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# Anticancer Activity of *Euphorbia fischeriana* Root Extract in Human Breast Cancer: An *In-vitro* Investigation

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**Abstract:- In the current work, the phytochemical profile of *Euphorbia fischeriana* root extract was characterized on a qualitative and quantitative level, and then their anticancer activity was proven in human breast cancer cells by MTT and morphological evaluation. Qualitative phytochemical analysis showed that *E. fischeriana* root extract contains flavonoids, quinones, terpenoids, triterpenoids, phenolics, carbohydrates, proteins, tannins, alkaloids, glycosides, and steroids. A quantity of 1 gm of *E. fischeriana* root extract was found to contain 18.29 ± 0.86 mg GAE of total phenolics and 26.10 ± 1.71 mg QE of total flavonoids. Also, the antioxidant activity of *E. fischeriana* root extract was revealed by DPPH and ABTS assays, and the $EC_{50}$ value (concentration required to scavenge 50% of free radicals) of *E. fischeriana* root extract was 72.59 ± 6.82 and 78.91 ± 5.70 µg/mL in DPPH and ABTS free radical scavenging activity, respectively. In an anticancer assay, the $IC_{50}$ value (concentration required to inhibit 50% of cell viability) of *E. fischeriana* root extract on human breast cancer (MDA-MB-231) was determined as 104.04 ± 3.27 µg/mL. Micromorphological analysis revealed that *E. fischeriana* root extract-treated human breast cancer (MDA-MB-231) cells demonstrated cellular membrane breakdown, cellular debris leakage, and the formation of apoptotic bodies. Thus, *E. fischeriana* root extract has potential anticancer action on human breast cancer (MDA-MB-231) cells. The study concluded that *E. fischeriana* root extract could be highly applicable as an antioxidant and anticancer agent in the biomedical industry.**

***Keywords:-*** *Euphorbia fischeriana, Phytochemical Profile, Anticancer, Breast Cancer, MDA-MB-231 Cells.*

## I. INTRODUCTION

The second leading cause of death in the world is cancer. Overall, the incidence of cancer has risen; as a result, it is a severe issue that has an impact on the health of all human cultures. Regrettably, at the tissue level, it is a range of diseases, and this variability makes a precise diagnosis and effective therapy extremely difficult. In men, the prostate, lung and bronchus, colon and rectum, and urinary bladder, correspondingly, have the most significant percentages of cancer kinds. The breast, lung, bronchi, colon, rectum, uterine corpus, and thyroid are the areas of the female body where cancer is most common. According to the data, the two cancers that affect men and women most frequently are prostate and breast cancer, respectively. Blood cancer, tumors of the brain, and cancers of the lymph nodes, respectively, account for the highest percentage of cancer cases in children. The development of cancer is caused by a succession of gene changes that alter how cells operate. Evidently, chemical substances have a part in the development of cancerous cells and gene alterations. Smoking also contains a number of chemical substances known to cause cancer and lung cancer. The cytoplasm and nucleus of cells are interestingly affected directly or indirectly by environmental chemicals having carcinogenic qualities, which results in genetic diseases and gene alterations. Another 7% of all cancers are caused by carcinogenesis agents such as viruses, bacteria, and radiation. In general, cancer alters cellular interactions and causes critical genes to malfunction. The cell cycle is affected by this perturbation, which causes aberrant proliferation. Under normal circumstances, proto-oncogenes are in charge of cell division and proliferation; nevertheless, they transform into oncogenes during genetic mutation, which is the riskiest for cell survival. Furthermore, the absence of tumor suppressor genes causes unchecked cell division. Additional knowledge has been gathered that can be helpful for early diagnosis and appropriate treatment with the use of technical advancements in bioinformatics and molecular approaches. The negative effects of medications on cancer patients can be anticipated and, in some cases, managed (Hassanpour & Dehghani, 2017; Ferlay et al., 2021).

The molecules derived from medicinal plants are more tolerable and non-toxic to normal human cells, giving them a number of advantages over chemical products. Radiotherapy and chemotherapy are currently available for cancer treatments, but they have a number of side effects that can gravely harm a patient's health, including neurological, cardiac, renal, and pulmonary toxicity. As a result, it is necessary to design a novel approach that uses a medicine that is more effective and less harmful than those that are currently on the market. Numerous studies have been conducted on naturally occurring substances that exhibit to kill cancer cells and are known to have cytotoxicity. Because of these benefits, medicinal plants are

in high demand, and a number of species have been chosen and vetted for the production of cancer medications. Numerous research has discovered how medicinal plants can be used to prevent and treat cancer. Recently, there has been a rise in interest in the research of plant-derived materials as anticancer compounds (Tariq et al., 2017).

In the present study, the anticancer potential of root extract from *Euphorbia fischeriana* was investigated. More than 2000 species make up the largest genus in the Euphorbiaceae family, Euphorbia. Folk medicine practitioners have utilized some members of the genus Euphorbia to treat conditions like gonorrhea, migraines, intestinal parasites, and warts. One of these is the perennial herb Euphorbia fischeriana, which may be found in the dry grasslands of Siberia, Mongolia, China, Korea, and Japan. It can reach a height of 30 cm. The root is enormous and resembles a humanoid shape. The stem is laticiferous, crimson, terete, dichotomously branched, and meaty. White as snow, the latex (Ma et al., 1997; Sun & Liu, 2011).

In the present study, the qualitative and quantitative phytochemical profile of *E. fischeriana* root extract was determined, and following their anticancer activity was established in human breast cancer cells by MTT and morphological assessment. The antioxidant activity of *E. fischeriana* root extract was determined by DPPH and ABTS assays.

## II. MATERIALS AND METHODS

### A. Chemicals and Reagents

Distilled water, ethanol, Whatman® grade 1 filter paper, gallic acid, potassium chlorate, hydrochloric acid, sodium hydroxide, isopropyl alcohol, conc. sulfuric acid, Folin-Ciocalteau, ferric chloride, aluminum chloride, lead acetate, Molisch's reagent, pyridine, silver nitrate, chloroform, glacial acetic acid, sodium chloride, quercetin, copper sulfate solution, Mayer's reagent, and other reagents used in the test were obtained from Merck, Bengaluru, India. Dulbecco's modified Eagle medium (DMEM), cisplatin, ABTS, fetal bovine serum (FBS), phosphate buffered saline of pH 7.4 (PBS), MTT reagent, dimethyl sulfoxide (DMSO), and DPPH were purchased from Sigma-Aldrich, Bengaluru, India. The plasticware was purchased from Nunc, Bengaluru, India.

### B. Collection of Plant Sample and Preparation of Root Sample

Roots from *Euphorbia fischeriana* were collected from Sri Venkateswara University, Tirupati, India. The roots were washed with clean water to remove dirt and dust. After that, the roots were rinsed with distilled water and allowed to drain the water from the roots for some time. Then the roots are shadow dried for one week to remove moisture from them. After drying the roots are powdered with the help of an electrical chopper to acquire fine powder. Then, using the cold maceration method, 100 g of *E. fischeriana* root powder was dissolved in 100 mL of ethanol. After three days of maceration, the filtrate was filtered through Whatman® grade 1 filter paper and lyophilized at -40 °C to concentrate. The extract of *E. fischeriana* powder was used for additional research after being stored at 4° C in a sterile airtight bag.

### C. Qualitative Phytochemical Analyses

Phytochemical screening of the aqueous solution of *Euphorbia fischeriana* root used for qualitative screening of phytochemical compounds viz., alkaloids, flavonoids, quinones, glycosides, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, and proteins in accordance with the standard methods described by Kokate, (1988).

- *Detection of Flavonoids*

The concentrated sulfuric acid was applied in tiny drops to the *E. fischeriana* root extract (1 mg/mL). The orange color that was seen indicates the presence of flavonoids. Furthermore, the ammonia test is used to determine the presence of flavonoids; in this test, *E. fischeriana* was combined with a minute amount of potassium chlorate and a drop of hydrochloric acid. The sample was then dried using an evaporator before the residue was subjected to ammonia vapor. In this test, purine alkaloids give off a pink color. Additionally, flavonoids were examined using a sodium hydroxide test. In short, 10 mL of distilled water was added, the mixture was agitated, and 3 mL of *E. fischeriana* root extract (1 mg/mL) was pipetted out. 1 mL of 10% sodium hydroxide is then added to the liquid after that. Yellow color is a positive indicator of flavonoids.

- *Detection of Quinones*

For the quinones test, sulfuric acid was used. 10 mg of root extract that had been dissolved in 10 mL of isopropyl alcohol received one drop of strong sulfuric acid. Quinones were present as shown by the production of red color.

- *Detection of Terpenoids*

For the terpenoids test, the Salkowskis test was used. Briefly, after treating the aqueous *E. fischeriana* root extract (1 mg/mL) with 2 mL of chloroform and a few drops of concentrated sulfuric acid, the presence of a reddish-brown color suggests the presence of terpenoids.

- *Detection of Phenols*

Ferric chloride and lead acetate assays were used for testing phenols. For the ferric chloride test, 10 mL of distilled water and 10 mg of *E. fischeriana* root extract were combined. Next, 5% of the ferric chloride solution was added dropwise. The development of a blackish-green or dark-blue color was used as a marker for the presence of phenols. In the lead acetate test, 5 mL of 10% lead acetate was added to 10 mg of *E. fischeriana* root extract. Phenols have been identified when a white precipitate formed.

- *Detection of Saponins*

In the foam test, 5-10 mL of distilled water was added to the graduated cylinder after adding 10 mg of *E. fischeriana* root extract, and the tube was shaken for 15 min. The formation of a 2 cm thick foam layer suggests the presence of saponins. In the emulsion test, a few drops of olive oil were added to the *E. fischeriana* root extract (10

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mg) along with 10 mL of water and shaken for some time. Saponins were observed by the formation of an emulsion.

➢ *Detection of Tannins*

In the ferric chloride test, a test tube containing the root extract of *E. fischeriana* (10 mg) was filled with 10 mL of distilled water, boiled, and then added. After adding a few drops of 10% ferric chloride, the ferric chloride became green, indicating the presence of tannins. Another test, the matchstick test involved dipping a wooden matchstick in the root extract of *E. fischeriana*, letting it air dry for a while, and then dipping it in a concentrated hydrochloric acid solution. As the matchstick warmed up next to the flame, purple color was visible, proving the tannins were there.

➢ *Detection of Carbohydrates*

Molisch's Test was used here. *E. fischeriana* root extract was added to 1 mL of Molisch's reagent and 1 mL of conc. sulphuric acid, a reddish ring was formed indicated the presence of carbohydrates.

➢ *Detection of Protein*

In the Biuret test, the aqueous extraction of *E. fischeriana* root extract was treated with 1 mL of 40% sodium chloride solution and 2 drops of 1% copper sulfate solution. Then Appearance of violet color indicates the presence of proteins.

In the Xanthoproteic test, when 1ml of conc. sulphuric acid was added to the *E. fischeriana* root extract, and a white precipitate was produced; however, when it was heated, a yellow precipitate developed. The color turned orange after the addition of 2 drops of ammonium hydroxide, showing the presence of proteins.

➢ *Detection of Alkaloids*

In Mayer's test, *E. fischeriana* root extract was treated with 4 mL of 1% hydrochloric acid and was warmed and filtered and then a few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

In Wagner's test, Wagner's reagent was used to treat the *E. fischeriana* root extract, and the formation of a reddish or brown precipitate indicates the presence of alkali.

➢ *Detection of Glycosides*

In the sulphuric acid test, the aqueous *E. fischeriana* root extract was treated with 1 mL of glacial acetic acid, and 5 mL of ferric chloride was added. Also, a few drops of conc. sulphuric acid was added to the solution. The greenish-blue color solution was formed, which indicates the presence of glycosides.

In the Keller – Kiliani test, to 2 mL of ethanolic *E. fischeriana* root extract, 2 mL of glacial acetic acid, and 0.5 mL of 1% ferric chloride were added. A 1mL conc. sulfuric acid solution was then applied to it. The greenish-blue color showed the presence of cardiac glycosides.

In Tollen's test, an equal volume of pyridine and metallic silver nitrate, the *E. fischeriana* root extract was added. The glycosides are represented by the silver liberation that was revealed by the development of silver mirrors on the tube walls.

➢ *Detection of Steroids and Triterpenoids*

In Lieberman-Buchard's test: Following the addition of 2 mL of chloroform and 1 mL of concentrated sulfuric acid, 1 mL of ethanolic *E. fischeriana* root extract was treated with 0.5 mL acetic anhydride, boiled, and cooled. A reddish-brown ring that formed at the level where the two liquids separated served as evidence that steroids were present. Triterpenoid's presence indicates when a deep red color forms.

*D. Quantitative Phytochemical Analysis*

➢ *Estimation of Total Flavonoids*

The amount of total flavonoids in *E. fischeriana* root extract was calculated using the aluminum chloride calorimetric test (Gunti et al., 2019). Briefly, 0.15 mL of a 5% $NaNO_2$ solution was added to 2 mL of distilled water, along with a diluted sample solution of *E. fischeriana* root extract (up to 10 mg) in distilled water. After adding 0.15 mL of a 10% aluminum chloride solution and letting the mixture allow for 10 min, 2 mL of a 4% NaOH solution was added. The final volume was quickly adjusted to 5 mL by adding water. The mixture was thoroughly mixed and then allowed to stand for a further 15 min. The mixture was completely amalgamated and then allowed to stand for an additional 15 min. The mixture's absorbance at 510 nm was calculated using a plate reader (Synergy H1, BioTek, USA) and compared to a water blank. Quercetin was used as standard flavonoid. A quantity of mg QUE/g (mg quercetin equivalents per gram of *E. fischeriana* root extract) was used to express the results.

➢ *Estimation of Total Phenolics*

The Folin-Ciocalteau colorimetric technique was used to ascertain the total phenol concentration of *E. fischeriana* root extract (Gunti et al., 2019). Briefly, up to 10 mg of *E. fischeriana* root extract had been dissolved in 2 mL of distilled water, 1 mL of Folin–Ciocalteau reagent, and 1 mL of sodium carbonate was added to the reaction tube. The reaction mixture's absorbance at 750 nm (Synergy H1, BioTek, USA) was measured after it had been incubated for two hrs at room temperature. Gallic acid was used as a standard phenolic. Gallic acid equivalents per gram of *E. fischeriana* root extract (mg GAE/g) were used to calculate the total phenolic content of the *E. fischeriana* root extract.

*E. Antioxidant Activity*

➢ *DPPH Assay*

Using a standard DPPH assay, the ability of *E. fischeriana* root extract to scavenge free radicals was evaluated (Iordache et al., 2023). Briefly, ethanol was used to generate a 0.3 mM DPPH solution, and different quantities of *E. fischeriana* root extract (up to 100 µg/mL)) were added to the DPPH (100 µL) solution. After being

vortexed, the mixture was incubated for 30 min at 27 °C in the dark. Without the *E. fischeriana* root extract, the DPPH solution was regarded as a blank. The reaction mixture was vortexed and the absorbance of the solution was assessed using a microplate reader (Synergy H1, BioTek, USA) at 517 nm, results were compared to a control. The reference standard used was ascorbic acid. The following formula was used to express the results.

$$\text{DPPH free radicals scavenging activity (\%)} = \frac{C_{OD} - T_{OD}}{C_{OD}} \times 100$$

Where, terms '$C_{OD}$' and '$T_{OD}$' in the equation refer to the control and test absorbance, respectively.

➢ *ABTS Assay*

One of the most popular antioxidant assays for evaluating free radicals is this one (Iordache et al., 2023). Briefly, ABTS (7 mM) and potassium persulfate (2.5 mM) reacted in a 1:1 (v/v) ratio to produce a working solution of $ABTS^{+}$ radicals. After about 15 hrs, the reaction mixture was left at room temperature in the dark. Ethyl alcohol was used to dilute this solution until an absorbance of 0.70 at 734 nm was noted. The different concentrations of *E. fischeriana* root extract and ABTS solution (200 µL) were then added to each well of the microplate. After 30 minutes of dark incubation, the mixture's absorbance at 734 nm was determined (Synergy H1, BioTek, USA). The standard utilized was ascorbic acid. The following formula was used to express the results.

$$\text{ABTS free radicals scavenging activity (\%)} = \frac{C_{OD} - T_{OD}}{C_{OD}} \times 100$$

Where, terms '$C_{OD}$' and '$T_{OD}$' in the equation refer to the control and test absorbance, respectively.

*F. Anticancer Activity*

Using the MTT assay and morphological observation of cells, the anticancer activity of *E. fischeriana* root extract was evaluated (Swaminathan et al., 2021). Selected cancer cell line human breast cancer (MDA-MB-231) was cultured in DMEM media supplemented with penicillin-streptomycin (1% v/v) solutions and FBS (10%). The cells were maintained at 37 °C and exposed to 5% $CO_2$. About, 90% of confluent cells were chosen, and they were then placed in DMEM media devoid of FBS at a density of $1.5 \times 10^4$ in 96-well cell culture plates and allowed to adhere overnight. *E. fischeriana* root extract was dissolved in DMSO (10 mg/mL) and test concentration was prepared in DMEM devoid of FBS, which had DMSO at 0.01% or less. The chosen cells were exposed to various concentrations of *E. fischeriana* root extract and incubation was carried out for 24 hrs at 37 °C and 5% $CO_2$. Following, the test solution was replaced with MTT solution (50 µL, which was prepared at 5 mg/mL in PBS) and left to incubate for 3 hrs at 37 °C and the formazan product's absorbance was measured at 570 nm using a microplate reader (Synergy H1, BioTek, USA). Subsequently, the cell viability of the *E. fischeriana* root extract treated sample was expressed with respect to control (100%). Cisplatin was used as a standard anticancer agent.

*G. Statistical Analysis*

Each test had been conducted over three times, and the data were expressed using the mean and standard deviation. ANOVA was used to analyze the data, then Dunnett's or Tukey's tests to determine statistical significance. The significance level was set at the 95% confidence interval ($p \leq 0.05$). The above-mentioned statistical analysis was carried out using the GraphPad Prism 9.0 trial version.

## III. RESULTS AND DISCUSSION

*A. Phytochemical Analysis*

There is no one uniform definition of phytochemicals that is agreeable to everyone; the term literally means "chemicals from a plant." However, this term is used to characterize plant-derived bioactive chemicals with potential health advantages in the field of nutrition. The majority of phytochemicals are secondary plant metabolites that can be found in a wide range of foods, medicines, and beverages, including fruit, vegetables, cereals, nuts, herbal medicines, wine, and cocoa/chocolate. Dietary intake of phytochemicals typically exceeds 1 g per day (Zhang & Virgous, 2019).

In the present study, qualitative phytochemical analysis showed that *E. fischeriana* root extract contains flavonoids, quinones, terpenoids, triterpenoids, phenolics, carbohydrates, proteins, tannins, alkaloids, glycosides, and steroids. However, *E. fischeriana* root extract doesn't have saponins (**Table 1**).

Table 1 Qualitative phytochemical profile of *E. fischeriana* root extract obtained by cold maceration technique.

| S. No | Compound (test) | | Result |
|---|---|---|---|
| 1 | **Flavonoids** | | |
| | | Sulfuric acid test | Positive |
| | | Ammonia test | Positive |
| | | Sodium hydroxide test | Positive |
| 2 | **Quinones** | | Positive |
| 3 | **Terpenoids** | | Positive |
| 4 | **Phenols** | | |
| | | Ferric chloride test | Positive |
| | | Lead acetate assay | Positive |
| 5 | **Saponins** | | |
| | | Distilled water test | Negative |

| | | | |
|---|---|---|---|
| | | Olive oil test | Negative |
| 6 | **Tannins** | | |
| | | Ferric chloride | Positive |
| | | Matchstick test | Positive |
| 7 | **Carbohydrates** | | Positive |
| 8 | **Proteins** | | |
| | | Biuret test | Positive |
| | | Xanthoproteic test | Positive |
| 9 | **Alkaloids** | | |
| | | Mayer's Test | Positive |
| | | Wagner's Test | Positive |
| 10 | **Glycosides** | | |
| | | Sulphuric acid test | Positive |
| | | Keller – Kiliani test | Positive |
| | | Tollen's test | Negative |
| 11 | **Steroids** | | Positive |
| 12 | **Triterpenoids** | | Positive |

Positive: Present. Negative: Absent.

The quantitative phytochemical profile of *E. fischeriana* root extract was revealed by estimating total flavonoids and total phenolics and results were depicted in **Figure 1**. A quantity of 1 gm of *E. fischeriana* root extract was found to contain 18.29 ± 0.86 mg GAE of total phenolics and 26.10 ± 1.71 mg QE of total flavonoids. The study concluded that *Fischeriana* root extract has a rich source of phenolic and flavonoid molecules, which could be highly useful as pharmacologically active compounds. In support of our study, Li et al., (2021) have reviewed the knowledge on *E. fischeriana* and reviewed its traditional, clinical, phytochemistry, and pharmacology applications.

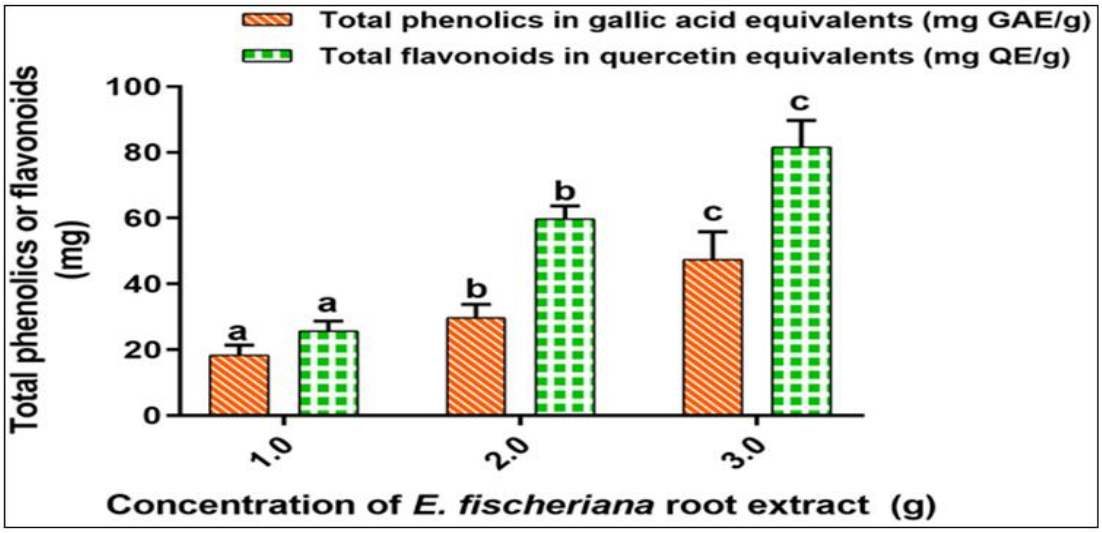


Fig 1 Total Phenolic and Total Flavonoid Content of *E. fischeriana* Root Extract Determined Dose-Dependently.

Each test had been conducted over three times, and the data were expressed using the mean and standard deviation. ANOVA was used to analyze the data, then Tukey's test to determine statistical significance. The significance level was set at the 95% confidence interval ($p \leq 0.05$). Bar graphs with separate alphabets are used to show the statistical significance in the particular study group.

*B. Antioxidant Activity*

Since oxygen is the final electron acceptor in the electron flow system that generates energy in the form of ATP, oxidation—the movement of electrons from one atom to another—represents a crucial component of aerobic life and our metabolism. However, when the electron flow decouples (transfer of unpaired single electrons), resulting in the production of free radicals, issues may occur. A free radical is a chemical entity with one or more unpaired electrons that is capable of independent existence. The molecules known as free radicals are very brittle. Reactive oxygen species (ROS) are substances that can oxidize biomolecules and are either radicals with at least one unpaired electron or reactive non-radical chemicals. As a result, oxidants or pro-oxidants are other names for these intermediates. The continual production of ROS during typical physiological processes makes it simple to start the peroxidation of membrane lipids, which results in the buildup of lipid peroxides. For appropriate cell activity, ROS at physiological amounts can be necessary. In addition, they have the power to harm important macromolecules like carbohydrates, lipids, proteins, nucleic acids, and

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polyunsaturated fatty acids. They might also harm DNA, which can result in mutations. ROS can trigger free-radical chain reactions that damage cellular macromolecules including proteins, lipids, and nucleic acids and eventually result in disease situations if they are not adequately scavenged by cellular components (Gülcin, 2012).

There has been a lot of interest recently in finding alternative, natural, and secure sources of antioxidants as well as in the hunt for natural antioxidants, particularly those with a plant origin. Antioxidants are frequently added to foods to stop the radical chain reactions of oxidation. They work by blocking the steps that start and spread the reaction, which delays the oxidation process. Research on the use of plant-derived antioxidants in food and human health has recently attracted increasing attention. Recent studies have shown that the beneficial effects of various foods and beverages, such as fruits, vegetables, tea, coffee, and cacao, on human health are caused by their antioxidant activity. The most popular techniques for determining the antioxidant capacity of food ingredients in vitro are evaluated and provided for this purpose (Munteanu & Apetrei, 2021).

By DPPH and ABTS antioxidant assays, the quantitative antioxidant potential of *E. fischeriana* root extract was revealed. The results are shown in **Figures 2 & 3**. The results showed that *E. fischeriana* root extract has dose-dependently scavenged the free radicals of DPPH and ABTS. The $EC_{50}$ value (concentration required to scavenge 50% of free radicals) of *E. fischeriana* root extract was 72.59 ± 6.82 and 78.91 ± 5.70 µg/mL in DPPH and ABTS free radical scavenging activity, respectively. In support of this study, Sun et al., (2023) have revealed the antioxidant activities of different extracts of *E. fischeriana*. The study concluded that *E. fischeriana* root extract has potential antioxidant activity and could be useful to treat oxidative stress-related disorders.

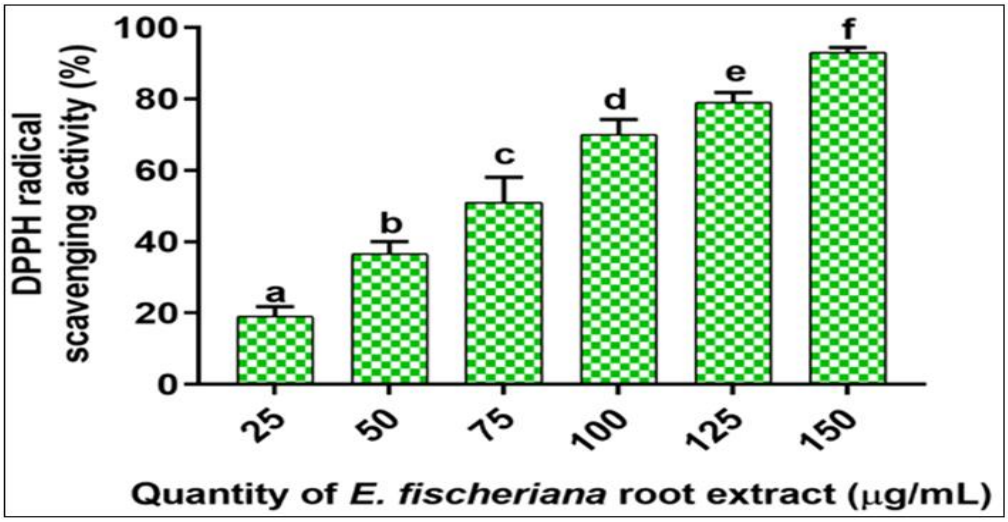


Fig 2 DPPH Free Radical Scavenging Activity of *E. fischeriana* Root Extract Determined Dose-Dependently.

Each test had been conducted over three times, and the data were expressed using the mean and standard deviation. ANOVA was used to analyze the data, then Tukey's test to determine statistical significance. The significance level was set at the 95% confidence interval ($p \leq 0.05$). Bar graphs with separate alphabets are used to show the statistical significance in the particular study group.

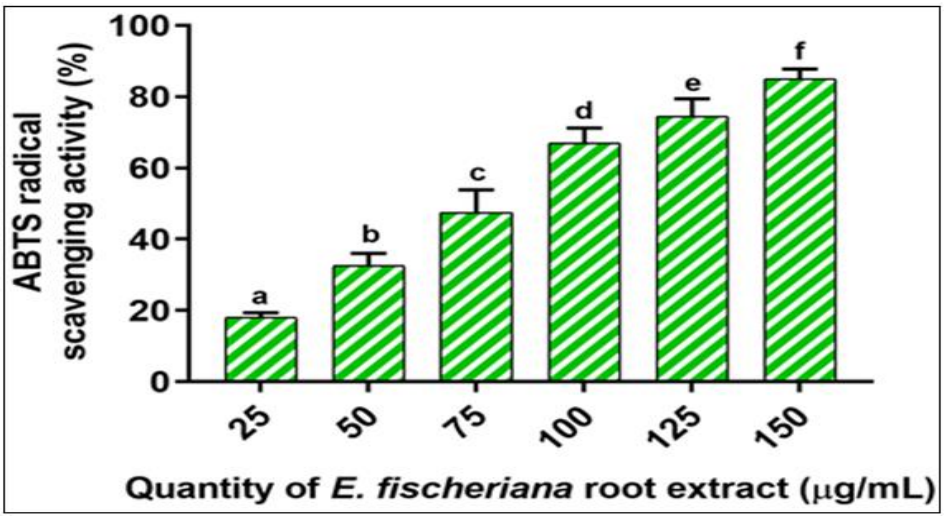


Fig 3 ABTS Free Radical Scavenging Activity of *E. fischeriana* Root Extract Determined Dose-Dependently.

Each test had been conducted over three times, and the data were expressed using the mean and standard deviation. ANOVA was used to analyze the data, then Tukey's test to determine statistical significance. The significance level was set at the 95% confidence interval ($p \leq 0.05$). Bar graphs with separate alphabets are used to show the statistical significance in the particular study group.

*C. Anticancer Activity*

Proteins that control the cell cycle regulate cell proliferation as a whole, ensuring that animals remain in the proper size and form. Failure of the regulatory processes has occasionally been shown to cause cells to divide indefinitely and eventually give rise to cancer. The second largest cause of mortality worldwide, cancer is a major health burden. Due to the participation of numerous elements during carcinogenesis, its treatment has become one of the most difficult tasks in recent times. Currently, surgery, radiation, and chemotherapy are the three main cancer treatment modalities. Each treatment modality has a specific role in the treatment or can be used alone or in conjunction with others. Chemotherapy is a common form of treatment for a variety of cancers in varying stages that involves the systemic administration of the chemical drug(s) to prevent or control the growth of cancer cells (Verma et al., 2020).

An integral component of ethnopharmacology is the thorough reporting of traditional knowledge and its experimental validation against humans and domestic animals. It might support the sustainable management, growth, and use of natural resources in the future. According to reports, more than 80% of people around the world rely primarily on complementary and alternative medicine, and over 60% of clinically used medications are made from natural sources. It is well known that chemotherapeutic medicines for cancer derived from natural materials, or their synthetic analogs, hold a crucial role due to their little or nonexistent side effects and host anti-multidrug resistance. Currently, the treatment of cancer involves the use of some effective plant-derived anticancer medications, including vincristine, vinblastine, irinotecan, etoposide, paclitaxel, camptothecin, and epipodophyllotoxin (Winston & Beck, 1999; Verma et al., 2020).

With 2000 species, Siddha (1121 species), Unani (751 species), and Tibetan (337 species), India has the most traditional knowledge in Asia when it comes to the use of plants and animals as medicine to treat various illnesses. Virtually every medication now used to treat cancer has been shown in studies conducted over the past 15 years to be able to cause apoptosis (Kaufmann & Earnshaw, 2020).

In the present study, the anticancer activity of *E. fischeriana* root extract on human breast cancer (MDA-MB-231) was revealed by MTT and morphological analysis. The MTT assay was carried out in a dose-dependent manner (**Fig. 4**). The $IC_{50}$ value (concentration required to inhibit 50% of cell viability) of *E. fischeriana* root extract on human breast cancer (MDA-MB-231) was determined as 104.04 ± 3.27 µg/mL. Micromorphological analysis revealed that *E. fischeriana* root extract-treated human breast cancer (MDA-MB-231) cells demonstrated cellular membrane breakdown, cellular debris leakage, and the formation of apoptotic bodies (**Fig. 5**). Contrarily, untreated control cells of human breast cancer (MDA-MB-231) had a smooth, monolayer shape. The results of the MTT experiment were compatible with the morphological characteristics. The results of the study suggest that *E. fischeriana* root extract has anticancer activity against human breast cancer (MDA-MB-231) cells. In support of our study, Jian et al., (2018) and Zhu et al., (2022) have confirmed the anticancer activity of different cancer cells. The study concluded that the anticancer potential of *E. fischeriana* root extract could be highly applicable in the biomedical industry.

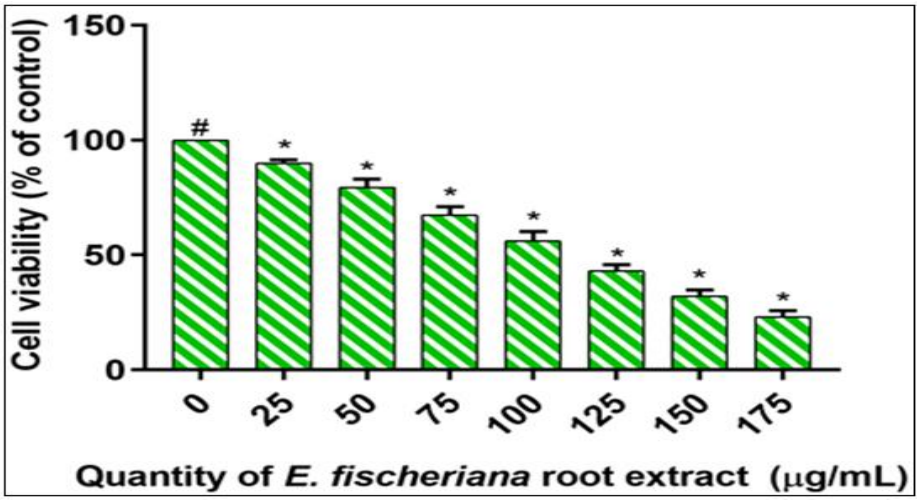


Fig 4 Dose-Dependent Anticancer Activity of *E. fischeriana* Root Extract by MTT Assay.

Each test had been conducted over three times, and the data were expressed using the mean and standard deviation. ANOVA was used to analyze the data, then Dunnett's test to determine statistical significance. The significance level was set at the 95% confidence interval ($p \leq 0.05$). Bar graphs in this study group with the symbols '#' and '*' indicate, respectively, that the test sample was not significant and significant in relation to the control.

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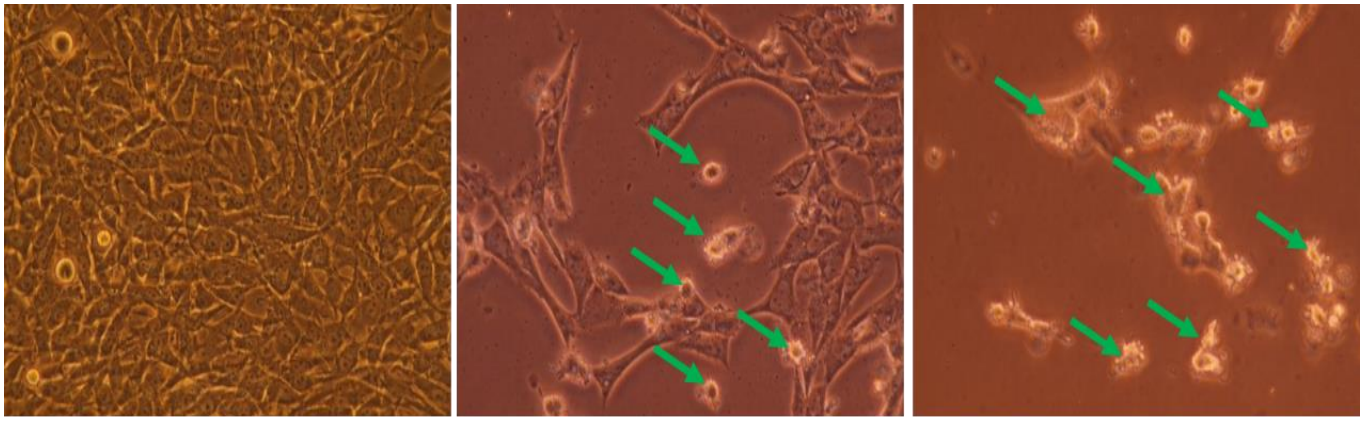


Fig 5 *E. fischeriana* root extract impact on the morphology of MDA-MB-231 cancer cells, a kind of human breast cancer. (A) Cells in control. (B) Cells that received 100 µg/mL of *E. fischeriana* root extract treatment. (C) Cells that received 150 µg/mL of *E. fischeriana* root extract treatment. The photographs were taken at 400x magnification. The development of apoptotic bodies, cellular debris leakage, and cell membrane breakdown are all depicted by the blue arrows.

The cells in this process are disassembled into numerous membrane-enclosed apoptotic bodies, which are eventually consumed by the body's phagocytic cells. This process is characterized morphologically by plasma membrane blebbing, cell shrinkage, and chromatin condensation. The activation of caspases, a class of cysteine-dependent aspartate-directed proteases, causes this biochemical method of cell death. The two main pathways lead to the transduction of signals, which in turn activates the initiator and executioner class of caspases. One pathway begins with the interaction of receptor-ligand (death receptors), and the other is linked to the release of cytochrome c from the damaged mitochondrial membrane (Budihardjo et al., 1999).

## IV. CONCLUSION

The qualitative and quantitative analysis showed that *E. fischeriana* root extract was abundant in primary and secondary metabolites, including flavonoids, quinones, terpenoids, triterpenoids, phenolics, carbohydrates, proteins, tannins, alkaloids, glycosides, and steroids. According to the quantitative analysis, *E. fischeriana* root extract has more phenolics than flavonoids. *E. fischeriana* root extract has exhibited potential antioxidant activity. Against the investigated human breast cancer (MDA-MB-231), the *E. fischeriana* root extract showed promising anticancer action. However, more proof of *E. fischeriana* root extract's anticancer potential will need to come from extensive molecular study. The investigation came to the conclusion that flavonoids and phenolics in *E. fischeriana* root extract had pharmacologically advantageous properties.

- *Conflict of Interest*

  No conflict of interest exists

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