Return the plate for incubation if the reading is low. Every experiment was supported by three spells. After calculating the average, it was compared to control test samples. Using the formula, the growth inhibition percentage was determined:

$$\% \text{ Growth Inhibition} = \frac{\text{Control OD} - \text{Treated OD}}{\text{Control OD}} \times 100$$

2.13.3 Estimation of Lactate Dehydrogenase Release Assay[15].
By examining NADH decrease during the pyruvate lactate transition, lactic dehydrogenase activity is spectrophotometrically evaluated at 340 nm in both culture media and cellular lysates. 50 mM Tris-HCl buffer (0.1ml), pH7.4 in addition to 0.1 ml Lysing the cells with 20 mM EDTA and 0.1ml of 0.5% SDS, followed by a 15-min centrifugation at 13,000xg. 0.2 milliliter of 1 M pyruvate, 0.2 milliliter of 0.2 mM NADH, as well as 33µlplant extract (50-250µg/ml in 48mM PBS) were added to the precipitate. In a UV spectrophotometer, a drop in NADH was detected at 340 nm after a 15-min incubation period.

2.13.4 DNA Fragmentation assay
Following the addition of escalating concentrations of EECV (10, 25, and 50 µg) to the EAC cells, which had been cultivated for 24hours at 37 degrees Celsius in a CO2 incubator containing 5% CO2. After the sample had been incubated, it was centrifuged at 10,000 rpm and 4 degrees Celsius. Three PBS washes were performed on the resultant pellet. After adding 0.1ml DMSO to the pellet, the mixture was thoroughly mixed using the vortexing technique. Additionally, 0.1ml SDS, along with 0.1ml TE buffer, had been added. The sample underwent centrifugation at 12,000 rpm at 4degrees Celsius. 40µl of the supernatant was exposed to a 2% agarose gel electrophoresis for visualizing DNA fragmentation.

2.14 IN-VIVO STUDIES
2.14.1 Experimental design
Animals were split into 5 groups, each consisting of 6 mice of each sex.
Group I- Normal Control
GroupII- Ehrlich Ascites Carcinoma cell line (1X106cells/mouse) (Disease Control)
GroupIII - Ehrlich Ascites Carcinoma cell line (1 × 10^6 cells/mouse) treated with
150mg /kg bw. Of EECV
GroupIV- Ehrlich Ascites Carcinoma cell line (1 × 10^6 cells/mouse) treated with
300mg /kg bw. of EECV
Group V - Ehrlich Ascites Carcinoma cell line (1 × 10^6 cells/mouse) treated with
Standard drug 5- Flurouracil (20mg/kg.bw)

EECV - Ethanolic extract of Canavalia virosa (Roxb) Wight and Arn
After 24 hours following EAC inoculation, the plant extract treatment began. The plant extract had been given orally to Group III and IV animals for 14 days at three

distinct dose levels: 150, 300, and 300 mg/kg bw. Group V mice were injected intraperitoneally with 5-fluorouracil for 14 days. Following the experiment, the ascites fluid was collected, and the animals were sacrificed via cervical decapitation. Collected blood used for performing biochemical tests. After being removed, liver tissue was homogenized using 0.1M phosphate buffer (pH 7.4), then rinsed using ice-cold saline. Its glycoprotein content as well as levels of antioxidants were determined.

2.14.2 Survival time [16]

Swiss albino mice were infected with 1 × 10^6 cells/mouse on day 0 and then split into 6 groups of 6 each. Groups III, IV, and V received dosages of 150 and 300 mg/kg bw of EECV for 14 days. Group II animals received normal saline as a tumor control. The % increase in life span had been evaluated by employing formulamentioned below.

ILS (%) = {(mean survival time of treated group/mean survival time of control group)-1} X 100.

MST = (Day of first death + day of last death)/ 2.

2.14.3 Tumour volume

After the mice had been dissected, ascitic fluid through the peritoneal cavity had been extracted.A graduated centrifuge tube was used to measure the volume, and then centrifuging at 1000 rpm for 5 minutes was used to calculate the packed cell volume.

2.14.4 Tumor Cell Count

Following extraction, the ascitic fluid had been diluted 100 times employing a WBC pipette. The number of cells in 64 small squares had been counted utilizing drop of diluted cell solution in a Neubauer counting chamber.

2.14.5 Viable/non-Viable Tumor Cell Count

A WBC pipette was used to dilute the ascitic fluid 100 times. Trypan blue dye, which is 0.4% in normal saline, has been employed to stain cells. Stain-absorbing cells were regarded as nonviable, whereas the non-absorbing cells were regarded as viable. Thus, the number of viable along with nonviable cells, had been determined.

$$\text{Cell count} = \frac{\text{No of cells X Dilution}}{\text{Area X Thickness of liquid film}}$$

2.15 Hematology

2.15.1 Determination of Hemoglobin[17]

The 0.01N hydrochloric acid was added to Sahli's pipette until it reached the 20-mark. After that, 20µlblood was added, thoroughly mixed, and kept at room temperature for 10 minutes. Distilled water had been used to dilute the mixture until the color matched the standard provided by Sahil's hemoglobin meter. The hemoglobin level was stated as (g%).

2.15.2 Determination of Red Blood Cell Count

The RBC pipette was filled with 20µl of blood. RBC diluting fluid was used to dilute it to the appropriate level. The blood was diluted 200 times. After using cotton to clean the haemocytometer, the counting chambers were examined under a microscope. After that, the counting chamber was put on stage and filled with diluted blood. The quantity of red blood cells was recorded and documented. Millions of cells/mm3 were used to express the outcome.

2.15.3 Determination of Total Count of WBC [18]

A WBC pipette was used to collect 20µl of blood. WBC dilution fluid was used to dilute it to the appropriate level. The blood was diluted 20 times. After using cotton to clean the hemocytometer, the counting chambers were examined under a microscope. After that, the counting cups were put on the microscope's stage and filled with diluted blood. White blood cell count was measured. After calculating the counter-vailing white blood cells, the outcome was given as millions of cells/mm3.

2.16 Estimation of Nucleic Acids Content

2.16.1 Extraction of Nucleic Acids

Nucleic acid extraction was done by Schneider's recommendations [19]. 500 mg of liver tissue had been homogenized using 5.0mlof ice-cold distilled water. The proteins and nucleic acids were allowed to fully precipitate by mixing 5% TCA with 5.0 ml of ice homogenate and keeping it on ice for 30 min. After centrifuging the mixture, ice-cold 10% TCA was used three times to wash the precipitate. To get rid of the lipids, it was then processed with 95% ethanol. Nucleic acids and proteins were quantitatively separated by dissolving the resultant precipitate in 5 mM NaOH and heating it to 90 degrees Celsius for 15 min while shaking it frequently. The amount of DNA and RNA in the solution (nucleic acid extract) was estimated.

2.16.2 Estimation of DNA by diphenylamine reaction [20].

The volume was increased to 3 ml by adding distilled water after pipetting off 0.5 ml of the test solution and aliquots of standards. The sample received the same treatment. The DPA reagent, which contained 5ml each tube, was submerged in a boiling water bath for 10 minutes. At 600 nm, the colour's intensity had been measured after cooling.

2.16.3 Estimation of RNA reaction [21].

The volume was adjusted with water to reach 2.5 ml after pipetting a series of standard (0.5-2.5) ml and 0.5 ml test solutions into the test tubes. Each test tube was filled with 3mlBial's reagent. Boiled for one hour in a water bath. The green colour's intensity was determined at 680nm.

2.17 Serum Heapatic Marker Enzymes

1ml buffered substrate and one milliliter of serum were added to a series of tubes, incubated for 15 minutes at 37 degrees Celsius. Incubation period was prolonged by an additional 15min following the addition of 0.2ml of NAD solution. After adding 0.1 ml DNPH reagent for stopping the reaction, the tubes were incubated at 37 degrees

Celsius for an additional 15min. Following the reaction's termination with DNPH, blank tubes were filled with 0.1ml of serum. A Systronics 119 UV Spectrophotometer was used to measure the color that was produced at 420nm after adding a 7.0ml solution of 0.4N sodium hydroxide. The same method had been used to examine appropriate aliquots of the standards. The U/L expression of the enzyme activity

2.17.1 Assay of Aspartate Transaminase(AST) [22].
The two tubes, designated "Test" and "Blank," were filled with 0.5 ml buffered substrate each and stored at room temperature for a few minutes. The "Test," after adding 0.1mlserum, had been incubated for 60 minutes at 37 degrees Celsius. It had been directly heated on the burner until turbidity had taken place, then allowed to cool before 0.5ml DNPH had been added. It was mixed and stored for 30 minutes at room temperature. 5ml of 0.4N NaOH had been added, mixture had been incubated for 10min. Optical density had been measured using a green filter (520 nm).

2.17.2 Assay of Alanine Transaminase (ALT) [22].
The buffered substrate was added to the two tubes marked "Test" and "blank" in amounts of 0.5ml each, and allowed to rest at room temperature for a few minutes. There were 0.4 ml of serum given to the "Test". After that, the tubes are incubated at 37 degrees Celsius for 30 minutes. Pour 0.5 ml of DNPH. Incubate for 20 min at room temperature after thoroughly mixing. 0.4N sodium hydroxide (5 ml) was then added. Using a green filter (540 nm), the optical density was measured after 5 minutes.

2.18 Analysis of Hepatic Glycoprotein Contents
2.18.1 Estimation of Protein-bound Hexose [23].
Alcohol was used to precipitate 1 milliliter of homogenate. 2ml of 4N HCl had been employed to hydrolyze the alcoholic precipitate for four hours at 100°C. Sodium hydroxide was used to neutralize the hydrolyzed substance. 1 ml of solution was created by adding 0.5ml water along with various standard concentrations to 0.5 ml of neutralized sample. After setting each tube on ice, 2.0ml Orcinol-Sulphuric acid reagent had been gradually added. For 15min, tubes were heated to 80 degrees Celsius. Tubes were cooled in ice bath before the color intensity at 540nm had been estimated. Amount of hexose had been evaluated from absorbance employing a standard graph. Hexoses were measured in mg/g of dry defatted tissue.

2.18.2 Estimation of Protein Bound Hexosamine[24].
Using alcohol, the tissue homogenate was precipitated. At 100°C for four hours, 2 ml of 4N HCl was used to hydrolyze the alcoholic precipitate. Sodium hydroxide was utilized to neutralize the hydrolyzed substance. The neutralized sample had been mixed with 0.8ml acetyl acetone reagent (0.6 ml). Water (0.8 ml) is contained in the blank. A hot water bath was used to heat the tubes for half an hour. After the tubes were completely cooled, 2.0ml Ehrlich reagent had been added. At 540 nm, the color developed was measured after each tube had been thoroughly shaken. Using a standard graph, the amount of hexosamine was estimated. mg/g of dried defatted tissue containing hexosamine.

2.18.3 Estimation of Sialic Acid [25].
The test tube included 0.2ml hydrolyzed sample, while the control tube had 0.2ml distilled water. Sodium meta per iodide reagent (0.2ml) had been put into each test tube and left at room temperature for 30 min. 0.2 ml Sodium Arsenate Reagent, along with 0.3 ml ThioBarbutric Acid, had been added to each test tube. 3 ml of acidified butanol was added. In a boiling water bath, tubes were heated for 15 minutes. Following centrifugation, the pink butanol phase's absorbance at 540 nm was evaluated.
The amount of sialic acid had been estimated by employing a standard graph. The amount of sialic acid in each gram of tissue was expressed in mg.

2.18.4 Estimation of Protein Bound Fucose[26].
The tissue homogenate (0.1ml) was precipitated using ethanol. The precipitate was dissolved in 0.1N sodium hydroxide, one milliliter. Using 1 m of water and varying concentrations of fucose, a blank, and a standard were formed. After immersing each tube in an ice bath, 4.5ml cold sulphuric acid water reagent was added. After that, the tubes remained for 30 min in a hot water bath. A solution of 3% cysteine hydrochloride (0.1ml) was added. RT for 75 minutes was used to incubate the tubes in the dark. The color developed was determined at 396nm. Once calculated using a standard graph, the amount of fucose had been expressed as mg/g of dry defatted tissue.

2.19 In-Vivo Antioxidant Activity
2.19.1 Estimation of Lipid Peroxide [27].
1.5ml of 20% acetic acid, along with 0.2ml tissue homogenate, made up the combination. After adding 1.5 ml TBA along with 0.2ml SDS, 4ml water was used to make up the volume. Allow the tubes an hour to incubate in a water bath. The butanol pyridine reagent (5 ml) was added after cooling. 10 minutes were spent centrifuging the material. Two layers were separated. At 530 nm, the extracted phase's pink color has been assessed.

2.19.2 Estimation of Reduced Glutathione [28].
8ml of 0.02M EDTA was used to homogenize 200 mg of liver in an ice bath. Aliquots of 0.2ml homogenate had been combined with 1.0ml of10% TCA along with 4.0ml distilled water in test tubes. For 5 minutes, tubes had been centrifuged at roughly 3000 rpm. At 412 nm, absorbance had been evaluated in accordance with the reagent blank after 2ml of DTNB[5,5'-dithiobis-(2-2-nitrobenzoic acid)] was mixed with half a milliliter of shaken supernatant solution.

2.19.3 Assay of Glutathione Peroxidase (GPX) [29].
Reaction mixture, comprising 0.2ml of EDTA, sodium azide, reduced glutathione, H2O2, 0.4ml phosphate

buffer, as well as 0.1ml tissue homogenate, had been incubated at 37 degrees Celsius for 10min. After stopping the reaction with 0.5 ml TCA, the tubes were centrifuged at 2000rpm. Colour produced at 420nm was evaluated after adding 4 ml disodium hydrogen phosphate along0.5ml DTNB to 0.5ml supernatant. Activity of glutathione is expressed as mg of reduced glutathione/min/mg/g of tissue.

2.19.4 Assay of Superoxide Dismutase [30].
After adding 0.1ml tissue homogenate to tubes having 0.75ml ethanol along with 0.15ml chloroform (chilled ice), the tubes were centrifuged. Add 1ml buffer along with 0.5ml EDTA solution to 0.5 mL supernatant. Reaction had been initiated by adding 0.5ml epinephrine, along with a rise in absorbance at 480nm,which was monitored.

2.19.5 Assay of Catalase [31].
Twenty microliters of tissue homogenate were mixed using 1.5ml phosphate buffer. 0.5ml hydrogen peroxide was added to begin the enzyme process. Tubes were filled with 0.25ml potassium dichromate, left in a boiling water bath for 10 min. For 3 minutes, absorbance reduction at 240nm was estimated every 60 seconds after turning green. An enzyme blank had been employed alongside 1.0ml distilled water in place of H2O2.

Results
Therefore, the common plant kattuthampattan, which is botanically comparable to Canavalia virosa (Roxb) Wight and Arn., was chosen for the current investigation. The anticancer activity of the selected plant material's ethanolic extract was investigated in vitro as well asin vivo using Ehrlich Ascites carcinoma.The purity of the test substance used in the investigation was demonstrated by, which showed that the plant material's ash content was 8.75% and that the acid-insoluble ash content was 1.35%.

Table 1: Physicochemical Constants

S. No	Parameters	Value % W/W
1	Loss on drying	8.9
2	Total Ash Content	8.75
3	Water-soluble Ash	4.8
4	Acid-insoluble Ash	1.35

Table 2:Successive Extractive Values

S. No	Parameters	Value % W/W
1.	Hexane	1.58
2.	Chloroform	2.8
3.	Ethyl acetate	1.4

Table 2 showed that the extractive values for chloroform (2.8%) were greater than those for hexane and ethyl acetate, indicating the presence of polar compounds like flavones and phenol in the plant drugs.

Table 3: Solubility

S. No	Parameters	Value % W/W
1.	Water	21.4
2.	Alcohol	16.4

It was discovered that the plant powder was more soluble in water than in alcohol (Table 3).

Table 4: Fluorescence Analysis of Drug Powder

S. No	Treatment	Canavalia virosa (Roxb) Wight &Arn			
		DAY light (24 hrs)	UV light (24 hrs)	DAY light (48 hrs)	UV light (48 hrs)
1	Drug Powder	Green	Green	Green	Green
2	Drug powder+ Hexane	Green	Yellow	Light brown	Pink

3	Drug powder+ Benzene	Light green	Yellowish green	Brown	Orange
4	Drug powder+ Chlorofo rm	Green	Green	Green	Orange
5.	Drug powder+ Ethyl acetate	Dark green	Green	Greenish Brown	Pink
6.	Drug powder+ alcohol	Green	Green	Green	Orange
7.	Drug powder+ acetone	Green	Dark green	Green	Pink
8.	Drug powder+ 50% H2SO4	Dark brown	Brown	Brown	Green
9.	Drug powder+ 1NHcl	Light green	Green	Green	Green
10.	Drug powder+ alc.1N NaOH	Green	Dark green	Green	Brown
11.	Drug powder+ aq.1N NaOH	Light green	Green	Green	Green
12.	Drug powder+ water	Green	Green	Green	Green

Table 4 displays the drug powder's fluorescence analysis. When drug powder containing compounds mentioned above was exposed to daylight and ultraviolet light, it showed fluorescence behavior that produced various shades of green,orange, and yellow colours. Alkaloids and flavones are indicated by the orange and yellow fluorescence. Sterols are present because of the green fluorescence.

Table 5: Preliminary Phytochemical Screening of Drug Powder

S. No	Test for	Reaction	Observation
1.	Terpenoids	Tin + Thinoyl chloride	-
2.	Flavones	Mg bits + HCL	+
3.	Steroids	Acetic anhydride+ Sulphuric acid	+
4.	Glycosides	Anthrone+H2S04	-
5.	Sugar	Fehling's A+, Fehling's B, and slightly heated in a water bath.	+

7.	Alkaloids	Dragendroff's reagent	+
8.	Quinines	Conc. H2SO4	+
9.	Phenols	Sodiumcarbonate+folins phenol	+
10.	Tannins	Lead acetate solution	+
11.	Saponins	Water shake	+
12.	Coumarin	10% NaoH	+

Table 5 shows the results of the test drug's initial photochemical screening, which showed that there were no terpenoids present but that there were steroids, alkaloids, flavones, phenols, coumarins, glycosides, sugar tannins, and saponins. Chloroform, hexane, and water extracts of the drugs powder Canavalia virosa (Roxb) Wight and Arn'sinitial phytochemical test of several preparations included alkaloids in chloroform, hexane, and water extract. Alkaloids, phenols, quinines, steroids, coumarins, and tannins were detected in the ethanol extract.

Table 6: Preliminary Phytochemical Screening of Various Extracts of Canavalia Virosa (Roxb) Wight &Arn

S. No	Test for	Hexane	Chloroform	Ethyl acetate	Ethanol	Water
1	Terpenoids	-	-	-	-	-
2	Flavones	-	-	-	+	+
3	Steroids	+	+	+	+	+
4	Sugar	-	-	-	+	+
5	Alkaloids	+	+	-	+	+
6	Quinines	-	-	-	+	+
7	Phenols	+	+	+	+	+
8	Tannins	-	+	-	+	-
9	Saponins	-	-	-	+	-
10	Coumarin	-	+	-	+	+

Three significant secondary metabolites were quantitatively estimated, and the results are listed in Table 7.

Table 7: Quantitative Analysis of major secondary metabolites and vitamin-C

S.No.	Particulars	Amount (mg/g)
1	Phenol	60
2	Flavanoids	2.5
3	Alkaloids	180
4	Vitamin C	19.1

It was discovered that alkaloid concentration was greater than that of flavonoids and phenols. A moderate amount of vitamin C is also included in the plant drugs. The secondary metabolites.The alkaloid concentration was 180 mg/g, which was greater than the flavonoid and phenol contents, which were 2.5 and 60 mg/g, respectively, as demonstrated in Table 7. The result showed that the high alkaloid content of the plant drug, as investigated, may provide anticancer effects. The test drug's moderate vitamin C content may be a contributing factor to its antioxidant properties

Figure 1: DPPH Scavenging Assay of Canavalia Virosa (Roxb) Wight &Arn

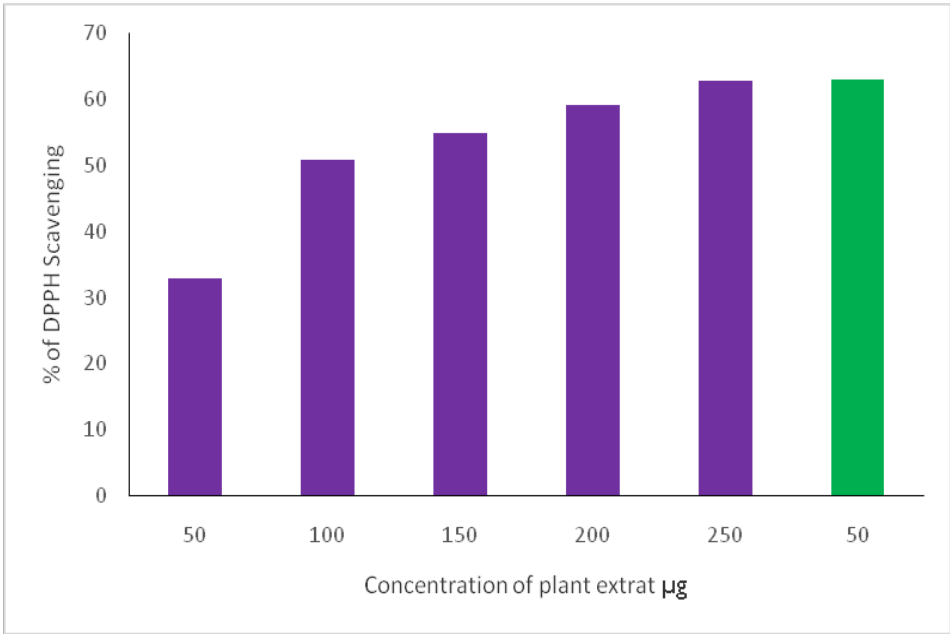


Figure 2shows that as the test drug concentration (50-250µg/ml) grew steadily, the proportion of DPPH scavenging correspondingly increased. The results were compared to ordinary ascorbic acid.IC50 had been estimated to be 95µg/ml.

Figure 2: Reducing Power ofCanavalia Virosa (Roxb) Wight &Arn

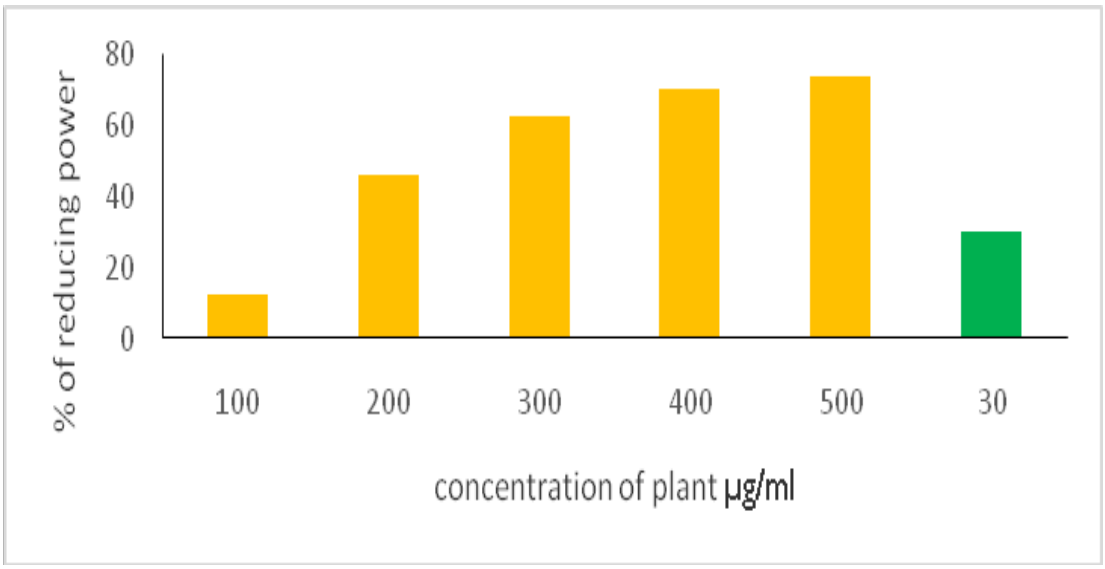
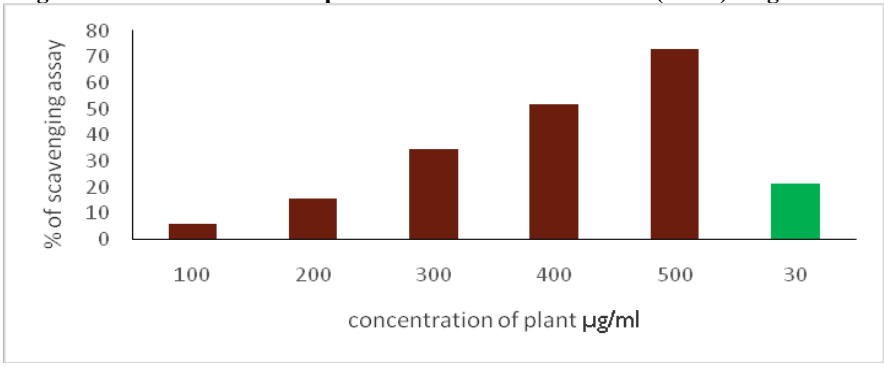


Fig. 2 displayed the reducing power effect of selected plant, in that 500 µg/ml represented the larger percentage (74%) of reducing power. It was found that when the concentration of the plant drugs rose, the result scaled continuously. Outcomes were compared to standard ascorbic acid. It was found that 225µg/ml was the IC50 value.

Figure 3: ABTS+ Inhibition potentials of Canavalia virosa (Roxb) Wight &Arn



The inhibitory potential of selected plant on ABTS radical was shown in Fig 4. The low concentration (100µg/ml) exhibited 5.76 %, At high concentrations (500 µg/ml), however, ABTS+ radical inhibition potentials showed 73% activity. Comparable results were obtained with standard ascorbic acid. It was discovered that the IC50 value was 395 µg/ml. EAC cell lines' cytotoxic response to EECV was recorded.

3.1Anticancer Activity of Canavalia Virosa (Roxb) Wight &Arn

Table 8 - Cytotoxic Effect of EECV on EAC Cell Line (Trypan Blue Method)

Concentration of EECV (µg/ml)	Viable cells (%)	Death cells (%)
Control	96	3.8
100	77	22.6
250	69	30.78
500	63	36.5
750	36	63.2
1000	31	68

The percentage of dead cells increased statistically between 100-1000µg/ml of EECV(Table 8), although the percentage of viable cells decreased as the amount of plant extract increased. Cytotoxicity was detected at 100 µg/ml at 22.7% (24 hours) and 23.9% (48 hours), and at a higher concentration of 500 µg/ml at 62% (24 hours) and 77.6% (48 hours). Dead cell = Stained with Trypan blue dye, Viable cell = Not stained with Trypan blue dye

Table 9- Cytotoxic Effect of Eecv Against Eac Cell Line (Mtt Assay)

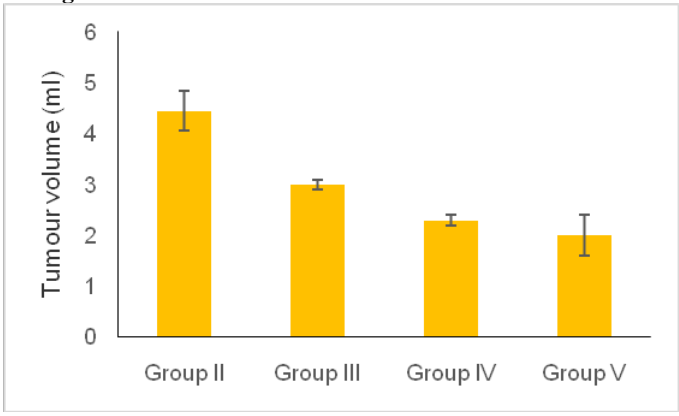
S.NO	Concentration µg	% Dead cell 24 hrs	%Dead cell 48 hrs
1	100	22.7	23.9
2	200	41.58	44.4
3	300	48.5	53.3
4	400	53.4	60
5	500	62	77.6

Table 9 presents the findings of an analysis of the cytotoxic effect of Canavalia virosa (Roxb) Wight &Arn's ethanol extract using MTT assay. The 48-hour incubation study proved to be more successful than the 24-hour incubation trial.

3.2 In Vivo Studies:

The results ofin vivo studies on the examination of the cytotoxic impact of the ethanol extract of Canavalia virosa (Roxb.) Wight & Arn are shown in the figure. Animals with tumors had longer lifespans. The animals that received 300mg/kg bw of EECV had a 56.6% longer life span than the tumor-bearing animals (Fig. 13). 5-fluorouracil, a common drug, extended life expectancy by 80.92%. The tumor development response was anticipated inTable 15

Figure 4:Effect of EECV Treatment on Tumour Volume



The experimental animals produced more ascites fluid (tumor volume) after receiving the EAC injection; however, the tumor volume decreased after receiving the test drugs at two different dosage levels. Additionally, the test drugs declined viable cell count indose-dependent way while increasing non-viable cell counts.

Table 10: Effect of EECV on Hematological Parameters

Particulars	Hb (g %)	RBC count 106 cells/mm3	WBC 103 cells/mm3
Group I	15.6±0.5	4.7±0.3	6.7±0.8
Group II	9.9±0.5*	2.3±0.2*	18.8±1.4*
Group III	12.5±0.6	3±0.1	15.6±0.5
Group IV	14.2±0.2**	3.45±0.1**	10.9±1**
Group V	14.8±0.6**	3.9±0.1**	8.1±0.6**

indicated that when tumor-bearing animals were given two distinct concentrations of 150 and 300 mg/kg bw, their hemoglobin (9.9±0.5%) and RBC count (4.75±0.3x106cells/mm3) would increase. The plant medicine EECV
raised both of these parameters. When compared to tumor control animals, the WBC count was shown to have dropped throughout therapy. It was discovered that plant extract at greater doses (300mg/kg bw) was more effective.

Table 11: Effect of EECVon Hepatic Nucleic Acid Levels

Groups	DNA mg/g of tissue	RNA mg/g of tissue
Group I	11.2±0.8	12.5±1.2
Group II	31±1.5*	26±0.8*
Group III	23.5±0.6	22.8±0.8
Group IV	18.5±0.7**	19.5±1**
Group V	13±0.5**	13.5±1.2**

Table 11showed that, in comparison to normal animals, the disease control (tumor animal) had a higher DNA and RNA content.Two distinct doses of EECV concentration (150mg/kg bw as well as 300mg/kg bw) were administered during therapy. Successfully lowered the hepatic tissue's amounts of RNA and DNA. Animals in Group II had greater levels of serum hepatic marker enzymes like ALT, AST, and LDH than animals in Group I. For 14 days, the animals in Groups III and IV received two distinct EECV concentrations (150 and 300 mg/bw). It was discovered that the effect was dose-dependent and that this considerably decreased blood hepatic marker enzymelevels. The usual drug 5 FU (20 mg/bw) also restored increased hepatic serum enzymelevels.

Table 12: Effect of EECVon Serum Hepatic Marker Enzyme Levels

Groups	ALT (U/L)	AST (U/L)	LDH(U/L)
Group I	68.0±3.8	68.0±4.9	487.3±8.43
Group II	269.2±11*	138.7±2.7*	912.8±7.46*
Group III	175.5±3.78	112.2±2.5	825.4±5.16
Group IV	118.2±2.2**	86.2±2.9**	713.4±10.14**
Group V	85.5±5.2**	74.7±2.6**	822.19±1.49**

Table 12 showed that Hexose hexosamine, fucose, and sialic acid—carbohydrates associated with membranes—were shown to be more prevalent in the liver tissue of tumor-bearing animals than in healthy ones. The membrane-bound carbohydrates were successfully reduced in the experimental animals (Groups III and IV). Afterward,they were given two distinct dose levels of EECV orally for 14 days.The membrane-bound carbohydrates in the animals in Group V that received the standard 5 FU also significantly decreased.

Table 13: Effect of EECV on Hepatic Glycoprotein Level

Groups	Hexsose (mg/g of defatted tissue)	Hexosamine (mg/g of defatted tissue)	Fucose(mg/g of defatted tissue)	Sialic acid(mg/g of defatted tissue
Group I	42±2	11.8±1.4	48±2.5	12.6±1.3
Group II	81.7±2.6*	25.5±1.4*	81.2±2.2*	48.6±1.7*
Group III	62.1±1.2	18.4±0.7	69.7±2.2	33.4±0.7
Group IV	54±0.8**	13.9±0.3**	55.2±2.2**	23.4±1.5**
Group V	50±2.1**	12.1±0.4**	53.5±1.2**	17.8±1**

The enzymatic and non-enzymatic state of experimental animals was predicted by Table 20. Lipid peroxides (LPO), a crucial measure of antioxidant status, were shown to be greater in animals that carried EAC. The enzymatic antioxidants encompassing GPX,SOD, as well as Catalase, along with non-enzymatic antioxidant reduced glutathione, were shown to be decreased. Following treatment with two EECV concentrations (150 and 300mg/kg bw), enzymatic as well as non-enzymatic antioxidant systems that had been impacted by the oxidative stress brought on by the induction of the EAC cell line were successfully recovered.

4. DISCUSSION

4.1 Physicochemical analysis and extractive profile.

Standardization of medicinal plant material is required in order to assure reproducible and high-quality herbal preparations. The physicochemical analysis of Canavalia virosa leaf powder was found to be acceptable for purity and very low in contamination from inorganic substances. With an overall ash content of 8.75% & acid insoluble ash of a mere 1.35%, the powder contains negligible siliceous matter, which is proof of its authenticity and cleanliness. These values are comparable to values conducted for other species of pharmacologically important species of the Fabaceae and are within pharmacopoeial limits [5]. Successive solvent extraction showed that the best extractive yield was chloroform (2.8%) when compared with hexane and ethyl acetate solvents. This provides a suggestion for enrichment with moderately polar bioactive constituents. Solubility tests were conducted, and they showed higher aqueous solubility (21.4%) compared to alcoholic solubility (16.4%), which is an indication of the presence of polar phytochemicals such as alkaloids, phenolics, and glycosides, which are compounds that are often associated with anticancer activities [3].

4.2 Fluorescence-based analysis and composition of phytochemicals.

Fluorescence examinations were conducted using the powdered drug treated with various reagents, revealing characteristic emissions in green, yellow, orange, and pink colors both in daylight and under UV light. These emission patterns indicate the presence of alkaloids, flavonoids, sterols, and phenolic compounds, which serve as widely recognized diagnostic markers in pharmacognostic studies [6].

Preliminary phytochemical screening results confirmed the presence of flavonoids, alkaloids, phenols, tannins,

saponins, coumarins, steroids, and quinones in the ethanolic extract. The absence of terpenoids suggests that the selective phytochemical profile is dominated by nitrogen-containing compounds and polyphenols. Among the solvents evaluated, ethanolic extracts exhibited the most extensive phytochemical spectrum, making them a suitable choice for biological evaluation. These same phytochemical patterns have been associated with the anti-cancer activity of the plant extracts [7].

4.3 Quantitative estimation of secondary metabolites and vitamin C

Quantitative analysis indicated a significantly high content of alkaloids (180 mg/g), followed by phenols (60 mg/g) and flavonoids (2.5 mg/g). Alkaloids are well-documented for their cytotoxic and apoptosis-inducing properties, which occur through mechanisms such as DNA intercalation, microtubule inhibition, and cell cycle arrest [32]. Additionally, the moderate level of vitamin C (19.1 mg/g) may contribute to antioxidant defense by scavenging reactive oxygen species (ROS), subsequently modulating redox-sensitive signaling pathways that are involved in tumor progression [33]. The predominance of alkaloids in C. virosa, as indicated by the data, supports its observed antiproliferative effect and aligns with previous findings that alkaloid-rich extracts exhibit strong antitumor effects against ascitic carcinoma models [3].

4.4 In vitro antioxidant activity

The ethanolic extract of C. virosa demonstrated concentration-dependent free radical scavenging activity across various in vitro models of free radicals. In the DPPH assay, the IC50 indicated a 95% efficiency concentration of DPPH+. This suggests a strong ability to donate hydrogen. Results from the reducing power and ABTS+ assays further confirmed the presence of electron-donating properties, effectively neutralizing both lipid- and water-soluble radicals.

Oxidative stress significantly contributes to carcinogenesis, leading to DNA damage and promoting tumor cell survival. Antioxidants derived from plant sources can mitigate these effects by restoring redox balance [34]. The antioxidant potential of C. virosa is likely due to its phenolic and alkaloid constituents, which work synergistically to suppress oxidative damage.

4.5 In Vitro Cytotoxic and Antiproliferative Activity

Cytotoxicity against Ehrlich Ascites Carcinoma (EAC) cells was dose-dependent, as indicated by the exclusion of trypan blue. Cells exhibited a 68% mortality rate at a concentration of 1000 µg/mL, suggesting significant membrane-damaging activity. The antiproliferative effects were further confirmed by the MTT assay, which recorded a 77.6% growth inhibition at a concentration of 500 µg/mL after 48 hours. An increase in lactate dehydrogenase (LDH) release and DNA fragmentation also supported the evidence of compromised membrane integrity and induced pseudolysis, consistent with earlier studies on plant alkaloids and flavonoids [35, 36].

4.6 In-vivo antitumor efficacy

In vivo studies in EAC-bearing Swiss albino mice showed significant antitumor effects. A dose of 300 milligrams per kilogram of body weight increased mean survival time by 56.6 percent compared with tumor controls and decreased ascitic tumor volume and viable cell count. These outcomes were dose-dependent and were similar to those obtained with a standard chemotherapy drug (5-fluorouracil). EECV also restored altered hematological parameters such as hemoglobin, RBC, and WBC count, indicating protection against tumor-catered myelosuppression. Hepatic markers (ALT, AST, LDH) and glycoprotein membranes were normalized, showing suppression of tumor-induced hepatic dysfunction.

4.7 Conventionalization of antioxidant and glycoprotein profiles.

The tumor-bearing mouse had higher lipid peroxidation and lower antioxidant enzymes, indicating that oxidative stress is greater. EECV therapy reduced the LPO and recovered the GSH, GPx, SOD, and catalase enzymes, confirming the innate defense against antioxidants. A reduction in hepatic glycoproteins, i.e., hexose, hexosamine, fucose, and sialic acid, suggested an inhibition of tumor cell proliferation and metastasis, as altered glycosylation is a hallmark of malignancy [37]. Collectively, the alkaloid-rich phytochemical profile of C. virosa appears to function by inducing apoptosis through oxidative stress-mediated mitochondrial dysfunction and oxidative stress restoration, inhibiting aberrant glycoprotein synthesis. These are combined effects that are likely responsible for the antitumor activity and superior survival seen.

5. CONCLUSION

The present study gives compelling experimental evidence that the ethanolic extract of Canavalia virosa (Roxb.) Wight &Arn. has great anticancer activity against Ehrlich Ascites Carcinoma. The observed effects are mediated by apoptosis induction, oxidative stress suppression, hematological and biochemical parameter normalization, as well as tumor growth and proliferation inhibition. A high alkaloid content, which is supported by phenolic and antioxidant constituents, appears to play a central role in the biological efficacy of the extract. These findings present solid scientific support for the traditional use of C. virosa and indicate that it is a promising candidate for natural source development of new plant-based anticancer agents. However, subsequent studies on isolation of bioactive compounds, elucidation of molecular mechanisms, and toxicity profiling are warranted by way of forwarding C. virosa towards clinical application. Future investigations should be based on isolation and structural characterization of bioactive alkaloids from C. virosa, followed by molecular docking and molecular pathway-specific validation studies. Toxicological profiling and pharmacokinetic evaluation will be the key to bringing this plant to preclinical and clinical development.

ACKNOWLEDGEMENTS
The authors of this article wish to convey their appreciation for the management of Srimad Andavan Arts and Science College (autonomous), Tiruchirappalli, Tamil Nadu, India, for providing research facilities, as well as the Dean of Life Sciences and Head and faculty of teaching and non-teaching of the Department of Biochemistry.For assistance in completing this research project.

COMPETING INTERESTS
The authors have no conflicts of interest to disclose.

AUTHORS' CONTRIBUTIONS
Bhuvaneswari formed the design of the study, sample assets, analysis, explanation of data, and framing of the manuscript. Sivasubramanian and Sridharan have read and authorized the final version of the manuscript.

ETHICAL APPROVAL
All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

REFERENCES

1. vanithaPudata, et al. (2011). Cancer biology and chemotherapy overview.International Journal of Pharmaceutical Sciences Review and Research, 6(2), 12–18.
2. G, Samuelson (1999), "Drugs of Natural Origin: A Textbook of Pharmacognosy", 4th Ed., Stockholm, Swedish Pharmaceutical Press.
3. Cragg, G. M., & Newman, D. J. (2013). Natural products: A continuing source of novel drug leads.Biochimica et BiophysicaActa (BBA) – General Subjects, 1830(6), 3670–3695. https://doi.org/10.1016/j.bbagen.2013.02.008
4. Gamble, J. S. (1997). Flora of the Presidency of Madras.Bishen Singh Mahendra Pal Singh.
5. Anonymous. (2001). The Ayurvedic Pharmacopoeia of India (Vol. I). Government of India, Ministry of Health & Family Welfare
6. Chase, C. R., & Pratt, R. J. (1949). Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification.Journal of the American Pharmaceutical Association, 38(6), 324–331. https://doi.org/10.1002/jps.3030380612
7. Brindha, P., Sasikala, B., &Purushothaman, K. K. (1981). Pharmacognostic studies on Merugankizhangu.Bulletin of Medico-Ethnobotanical Research, 3, 84–96.
8. Malick CP, Singh MB. 2008, Estimation of Phenols, In: Plant Enzymology and Histo-enzymology New Delhi, KalyaniPub, 286.
9. Ferguson NM. 1956, In: A Textbook of Pharmacognosy. New Delhi: Mcmillan company; p: 191.
10. Sadasivam, 1996 Determination of total carbohydrate by Anthrone method, Biochemical methods, : (2) 8-9.
11. Gyamfi, M. A., Yonamine, M., & Aniya, Y. (2002). Free-radical scavenging action of medicinal herbs from Ghana.General Pharmacology, 32(6), 661–667. https://doi.org/10.1016/S0306-3623(98)00238-9
12. Gothoskar, S. V., Ranadive, K. J., & Shah, V. C. (1971). Experimental studies on Ehrlich ascites carcinoma.Indian Journal of Cancer, 8(1), 1–7.
13. Sheeja, K. R., Kuttan, G., &Kuttan, R. (1997). Cytotoxic and antitumor activity of Berberin.American Journal of Chinese Medicine, 25(2), 115–123. https://doi.org/10.1142/S0192415X97000125
14. Scudiero, D. A., Shoemaker, R. H., Paull, K. D., et al. (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity.Cancer Research, 48(17), 4827–4833.
15. Russo, A., et al. (2016). Lactate dehydrogenase assay in cell toxicity.Methods in Molecular Biology, 1601, 165–170. https://doi.org/10.1007/978-1-4939-6960-9_14
16. Kuttan, R., Bhanumathy, P., Nirmala, K., & George, M. C. (1990). Potential anticancer activity of turmeric (Curcuma longa).Cancer Letters, 29(2), 197–202. https://doi.org/10.1016/0304-3835(85)90159-4
17. Armour, F. E., Blood, F. R., & Belden, D. A. (1965). Manual for clinical laboratory procedures. McGraw-Hill.
18. Armour FE, Blood FR, Belden DA. 1965, The manual for laboratory work in mammalian physiology. Illinois Chicago: The University of Chicago Press; 3; 4-6
19. Schneider, W. C. (1957). Determination of nucleic acids in tissues by pentose analysis.Methods in Enzymology, 3, 680–684. https://doi.org/10.1016/S0076-6879(57)03440-5
20. Giles, K. W., & Myers, A. (1965). An improved diphenylamine method for estimation of DNA.Nature, 206, 93–95. https://doi.org/10.1038/206093a0
21. Endo, Y. (1970). Quantitative estimation of RNA by orcinol method.Analytical Biochemistry, 35(1), 160–164.
22. King, J. (1965). The transaminases: ALT and AST.Journal of Medical Laboratory Technology, 22, 97–104.
23. Niebes, P. (1972). Determination of enzymes and glycoproteins in tissues.ClinicaChimicaActa, 42, 399–408.

24. Wagner, W. D., et al. (1972). Protein-bound hexosamine estimation.Analytical Biochemistry, 46(1), 80–87.
25. Warren, L. (1959). The thiobarbituric acid assay of sialic acids.Journal of Biological Chemistry, 234, 1971–1975.
26. Dische, Z., &Shettles, L. B. (1948). A specific color reaction of methylpentoses and spectrophotometric micromethod for their determination.Journal of Biological Chemistry, 175, 595–603.
27. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.Analytical Biochemistry, 95(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3
28. Luck, H. (1963). Catalase. In Methods of Enzymatic Analysis (pp. 885–894). Academic Press.
29. Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., & Hoekstra, W. G. (1973). Selenium: Biochemical role as a component of glutathione peroxidase.Science, 179(4073), 588–590. https://doi.org/10.1126/science.179.4073.588
30. Maehly, A. C., & Chance, B. (1972). The assay of catalases and peroxidases.Methods of Biochemical Analysis, 1, 357–424.
31. Misra, H. P., &Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine.Journal of Biological Chemistry, 247(10), 3170–3175.
32. Wink, M. (2007). Molecular modes of action of cytotoxic alkaloids: From DNA intercalation, spindle poisoning, topoisomerase inhibition to apoptosis and multiple drug resistance.Alkaloids: Chemistry and Biology, 64, 1–47. https://doi.org/10.1016/S1099-4831(07)64001-2
33. Padayatty, S. J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J. H., Chen, S., Corpe, C., Dutta, A., Dutta, S. K., & Levine, M. (2003). Vitamin C as an antioxidant: Evaluation of its role in disease prevention.Journal of the American College of Nutrition, 22(1), 18–35. https://doi.org/10.1080/07315724.2003.10719272
34. Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress–induced cancer.Chemico-Biological Interactions, 160(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009
35. Elmore, S. (2007). Apoptosis: A review of programmed cell death.Toxicologic Pathology, 35(4), 495–516. https://doi.org/10.1080/01926230701320337
36. Russo, A., Cardile, V., Lombardo, L., Vanella, L., Acquaviva, R., & Genovese, C. (2016). Antioxidant activity and antiproliferative effects of plant-derived compounds: Evaluation by MTT and LDH assays.Methods in Molecular Biology, 1601, 165–170. https://doi.org/10.1007/978-1-4939-6960-9_14
37. Dennis, J. W., Granovsky, M., & Warren, C. E. (1999). Protein glycosylation in cancer: Mechanisms and functional implications.Biochimica et BiophysicaActa (BBA) – General Subjects, 1473(1), 21–34. https://doi.org/10.1016/S0304-4165(99)00167-1