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Antitumor efficacy of *Stachytarpheta mutabilis* methanolic extract in MCF-7 cell line xenografts

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ABSTRACT

Natural products are being increasingly considered as viable alternatives for anticancer therapy due to their inherent non-toxicity and safety profiles. The objective of this study was to investigate the *in vitro* and *in vivo* anticancer properties of the leaf extract of *Stachytarpheta mutabilis*. The preparation of methanol leaf extracts was conducted, followed by a subsequent analysis of phytochemical constituents. The cell viability assay was conducted using the MCF-7 cell line, and the results indicated that the extract exhibited cytotoxic effects on MCF-7, a cell line commonly used in breast cancer research. The analysis of the cell cycle revealed that cellular growth ceased at the Sub-G0 and G1 phase. The study employed methanol extracts at various concentrations to investigate the impact of tumour regression in a breast cancer model using athymic nude mice. The findings of the experiment conducted on athymic nude mice utilising a xenograft model revealed that the administration of a dosage of 400 mg/kg resulted in a significant inhibition of tumour growth, amounting to 57.88%. The results of the MTT3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay indicated that the methanol extract derived from *Stachytarpheta mutabilis* exhibited a statistically significant specificity ($p<0.005$) towards MCF-7 cells. The discovery of the anticancer activity and low toxicity of *Stachytarpheta mutabilis* extract is noteworthy, as no previous reports have documented such effects. In conclusion, this finding presents a valuable prospect for the future development of novel anti-cancer pharmaceutical candidates targeting the highly lethal breast cancer.

Keywords: MCF-7 cell line, MTT assay, Apoptosis, Cell cycle study, Athymic nude mice, Anticancer activity.

INTRODUCTION

Cancer continues to be a prominent global health issue. Therefore, it is imperative to identify novel pharmaceuticals that can serve as prototype compounds for effective cancer drug development. Herbal medicines have been observed to exhibit anticancer properties through various mechanisms, including the stimulation of antioxidant activity, inhibition of cancer-promoting hormones and enzymes, activation of DNA repair mechanisms, promotion of the synthesis of protective enzymes, and enhancement of immune function [1]. Phytochemicals and their derivatives play a significant role in the development of anticancer drugs. Throughout history, ethnobotanical herbs have been of significant importance in the therapeutic management of various types of human cancers [2]. There have been numerous reports on the extensive demonstration of anti-proliferation effects against various types of cancers by alkaloids, phenols, and flavonoids [3]. The extraction of anticancer drugs, including podophyllotoxins, camptothecin, vincristine, and taxol, primarily relies on plant sources. The taxonomic classification of the plant genus *Stachytarpheta* places it within the family Verbenaceae. The *Stachytarpheta* genus encompasses approximately 156 species that are distributed globally and possess bioactive compounds with significant pharmacological potential. According to reports, various components of *Stachytarpheta* spp., such as leaves, roots, and stems, exhibit promising properties with regards to their potential antibacterial, antifungal, anticancer, and anti-inflammatory effects. The ethnic tribes utilise *Stachytarpheta mutabilis* extensively in their traditional medicinal practises. Iridoid glycoside, 6 β-hydroxy ipolamide, lamiide, and saponin were detected in *S. mutabilis*. According to GC/MS analysis, the main constituents of *S. mutabilis* essential oil were 1-octen-3-ol, (Z)-3-hexen-1-ol, (Z)-4hexen-1-ol, linolenic acid, palmitic acid, octan-3-ol, and rosifoliol [4].

Considering the extensive medicinal properties associated with *Stachytarpheta mutabilis*, the objective of this study was to investigate the potential anticancer effects of a methanol leaf extract of *Stachytarpheta mutabilis* in athymic nude mice with induced cancer.

MATERIALS AND METHODS

Collection of the plant

The *Stachytarpheta mutabilis* plant used in the suggested investigation was acquired in June 2020 from Further Nursery along with Fields in Pune, India. The specimen voucher number 15 in the form of a botanical gardens was submitted in the Faculty of Botany at Gulbarga University in Karnataka, India, after verification was verified. The specimen is preserved in department of Botany.

Preparation of methanolic extract of *Stachytarpheta mutabilis*

The plant was processed into a delicate powder after being shade-dried. About 1,000 cc of methanol as well as 100 g of plant powder were stored for separation using a Soxhlet extractor [5]. A Whatman filter paper (Merck) was subsequently employed to filter and retrieve the extract. After using a rotating vacuum evaporator to evaporate the solvent, the extract was eventually purified and stored at 4°C until it was needed. Every chemical employed in this investigation was of analytical quality. The phytochemical examine was conducted using basic phytochemical techniques, which revealed a variety of phytochemicals.

Cell lines and cell culture preparation

The ATCC provided the MCF-7 cell line, and the stock cells were cultivated in DMEM supplemented with 10% FBS, 100 IU/ml of penicillin, and 100 µg/ml of streptomycin in a humidified environment with 5% CO_2 at 37ºC until confluency occurs [6]. Cell dissociating solution (0.2 percent trypsin, 0.02% EDTA, and 0.05 percent glucose in PBS Phosphate Buffered Saline) was used to separate the cell. The cells' vitality is examined and centrifuged. Additionally, a 96-well plate was seeded with fifty thousand cells per well, and the cells were incubated for 24 h at 37°C with 5 percent carbon dioxide (CO_2).

Cell viability Assay or MTT assay

The MTT assay was carried out according to standard procedure [7]. Using the correct medium including 10% FBS, the monolayer cell culture was trypsinized as well as the cell count was brought down to 5.0 x 105 cells/ml. one hundred milliliter of the diluted cell suspension (50,000 cells/well) was injected to each well of the 96-effectively microtiter plate. When a partial monolayer appeared after 24 h, the supernatant was flicked off, the monolayer was once again cleaned with medium, and 100 µl of various test drug concentrations were applied to the partial monolayer in microtiter plates. After that, the plates have been treated for 24 h at 37°C in an environment with a carbon dioxide concentration of 5%. Following incubation, 100 µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well, and the test solutions in the wells were disposed of. After removing the resultant solution, 100 µl of DMSO was introduced as well as the plates were gently shaken to dissolve the formazan that had formed. A microplate reader set to 590 nm was used for determining the absorbency. The formula was used to get the proportional rise restriction, and the dose-response graphs for each cell line were employed to obtain the concentration of test medication required to inhibit cell growth by fifty percent, or IC_{50} values.

Apoptosis Study

Using DMEM cell culture media, place 1 X 10^6 cells per well in a 6-well plate the day before apoptosis is induced [8]. The liquid-filled (dead) cell wells were pipetted out after around eighteen h. After being exposed to materials that caused apoptosis, cells were cultured for a full day. After removing the cells from the plate with a scoop, one milliliter of medium was added to each well, and the contents were transferred to identical Ria vials. After spinning the sample, the supernatant was removed. One by one, the cells were resuspended in 1 mL of 1X Binding Buffer after two cold PBS washes.

Cell Cycle Study

In a 6-well plate with 2 ml of medium, 1 x10^6 cells were planted and cultivated for 24 h. Following treatment alongside the suitable amounts of the produced samples in medium, cells were cultured for an additional twenty-four h [9]. Following cell harvesting, the cells were centrifuged for five minutes at room temperature at 2000 rotations per minute when the supernatant was disposed of while keeping the cell pellet. The cell pellet was resuspended in 2 milliliters of 1X PBS for washing. Under the same circumstances, the washing was done once more. The pellet was kept while the supernatant was disposed of. After reviving the cells in 300 µl of sheath fluid, 1 mL of cold 70% EtOH was added drop by drop while being gently shaken continuously, and then another 1 mL of chilled 70 percent ethylene oxide was applied all at once to fix the cells. After that, the cells were kept for the rest of the evening at 4 degrees Celsius. The cells were centrifuged for five minutes at 2000 rotations per minute after fixation. Two milliliters of cold 1X PBS were used to wash the cell pellet twice. A 450 µl sheath fluid containing 0.05 mg/ml PI and 0.05 mg/ml RnaseA was then used to reintroduce the cell pellet, which was subsequently incubated for 15 minutes in the dark. FACS Caliber (BD Biosciences, San Jose, CA) was used to calculate the proportion of cells in each stage of the cell cycle in populations administered a drug.

Cell culture and maintenance for Animal study

MCF-7 cells were purchased from ATCC, USA and cultured in DMEM medium containing 10% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 µg/ml streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO2 until confluence was achieved [10]. Cell viability was then measured after the removal of cells at the non-confluent stage using a hemacytometer. To obtain the number of cells required for tumor induction, an appropriate number of cells were transferred to T75 culture flasks.

Animal procurement and Housing

Three athymic bare-fur mice per cage were housed in individually ventilated cages (IVCs) after being purchased from Charles River, USA. The simulated animal room was kept at 22ºC (+3ºC) and had a relative humidity of 50–60%. artificial illumination cycle was a full twelve h of light followed by Twelve h of darkness. The feed was purchased from PMI Nutrition International in the United States. During the experiment, sterilized water was administered. The BSL-2 biosafety cabinet was employed for all experimental operations, and all equipment and substances were previously sterilized.

Tumour induction

Sub confluent MCF-7 cells were isolated, and a haemocytometer was used to count the number of viable cells. Following an economically feasible check, a single cell suspension containing 5x10^7 cells/ml was made in serum-free medium and combined 1:1 with Matrigel. A subcutaneous injection of two millilitres of cell solution was administered to each animal in the area above the right flank.

Treatment Groups and conditions

The tumor's length and width were measured every other day using vernier callipers, and its volume was calculated using the procedure below. After 2 weeks, when cancer growth reached the desired volume of 100-150 mm^3, tumor volumes were measured and randomly assigned to four groups, each consisting of three rats. Group V received an oral dose of the test sample at 600 mg per kilogram for 21 days, whereas Groups III and IV received oral doses of the sample at 200 mg per kilogram and 400 mg/kg, respectively. The positive control group was given 5 milligrams per kilogram of cisplatin and test sample, whereas the tumor control group was given the usual saline orally. These are common formulas for calculations-

Standard Formulas:

Tumour Volume:

$$\text{Tumour Volume} = \text{Tumour length} \times \frac{(\text{Tumour Width})^2}{2}$$

Percentage of tumor growth inhibition: (MTV- Mean tumour volume)

$$\%TGI = 1 - \frac{(MTV\ of\ treated\ mice)}{(MTV\ of\ tumor\ control\ mice)} \times 100$$

% Change in body weight:

$$\%\,\Delta\, B.wt. = \frac{(B.wt_{Day\,0} - B.wt_{Day\,24})}{B.wt_{Day\,0}} \times 100$$

Sampling and analysis

The study was carried out to evaluate the anti-tumour efficacy of test sample at 200 mg/kg, 400 mg/kg and 600 mg/kg. Athymic nude mice were inoculated with five million HCT-116 cells subcutaneously in a 1:1 Matrigel mixture. The animals were randomly sorted into 5 groups, each consisting of 3 animals with mean tumour size of 100-150mm^3 (Table 1). The treatment was carried out for 21 days with every alternate day dosing. Tumour control animals received vehicle orally. Cisplatin at 5 mg/kg body weight was administered intravenously to group 2. Mice in group 3 and group 4 were administered with test sample at 200 mg/kg and 400 mg/kg body weight orally every alternate day for 21 days. Mice in group 5 administered with test sample at 600 mg/kg. The tumour size measurements, body weight, feed intake was noted every alternate day for 21 days.

At the end of the study, mice were euthanized using sodium pentathiol overdose and organs were harvested for H&E staining.

RESULTS

Phytochemicals analysis

Phytochemical analysis of methanolic plant extract revealed the presence of phenols, flavonoids, steroids, saponins, alkaloids, tannins, glycosides, terpenoids.

In vitro antiproliferative activity

For *in vitro* antiproliferative studies, through MTT analysis, we got IC_{50} value 98.81 and 57% of cell inhibition as observation of cytotoxicity study. The standard vincristine was used for comparing the data. The IC_{50} value of vincristine was 19.7 (Figure 1).

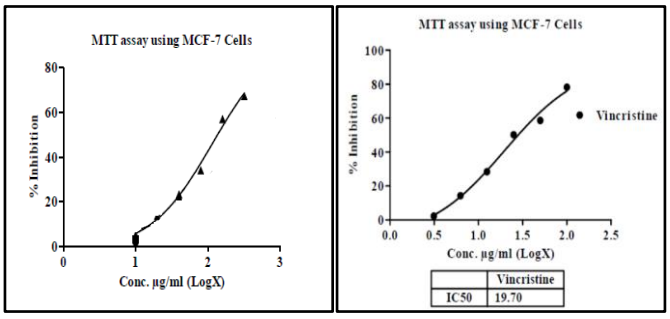


Figure 1: MTT assay and IC_{50} value

Cell cycle study

The treatment of MCF-7 cells at the concentrations of 80 µg/ml and 160 µg/ml with sample has shown S phase and G2M phase arrest of 6.8%, 24.71% and 18.54%, 24.67% respectively. Standard Vincristine at 25µM (22µg/ml) showed G2M arrest of 26.14% and S phase arrest of 26.36% respectively in MCF-7 cells (Figure 2 and 3).

One of the most significant ways that chemotherapy drugs stop tumor growth is by cell cycle arrest. In general, 2n (diploid) denotes the G0/G1 phase, 4n (tetraploid) denotes the G2/M phase, and the S phase falls between diploid and tetraploid.

Apoptosis Study

The chosen cell line, MCF-7, responded to *S. mutabilis* extracts with antiproliferative activity. Therefore, the ability of *S. mutabilis* extracts to induce cell death was examined in relation to their cytotoxic consequences. It has been demonstrated that natural products have cancer fighting and tumor fighting abilities through the following mechanisms: anti-angiogenesis, regulation of the cell cycle, induction of apoptosis, suppression of cell migration, proliferation, and metastasis. One essential characteristic needed for anticancer medication assessment is the activation of apoptosis in medicinal plant extracts [11]. Since necrotic cell death might result in aggravation during treatment, this is the recommended method of cell death. Chromatin condensation, cell membrane shrinking, and DNA fragmentation are the hallmarks of apoptosis. First, flow cytometry was used to examine apoptotic cell populations from cells treated with *S. mutabilis* extracts [11]. After 24 h, apoptosis was induced, according to flow cytometric annexin V FITC and PI analysis. Pure MCF-7 cells at two distinct quantities—80 µg/ml and 160 µg/ml—are depicted in Figure 4. In MCF-7, samples treated with 80 µg/ml and 160 µg/ml caused early apoptosis of 13.96% and 17.42%, respectively, and late apoptosis of 14.88% and 19.00%. In MCF-7 cells, standard vincristine at 22 µg/ml has demonstrated a 53.63% overall apoptosis.

Phosphatidyl-serine (PS) on the plasma membrane acts as a phagocyte identification site at the start of the apoptotic process [12]. The calcium-dependent protein annexin V may bind to free phosphatidyl-serine (PS) chains on the outermost layer of the membrane [13] as demonstrated in Table 2 and Figure 5, apoptosis was one of the primary mechanisms of cell death induced by the plant extracts at two dosages in this study.

In vivo study in athymic nude mice

The results suggest that the animals treated with Cisplatin showed a tumor growth inhibition up to 81.33% whereas, the test samples showed the inhibition up to 22.13% and 57.88 % at 200 mg/kg and400mg/kg respectively, 600 mg/kg sample showed around 49% as shown in the Figure 6.

Mean tumour weight of mice is shown in Figure 7. The mean tumour weight 0.38 g was least in the group IV, administered with 400 mg/kg dose and highest in 0.99 g in vehicle. In the group III (200 mg/kg) and group V (600 mg/kg) it was 0.5 gm and 0.45 gm respectively.

During the experiment, body weights of all mice in 5 experimental groups were observed on alternate days. It was observed that gradually weight of all mice from all the groups were decreasing in gradual manner. Group IV which was administered with 400 mg/kg dose has shown reduction in body weight of mice compared to vehicle and other groups. Group IV readings were similar as close to group administered with cisplatin (5 mg/kg) as shown in graph Figure 8. The body weight was reduced by 1 gm in each animal.

Organ weight of experimental mice was also observed particularly of lungs, liver, kidney, heart and spleen. The weight of each organ was reduced by 0.1 gm. In lungs, kidney and heart. The weight of liver was reduced by 1 gm in each group. Tumour growth inhibition was very less when treated with 600 mg/kg methanolic extract as compared to 200 mg/kg and 400 mg/kg extract. So, the optimum dose for this tumour growth inhibition was 400 mg/kg. In group (G4), mortality of one animal was observed as shown in Figure 9.

In histopathological studies of tumour cells showed necrotic area compared to apoptotic area. In vehicle group dense tumour cells were observed. In cisplatin treated group, necrosis observed along with the

reduction in the tumour cells. Necrosis of tumor cells and density is reduced compared to control group in 200 mg/kg. Tumor cells density is reduced with necrosis in 400 mg/kg. Similar results are obtained with 600 mg/kg where tumor cell density reduced with necrosis. Furthermore, an extremely thin layer of cells with a high number of blood vessels & reduced cell death and apoptosis were observed in the morphological characteristics of the tumours removed from untreated mice (Figure 10 and 11). In contrast, the cancerous growth tissues of animals given extracts had significant necrosis or a loss of cell compactness, along with numerous tumor cell pools and regions of poor blood vessel density.

Table 1: Treatment Groups and conditions

Group	Treatment	Dose (mg/kg bodyweight)	No. of animals
G–I	Tumor Control	Vehicle	3
G–II	Positivecontrol Cisplatin	5 mg/kg, i.v.	3
G–III	Testsample	200 mg/kg (PO)	3
G–IV	Testsample	400 mg/kg (PO)	3
G-V	Test sample	600 mg/kg (PO)	3
Total No. of animals			15

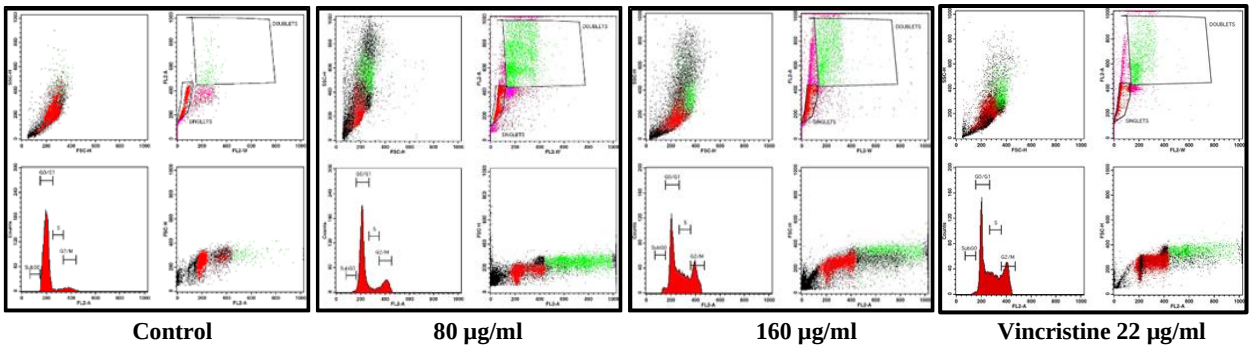


Figure 2: Cell cycle flow cytometry plots of MCF-7 treated with vincristine

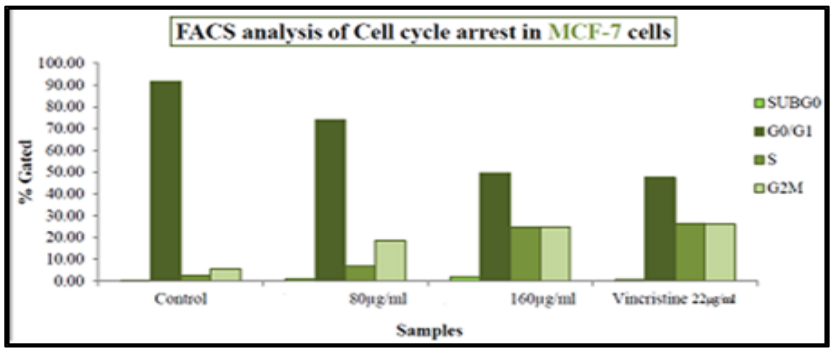


Figure 3: FACS analysis of Cell cycle arrest in MCF-7 Cells

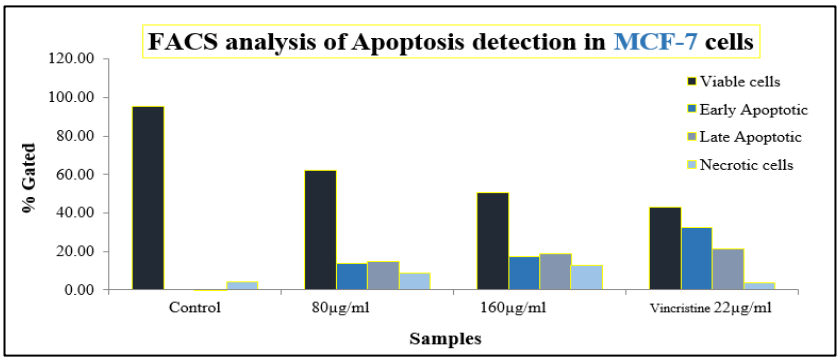


Figure 4: FACS graphical analysis of apoptosis detection in MCF-7 Cells

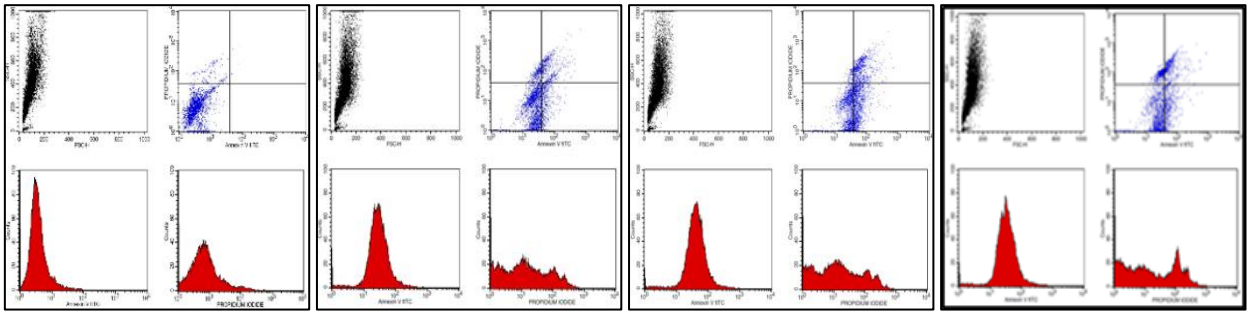


Figure 5: Flow cytometry plots of MCF-7 treated with vincristine and different concentrations

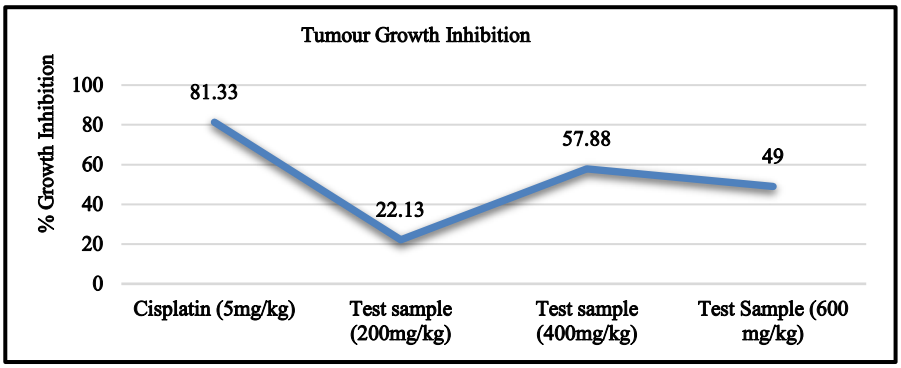


Figure 6: Tumour Growth Inhibition

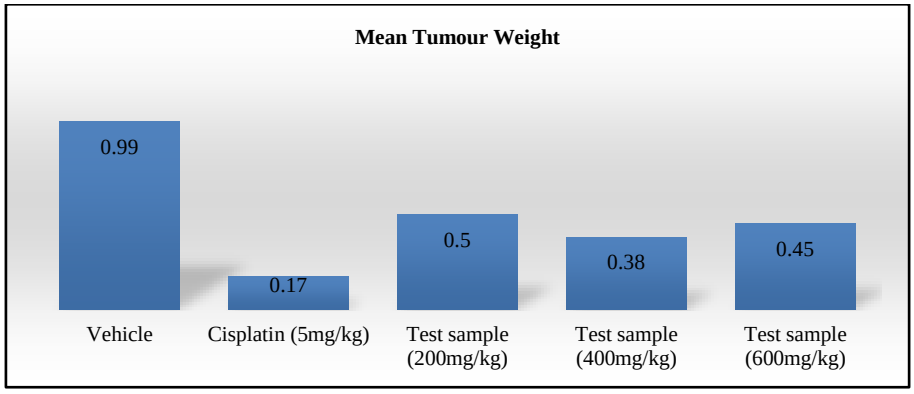
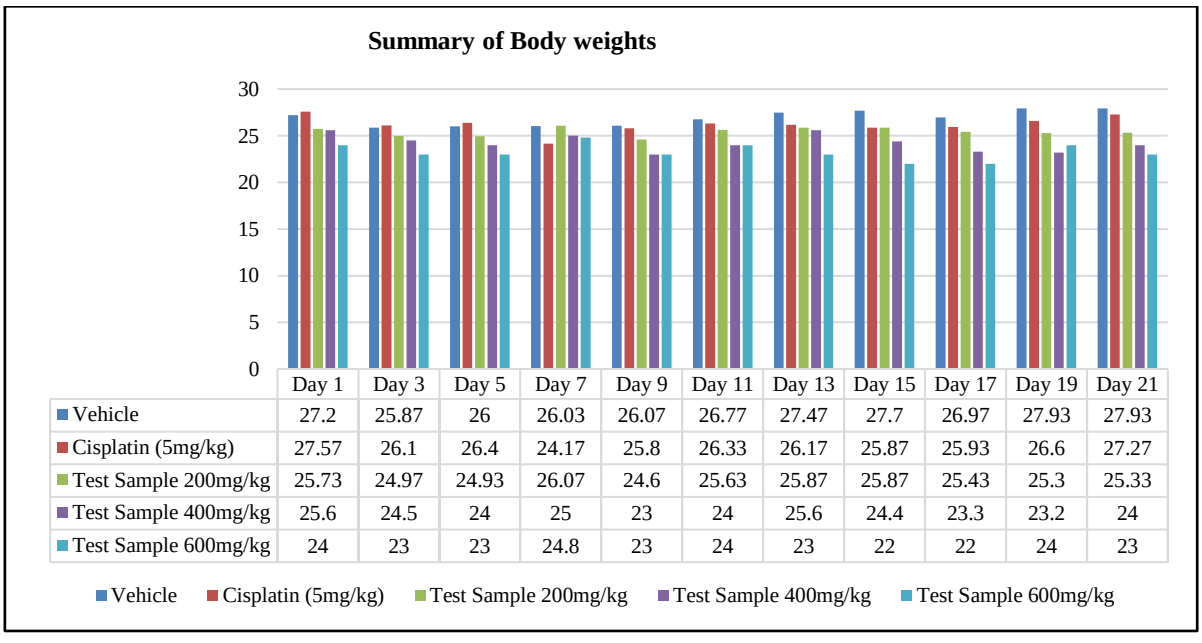
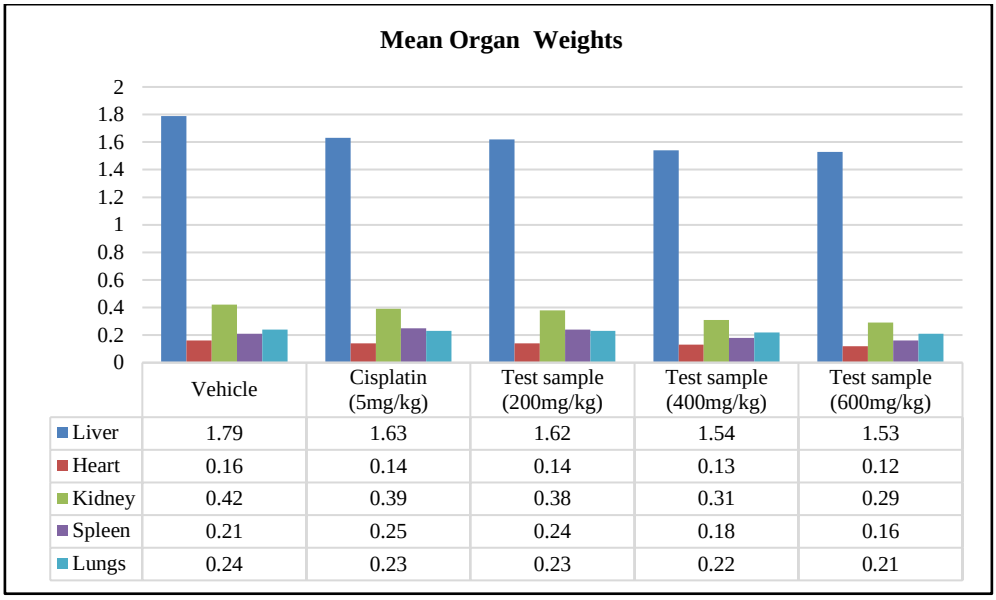


Figure 7: Mean Tumour Weight



	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17	Day 19	Day 21
Vehicle	27.2	25.87	26	26.03	26.07	26.77	27.47	27.7	26.97	27.93	27.93
Cisplatin (5mg/kg)	27.57	26.1	26.4	24.17	25.8	26.33	26.17	25.87	25.93	26.6	27.27
Test Sample 200mg/kg	25.73	24.97	24.93	26.07	24.6	25.63	25.87	25.87	25.43	25.3	25.33
Test Sample 400mg/kg	25.6	24.5	24	25	23	24	25.6	24.4	23.3	23.2	24
Test Sample 600mg/kg	24	23	23	24.8	23	24	23	22	22	24	23

Figure 8: Summary of body weights



	Vehicle	Cisplatin (5mg/kg)	Test sample (200mg/kg)	Test sample (400mg/kg)	Test sample (600mg/kg)
Liver	1.79	1.63	1.62	1.54	1.53
Heart	0.16	0.14	0.14	0.13	0.12
Kidney	0.42	0.39	0.38	0.31	0.29
Spleen	0.21	0.25	0.24	0.18	0.16
Lungs	0.24	0.23	0.23	0.22	0.21

Figure 9: Mean Organ Weights

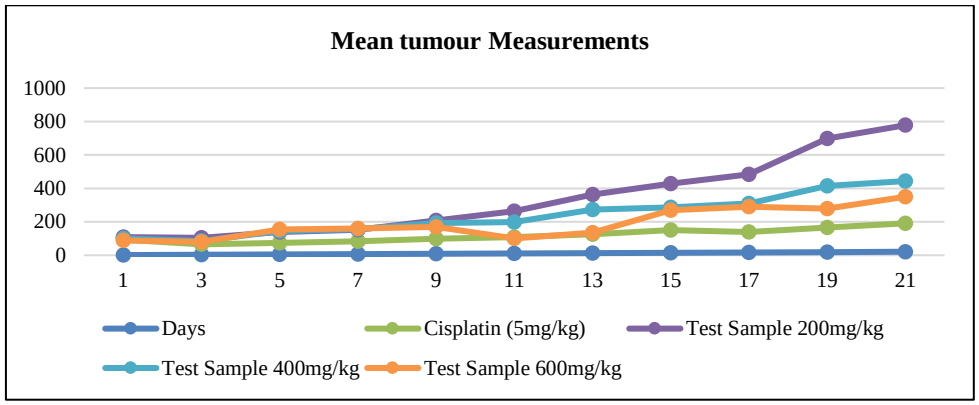


Figure 10: Mean Tumour Measurements

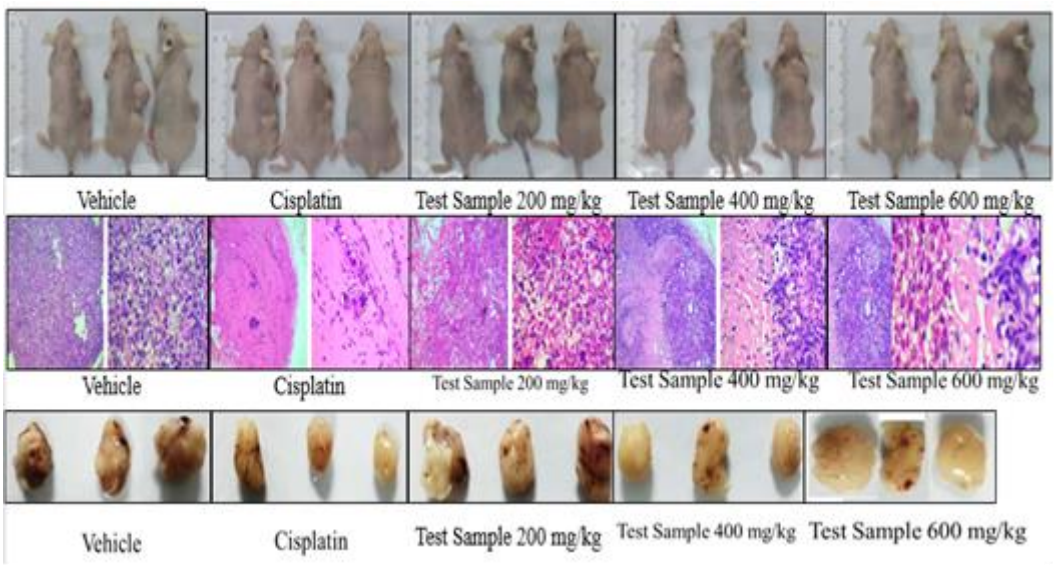


Figure 11: Mice treated with different concentrations and dissected tumours from treated mice along with histopathology of tumour

DISCUSSION

Cancer is currently regarded as one of the deadliest illnesses in both developed and developing nations, making it a significant public health issue. It is believed that a variety of external factors, such as smoking, exposure to chemicals and radiation, and pathogenic organisms, can contribute to the development of cancer. Furthermore, it is thought that intrinsic variables including hormonal immune problems and genetic abnormalities contribute to the development of cancer by acting as risk factors or causal factors. Public health is still heavily burdened by the scientific complexity of cancer treatment and recovery [14]. Terpenoids, phenolic acids, lignans, tannins, flavonoids,

quinones, coumarins, and alkaloids are just a handful of the numerous naturally occurring substances that can be found in plant sources. These compounds are known to have significant antioxidant properties and to play a significant role in the therapy of cancer. Numerous research has demonstrated the anti-inflammatory, anti-tumor, anti-mutagenic, and anti-carcinogenic properties of antioxidant compounds [15]. Natural substances with antioxidant capabilities can directly inhibit cell division and enhance immunity [16]. In the last ten years, the pharmaceutical industry's primary supply of pharmaceuticals has been natural substances. Nowadays, many people consider herbal therapies as additional treatments for a number of ailments, particularly cancer [17,18].

An investigation in 2022 regarding the anticancer potential of *J. adhatoda* leaf extract. The authors used the MTT assay to evaluate the cytotoxic activity of the extract against the MCF-7 cell line [12]. The *J. Adhatoda* leaf extract exhibited the strongest cytotoxic activity against MCF-7 when cells were exposed to a range of extract doses for one day. A considerable reduction in percentage viability of cells was noticed by the researcher since the extract gave an MCF-7 IC_{50} value of 161.57 µg/ml. A value of IC_{50} 98.81 µg/ml signifies the plant extract of *Stachytarpheta. mutabilis* has a 57% cell inhibition rate. The plant extract's IC_{50} values in MCF 7 were found to be 113.07 µg/mL. According to the study, cell growth inhibition (cytotoxicity) and cell survival decrease with increasing concentrations, indicating that these fractions may be used to create anticancer drugs. Similarly, our results on the *Stachytarpheta mutabilis* methanolic extract are consistent indicating that our extract has anticancer qualities. The early cytotoxicity of a methanolic extract from the *Fagonia arabica* plant on the MCF-7 cell line. Notable effects on increasing cell death and preventing cell development were noted by the researchers, and these effects were shown to be concentration-dependent [19].

The ability of the methanolic extract from *Lantena camera* to induce cell cycle arrest in G1 phase MCF-7 cells [20]. The G1 to S phase transition was effectively blocked with a significant number of cells arresting in G1. The maximum observed cell count at this arrest point was found to be 80.35%, with a concentration of 25 µg mL−1. No significant changes were observed in the S phase, but a reduction in the population of MCF-7 cells was shown in the G2/M phase. The methanolic extract prepared from *Stachytarpheta mutabilis* has been observed to cause specific cell cycle arrest at the G2/M stage, leading to a decrease by about 25% in the population of the affected cells. However, at the G1 stage, maximum cell blocking of 74% was recorded. The methanolic extract from *Holarrhena floribunda* leaves has effects on the evaluation of cell cycle. Findings from this experiment indicate that the extract causes significant impairment on the transition of cells to the subsequent phase thus efficiently inhibiting cell cycle progression. This would mean that the extract is interfering with protein synthesis, a very critical event in cell development from G1 phase to the S-phase [21]. p53 and MDM2 proteins play a role in the advancement of the cell cycle at G0/G1. The potential activity of extract affecting disturbance of protein was not tested in this experiment. This may be due to the presence of different phytochemical compounds such as flavonoids, alkaloids, terpenoids, and phenols in the extract, which can affect the cell cycle progression [22]. The methanolic extract from *Capparis zeylanica* was found to have the capacity to stop the cell cycle's progression, particularly in the MCF-7 cell line's S phase and G2/M phase. The outcomes are consistent with what we discovered when we applied the methanolic extract of *Stachytarpheta mutabilis* to the MCF-7 cell line [23].

The initial stages of apoptosis are distinguished by the relocation of phosphatidylserine (PS) from the inner layer of the plasma membrane to the outer surface [24]. The analysis of flow cytometry involves the utilisation of Annexin V/propidium iodide (AnnV/PI) staining, which relies on the capacity of the protein Annexin V to attach to phosphatidylserine (PS). This phospholipid is translocated to the outer leaflet of the cell membrane during the initiation of apoptosis. In living cells, phosphatidylserine (PS) is primarily found in the inner membrane leaflet. However, during the initiation of apoptosis, PS is relocated to the outer membrane leaflet, where it becomes accessible for binding with Annexin V. It was demonstrated that the inclusion of PI facilitated the differentiation of viable (AnnV−/PI−), early apoptotic (Ann V+/PI−), late apoptotic (Ann V+/PI+), and necrotic (AnnV−/PI+) cells [25]. The flow cytometry analysis of MCF-7 cells revealed that the application of *Stachytarpheta mutabilis* extract resulted in a noticeable transition of the cell population from a viable state to an apoptotic state. An assessment of apoptosis induction in MCF-7 breast cancer cells using *Euphorbia hirta*. The results revealed a higher proportion of early apoptotic cells in comparison to the late apoptotic phase [26]. The aforementioned observation exhibits a resemblance to the induced apoptosis observed in the methanolic extract of *S. mutabilis.*

In order to provide a more comprehensive understanding of the potential anti-cancer mechanism of *T. terrestris*, the methanolic extract was utilised for flow cytometry analysis, specifically focusing on the assessment of early and late apoptosis. The results indicated that the extract exhibited significant apoptotic activity in comparison to the MCF-7 cell lines that were not treated. Nevertheless, the extract exhibited a lower apoptotic effect compared to the standard drug, cisplatin. The vanillin derived from proso and barnyard millet has the ability to induce apoptotic cell death in the MCF-7 cell line during the early apoptotic phase [27].

Linalool exhibited anti-cancer activity on Breast cancer MCF-7 xenograft mice models. The xenograft model revealed that the oral administration of linalool at 100mg/kg resulted in significant growth inhibition of the breast cancer. This study supports the view that linalool has anti-tumour activities by inducing apoptosis and modulating the immune system [28].

T33 Extract's effectiveness on both in vitro and in vivo-cultured breast cancer cells. The authors demonstrated that T33 Extract could significantly increase autophagy in MDA-MB231 and MCF-7 cells, preventing the cells from proliferating. Additionally, the study T33 Extract was able to suppress the growth of xenografted MDA-MB231 and MCF-7 cells in nude mice. T33 Extract is made from *Radix Kansui, Rheum rhabarbarum, Paeonia lactiflora, Jiangbanxia, and Zhigancao*. An animal study demonstrated that T33 in both low (200 mg) and high (600 mg) doses, is effective in suppressing xenografted breast cancer cells in BALB/c nude mice [29]. *Stachytarpheta mutabilis* methanolic extract suppressed xenografted MCF-7 cell proliferation in athymic nude mice by apoptosis mechanism. The in vitro analysis of MCF-7 cells by apoptosis assay showed the maximum apoptotic activity.

CONCLUSION

According to the available evidence, *Stachytarpheta mutabilis* methanol extract significantly inhibits breast cancer cell lines. *Stachytarpheta mutabilis* would therefore be a prospective source of anticancer medications, requiring further investigation to find the lead compounds possessing anticancer qualities. The presence of phenols, flavonoids, steroids, saponins, alkaloids, tannins, glycosides, and terpenoids in the methanol extracts is often responsible for their anti-cancer properties. The results of this study offer a scientific justification for the use of the herb in traditional medicine to treat breast cancer. To ascertain the extracts' cytotoxicity and the ideal dosage for use in conventional medicine, further confirmation research is necessary.

Conflict of interest

The authors declared no conflict of interest.

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