International Journal of Zoology and Applied Biosciences ISSN: 2455-9571
Volume 10, Issue 6, pp: 98-116, 2025
https://doi.org/10.55126/ijzab.2025.v10.i06.012 http://www.ijzab.com

Research Article

COMPARATIVE EVALUATION OF ANTIOXIDANT AND ANTICANCER PROPERTIES OF *OCIMUM TENUIFLORUM* AND *CATHARANTHUS ROSEUS*

[1]*Ayesha Shaikh and [2]Sandhya Kadiru

[1]Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai, India
[2]Department of Zoology, Sophia College for Women (Empowered Autonomous), Mumbai, India

Article History: Received 5th September 2025; Accepted 22nd October 2025; Published 1st November 2025

ABSTRACT

Plants possess a remarkable ability to produce a wide range of secondary metabolites, many of which serve as potent antioxidants and anticancer agents. India, being rich in medicinal plant biodiversity, holds immense untapped potential in this field. Cancer remains one of the leading causes of mortality globally, with brain tumors in India showing an incidence rate of 5 to 10 per 100,000 population and a rising trend. The present study explores the antioxidant and anticancer properties of plant extracts i.e. *Ocimum tenuiflorum* (Krishna Tulsi) and *Catharanthus roseus* (Periwinkle). Reactive oxygen species (ROS), which are harmful by-products of normal aerobic metabolism, contribute significantly to cancer development. Antioxidants act as scavengers of these free radicals. The antioxidant potential of the plant extracts was evaluated using the phosphomolybdenum assay. The results revealed that *C. roseus* exhibited an antioxidant activity of 28 µg/ml ascorbic acid equivalent (AAE), while *O. tenuiflorum* showed activity at 23 µg/ml. To assess preliminary anticancer potential, the in vitro antimitotic activity of the extracts was tested using the *Allium cepa* root tip assay. The methanolic extract of *O. tenuiflorum* demonstrated stronger antimitotic activity compared to *C. roseus*, with observable mitotic abnormalities such as cell fragmentation, receding cell contents, and necrosis indicating its ability to inhibit cell proliferation in cancerous tissues.Further cytotoxic studies were conducted on glioma cells, where both plant extracts induced cell death. Dye exclusion tests comparing the extracts with cyclophosphamide on white blood cells (WBCs) showed that both extracts were cytotoxic to WBCs but less so than cyclophosphamide. *Ocimum tenuiflorum*, particularly and *Catharanthus roseus* exhibit antioxidant and preliminary anticancer activities, warranting further investigation for selective, safe anticancer applications.

Keywords: Antioxidants, Anticancer agents, *Ocimum tenuiflorum*, *Catharanthus roseus*, *Allium cepa*, Glioma cells.

INTRODUCTION

Natural products derived from plants, animals, and minerals have historically served as effective treatments for a wide range of human diseases. Among them, plants have played a critical role in traditional and modern healthcare systems. Although the plant kingdom includes an estimated 500,000 species, only about 1% have been phytochemically investigated for the discovery of novel bioactive compounds (Atanasov *et al*., 2015). Medicinal plants grow abundantly and have been utilized for centuries across various cultures to treat numerous ailments (Zhang *et al*., 2015). The use of herbal remedies dates back to the oldest civilization and has remained integral to traditional medicine systems worldwide, the scientific isolation and characterization of their active compounds only began in the early nineteenth century (Petrovska, 2012). These plant-based substances have since become integral to the development of modern pharmacotherapy. A prominent historical example includes the semi-synthetic derivation of aspirin from the bark of Salix alba (white willow) in 1899. The World Health Organization (WHO) estimates that approximately 80% of the global population relies on herbal medicines for primary healthcare needs (Yuan *et al*., 2016). India, with its rich biodiversity and traditional systems like Ayurveda, remains a major hub of medicinal plant use. Similarly, in countries such as China and parts of Europe, herbal medicine continues to play a central role in healthcare delivery.

*Corresponding Author: Ayesha Shaikh, Assistant Professor, Department of Microbiology, Sophia College for Women (Empowered Autonomous), Mumbai, India Email: ayeshass786@gmail.com.

Plants are known to biosynthesize a vast array of secondary metabolites, including alkaloids, glycosides, terpenoids, flavonoids, steroids, tannins, and coumarins. Many of these compounds are potent antioxidants. Oxidative stress, caused by free radicals generated during normal metabolic activities, has been implicated in various acute and chronic disorders such as cancer, diabetes, atherosclerosis, immunosuppression, and aging. Antioxidants neutralize these free radicals, thereby preventing cellular damage (Sathisha *et al*., 2015). Numerous studies have shown that the antioxidant content of medicinal plants can play a significant role in reducing the risk of degenerative diseases. In cancer treatment, antioxidants are increasingly valued for their potential to enhance survival rates, mitigate side effects, and reduce the oxidative stress associated with chemotherapy. The integration of antioxidant-rich plant extracts into cancer treatment regimens is being explored as a promising strategy to improve patient outcomes and reduce therapeutic toxicity.

Cancer remains a major global health challenge and is the second leading cause of death worldwide. It is characterized by uncontrolled cellular proliferation, often resulting in tumor formation and metastasis. Brain tumors, particularly in children, are among the most common and deadly forms of cancer, accounting for a significant percentage of cancer-related mortality (Suresh *et al*., 2017). Although conventional treatments such as chemotherapy and radiation exist, they are often accompanied by severe side effects, including immune suppression and general health deterioration. Thus, there is a critical need for alternative therapies that are both effective and less toxic. Plant-based therapeutics have emerged as a compelling alternative in this context. Among the most notable medicinal plants are *Ocimum tenuiflorum L*. (commonly known as Krishna Tulsi or Holy Basil) and *Catharanthus roseus* (Periwinkle). *O. tenuiflorum*, a member of the Lamiaceae family, has demonstrated a wide range of pharmacological properties, including immunomodulatory, anti-inflammatory, hepatoprotective, cardioprotective, and anticancer effects (Rajesh *et al*., 2013; Cohen, 2014). Similarly, *C. roseus*, a plant native to Madagascar and widely used in traditional medicine, contains bioactive compounds with known anticancer activity, such as vincristine and vinblastine (Kabesh *et al*., 2015).

To evaluate the anticancer potential of these plants, it is essential to assess their effects on cancer cell lines. While various screening assays are available, many are cost-intensive and technically complex. The *Allium cepa* (onion) root tip assay offers a cost-effective and reliable method for initial cytotoxicity screening (Thenmozhi & Mahadevarao, 2011). Furthermore, glioma cell lines (e.g., C6 astrocytoma cells) provide a relevant model for studying brain tumor responses to plant extracts. Chemotherapy often leads to reduced white blood cell (WBC) counts, compromising the immune system and increasing susceptibility to secondary infections. Since WBCs can infiltrate inflamed tumor sites and contribute to immune surveillance, evaluating the protective effects of plant extracts on WBCs is also crucial. Dye exclusion tests can be employed to assess the cytotoxic or protective influence of these extracts on immune cells, helping determine their therapeutic viability. In this study, the antioxidant and anticancer properties of *Ocimum tenuiflorum* and *Catharanthus roseus* were explored through a series of in vitro assays, including antioxidant evaluation, cytotoxicity analysis on glioma cells, and WBC protection studies, aiming to establish their potential as plant-based agents in cancer therapy.

MATERIALS AND METHODS

Plant Material Collection

Fresh leaves of *Ocimum tenuiflorum* (Krishna Tulsi) and *Catharanthus roseus* (Periwinkle) were procured from a certified local nursery in Mumbai, India.

Preparation of Plant Extracts

The collected leaves were thoroughly washed with distilled water to remove dust and surface contaminants. They were then air-dried at room temperature in the shade to preserve bioactive compounds. Dried leaves were cut into small pieces and ground into a fine powder using a sterile electric grinder. The powdered material was stored in airtight, sterile containers at 4°C until further use.

Extraction Procedure

Crude extracts were prepared using the Soxhlet extraction method. For each plant, 5 grams of powdered leaf material was extracted with 100 mL of methanol. The extraction was carried out until the solvent in the siphon tube became colorless, indicating complete extraction. The resulting extract was concentrated by evaporating the solvent using a boiling water bath. The dried residue was weighed and reconstituted in methanol for subsequent analyses.

Preliminary Phytochemical Analysis

Preliminary phytochemical screening of *O. tenuiflorum* and *C. roseus* plant extracts was conducted using standard qualitative methods to detect major groups of secondary metabolites. The analysis was carried out using the following chemical tests:

Saponins

Foam test was performed by vigorously shaking the plant extract with water. Persistence of foam for 10 minutes indicated saponins.

Alkaloids

Three drops of Wagner's reagent were added to the extract. Formation of a brown/reddish-brown precipitate confirmed alkaloids.

Flavonoids

A few drops of 1 N NaOH were added to the extract. Yellow coloration that turned colorless upon addition of 1 N HCl indicated flavonoids.

Carbohydrates

Molisch's test was performed by adding alcoholic α-naphthol and concentrated H_2SO_4. Formation of a violet ring at the interface confirmed carbohydrates.

Glycosides

Modified Borntrager's test was carried out by treating the extract with 5% $FeCl_3$, boiling, cooling, and extracting with benzene followed by ammonia. A pink color in the benzene layer indicated anthraquinone glycosides.

Di-terpenes

Copper acetate solution was added to the extract. Emerald green color indicated the presence of diterpenes.

Triterpenes

Salkowski's test was performed using chloroform and concentrated H_2SO_4. A light-yellow color indicated triterpenes.

Phenolic Compounds

Ferric chloride (1.5%) was added to the extract. Bluish-black color confirmed phenols.

Tannins

The extract was treated with 1% gelatin in 0.8% NaCl. Formation of a white precipitate indicated tannins.

Proteins

Biuret test was performed by adding 10% NaOH and 0.7% $CuSO_4$ to the extract. A purplish-violet color indicated proteins.

Condensed Tannins

Acid butanol and iron reagent were added and the mixture was boiled. A cherry red color indicated condensed tannins.

Steroids

Salkowski's test was conducted using chloroform and concentrated H_2SO_4. A reddish-brown ring at the interface indicated the presence of steroids. All reactions were observed visually, and color changes or precipitate formation were recorded as qualitative indicators of the presence of specific phytochemical groups.

Determination of Antioxidant Activity (Phosphomolybdenum Assay)

The antioxidant activity of the plant extracts of *O. tenuiflorum* and *C. roseus* was evaluated using the phosphomolybdenum method as described by Prieto *et al*. (1999). In this assay, 0.6 ml of plant extract at different concentrations (50–250 µg/ml) was mixed with 6 ml of freshly prepared reagent solution containing sulphuric acid, sodium dihydrogen phosphate, and ammonium molybdate in equal proportions. To minimize the potential degradation of antioxidants due to light exposure, all tubes were kept in the dark, securely capped, and incubated in a water bath at 95 °C for 90 minutes. After incubation, the tubes were cooled to room temperature, and the absorbance of each solution was measured at 695 nm using a colorimeter against a reagent blank. The antioxidant activity was quantified by comparing the absorbance of the test samples to that of a standard curve prepared with ascorbic acid. Results were expressed as ascorbic acid equivalents (µg/ml). A higher absorbance indicated greater antioxidant activity.

Study of Mitotic Inhibition on Onion (*Allium cepa*) root tip

The antimitotic activity of plant extracts from *O. tenuiflorum* and *C. roseus* was evaluated using the *Allium cepa* root tip assay as described by Sehgal *et al*. (2006), with slight modifications. Onion bulbs were first germinated in tap water at room temperature under light conditions until roots reached a length of 2–3 cm. The water was replaced daily to ensure proper root growth. Once germinated, the bulbs were exposed to varying concentrations of plant extracts (50–250 µg/ml) for 24 and 48 hours at room temperature. Separate bulbs were maintained in distilled water as a negative control, while cyclophosphamide (50 µg/ml) and methanol served as positive and solvent controls, for *O. tenuiflorum* and *C. roseus* respectively.

At the end of the treatment period, the root tips were excised (2–3 mm), fixed in Carnoy's fixative, and hydrolyzed in 1N HCl at 60ºC for 12 mins. Following hydrolysis, the tips were stained with acetocarmine and incubated at room temperature for 10 minutes. The stained root tips were then gently squashed on a clean glass slide under a coverslip and observed. Microscopic observations were performed under 10x and 40x objectives. For each root tip, a minimum of 40 fields was examined to count the number of dividing cells at various mitotic stages (prophase, metaphase, anaphase, and telophase). The mitotic index (MI) was calculated using the following formula.

$$\text{Mitotic index (MI)} = \frac{P+M+A+T}{\text{Total cells observed}} \times 100$$

where P, M, A, and T are the numbers of cells in prophase, metaphase, anaphase, and telophase, respectively

All experiments were performed in triplicates. The results were analyzed statistically by calculating the mean ± standard deviation (SD). One-way ANOVA was employed to determine statistical significance between treated and control groups, followed by Holm's post-hoc correction. A p-value of less than 0.05 was considered statistically significant.

Cytotoxic Effects of *O. tenuiflorum* and *C. roseus* on Glioma Cells

The cytotoxic potential of *O. tenuiflorum* and *C. roseus* plant extracts was evaluated on C6 rat-derived astrocytoma cells. Glioma cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) under standard conditions (37°C, 5% CO_2).

Sub-culturing of Cells

Cells were trypsinized using cold trypsin (100 µl in 900 µl PBS), neutralized with DMEM + 10% FBS, and centrifuged at 3000 rpm for 3 minutes. The resulting pellet was resuspended in 100 µl of fresh DMEM.

Cell Counting

Cell viability and density were determined using a hemocytometer. A clean hemocytometer and coverslip were used to load the cell suspension, and cells were counted under 100x magnification. Treatment Protocol: Day 1: 4000 cells/100 µL were seeded onto 6-well plates containing sterile coverslips. Day 2: Cells were treated with the plant extracts at a concentration of 250 µg/ml. Untreated wells served as controls. Day 3: After 24 hours, morphological changes were observed and documented microscopically to assess cytotoxic effects. The experiment was conducted in triplicate. Data were recorded and analyzed to compare cell viability and morphological differences between treated and control groups.

Effect of the Prepared Plant Extracts of *O. tenuiflorum* and *C. roseus* on WBC Viability

Human peripheral blood was collected using a sterile syringe into Eppendorf tubes containing EDTA as an anticoagulant. For the isolation of leukocytes, 2 mL of the collected blood was carefully layered along the side of a sterile centrifuge tube containing 2 mL of Ficoll-Hypaque solution to ensure the formation of two distinct layers. The sample was centrifuged at 1000 rpm for 15 minutes at room temperature. Post centrifugation, the buffy coat formed at the interface was carefully aspirated using a sterile Pasteur pipette. To assess the cytotoxic effect of plant extracts on leukocyte viability, 10 µL of the isolated WBC suspension (buffy coat) was mixed with 10 µL of each plant extract (prepared at a concentration of 250 µg/mL). The mixture was incubated at room temperature for 30 minutes. After incubation, 10 µL of 0.4% Trypan Blue dye was added to each tube. The stained samples were then loaded onto clean, grease-free microscope slides and observed under high power magnification using a light microscope. Viable cells remained unstained (excluded the dye), while non-viable cells took up the blue stain due to compromised membrane integrity. Two control sets were included in the experiment: Positive control: WBCs treated with Cyclophosphamide (a known cytotoxic agent). Negative control: Untreated WBCs (only WBC suspension with no plant extract). The percentage of viable versus non-viable WBCs was recorded for each treatment and control. All experimental procedures were conducted in compliance with the guidelines and approval of the Ethics committee of Sophia College for Women, Mumbai.

RESULTS AND DISCUSSION

Methanolic extracts of two medicinal plants, *O. tenuiflorum* and *C. roseus*, were prepared and evaluated based on the amount of extract recovered after evaporation of the solvent. The percentage extract recovery was calculated to assess extraction efficiency.

Ocimum tenuiflorum: Weight of empty beaker (A): 187.12 g. Weight of beaker + extract (B): 190.11 g. Weight of extract recovered (B − A): 2.99 g. Weight of plant sample used: 5 g

Extract recovery (%) = Weight of the extract recovered after evaporation (g) x 100

Weight of the plant sample (g)

3 / 5 x 100 = 60%

Catharanthus roseus: Weight of empty beaker (A): 109.45 g, Weight of beaker + extract (B): 114.13 g, Weight of extract recovered (B − A): 4.68 g, Weight of plant sample used: 5 g

Extract recovery (%) =Weight of the extract recovered after evaporation (g) x 100

Weight of the plant sample (g)

4.68 5 x 100 = 93.6%

The extraction yield for *C. roseus* (93.6%) was significantly higher than that of *O. tenuiflorum* (60%). This difference could be attributed to variations in phytochemical composition, polarity of active compounds, and overall solubility in methanol. *C. roseus* is known to be rich in alkaloids, flavonoids, and phenolic compounds, many of which are methanol-soluble, contributing to the higher yield. The plant is a well-known source of anticancer alkaloids like vincristine and vinblastine, which are effectively extracted using polar solvents like methanol. *O. tenuiflorum* contains essential oils, terpenoids, and other bioactive compounds, but many of these may be less efficiently extracted with methanol alone, leading to a comparatively lower yield. This difference in extract recovery has practical implications. A higher yield may enhance the feasibility of using *C. roseus* extract in pharmacological or toxicological studies, especially in small-scale lab-based bioassays. However, a lower yield, as seen in *O. tenuiflorum*, does not necessarily indicate lower efficacy, since even small quantities of extract may contain

potent bioactive molecules. Thus, the preparation of plant extracts and the quantification of yield provide important preliminary data, guiding further evaluation of biological activity and standardization for experimental treatments.

The qualitative screening of methanolic extracts from *O. tenuiflorum* and *C. roseus* revealed the presence and absence of various phytochemical groups, as summarized in Table 1.

Table 1. Summary of phytochemical groups detected in methanolic extracts of *O. tenuiflorum* and *C. roseus* through qualitative assays.

Phytochemical Test	*O. tenuiflorum*	*C. roseus*
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Carbohydrates	-	-
Glycosides	-	-
Diterpenes	+	+
Triterpenes	+	-
Phenols	+	-
Tannins	-	-
Condensed Tannins	-	-
Proteins	-	-
Steroids	+	-

Key: (+) = Present; (−) = Absent

O. tenuiflorum extract tested positive for Saponins, Alkaloids, Flavonoids, Di-terpenes, Tri-terpenes, Phenols, and Steroids. *C.roseus* extract showed the presence of Saponins, Alkaloids, Flavonoids, and Di-terpenes, but was negative for Tri-terpenes, Phenols, and Steroids. The phytochemical profiles of the two plant extracts exhibit both similarities and distinct differences that may influence their biological activities: Both plants contain Saponins, Alkaloids, Flavonoids, and Di-terpenes, which are known for diverse pharmacological properties such as antimicrobial, anti-inflammatory, and antioxidant activities. The presence of these compounds in both extracts suggests potential overlapping bioactivities. The presence of Tri-terpenes, Phenols, and Steroids exclusively in *O. tenuiflorum* may confer additional therapeutic benefits, as these compounds have been linked to anti-inflammatory, anticancer, and immunomodulatory effects. For example, phenolic compounds contribute to strong antioxidant activity, which could enhance the plant's ability to modulate immune cell viability. *C. roseus* lacks these tri-terpenes, phenols, and steroids but still exhibits key bioactive classes like alkaloids, which include known anticancer compounds such as vincristine and vinblastine, explaining its widespread medicinal use. Neither extract contained carbohydrates, glycosides, tannins, condensed tannins, or proteins, indicating that these groups may not play a significant role in the observed biological activities or may be present in very low amounts. The preliminary phytochemical screening confirms that both plant extracts are rich in bioactive secondary metabolites. This supports their further investigation for biological effects, including the influence on white blood cell viability as part of immunomodulatory studies.

The antioxidant potential of *O. tenuiflorum* and *C. roseus* extracts was evaluated using the phosphomolybdenum assay. This method relies on the reduction of Mo (VI) to Mo (V) by antioxidant compounds under acidic conditions, resulting in a green phosphate/Mo(V) complex measurable at 695 nm. A standard curve was generated using ascorbic acid (10–100 µg/ml), and the antioxidant activity of the extracts was determined by extrapolating their absorbance values against this standard curve.

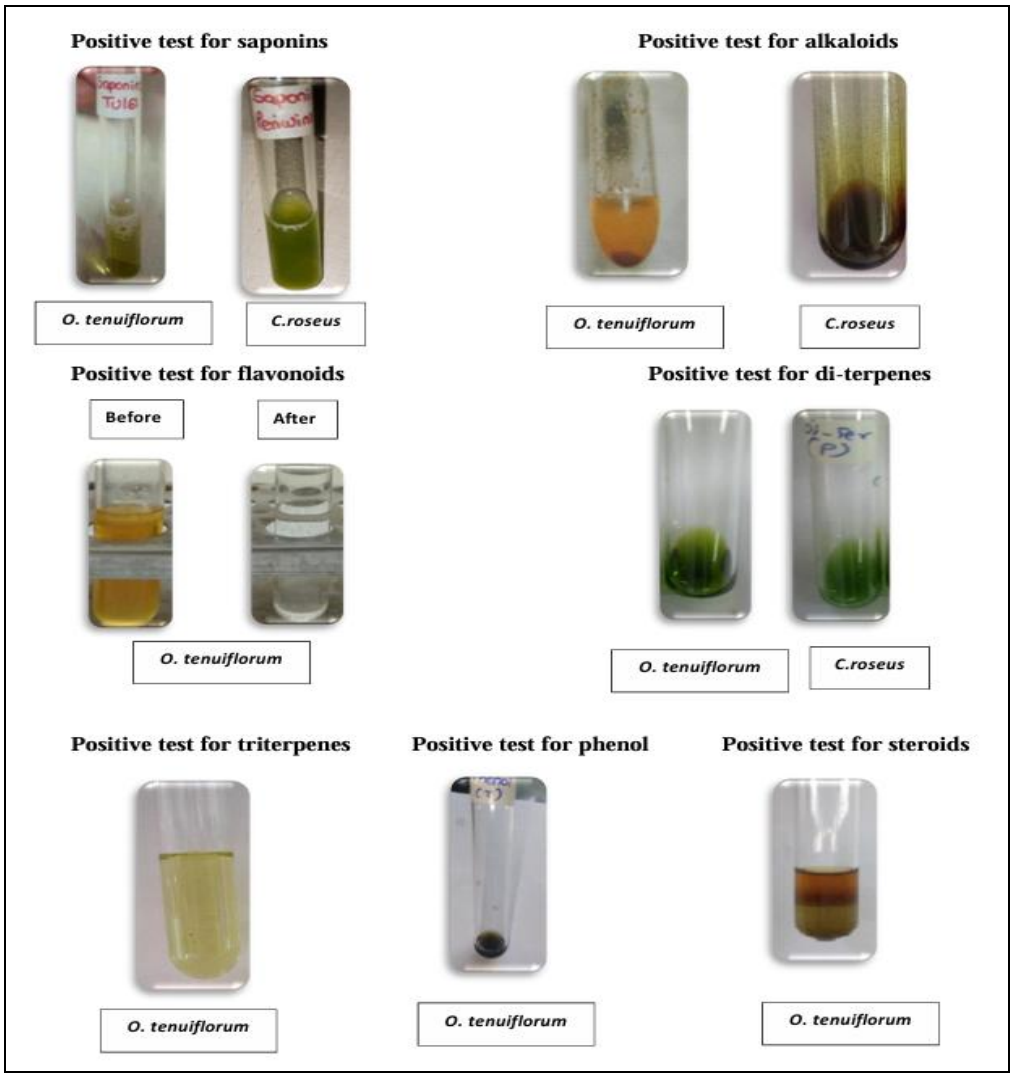


Figure 1. Preliminary phytochemical analysis of plant extracts.

Figure 2. Results of antioxidant activity by phosphomolybdenum assay showing concentration-dependent change to green coloration after incubation at 95 °C for 90 minutes**.**

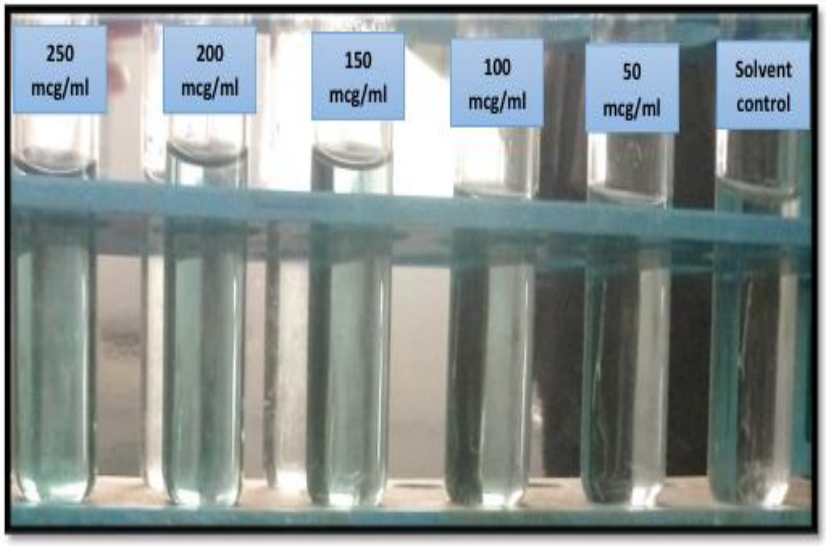


Figure 3. Antioxidant activity of *O. tenuiflorum.*

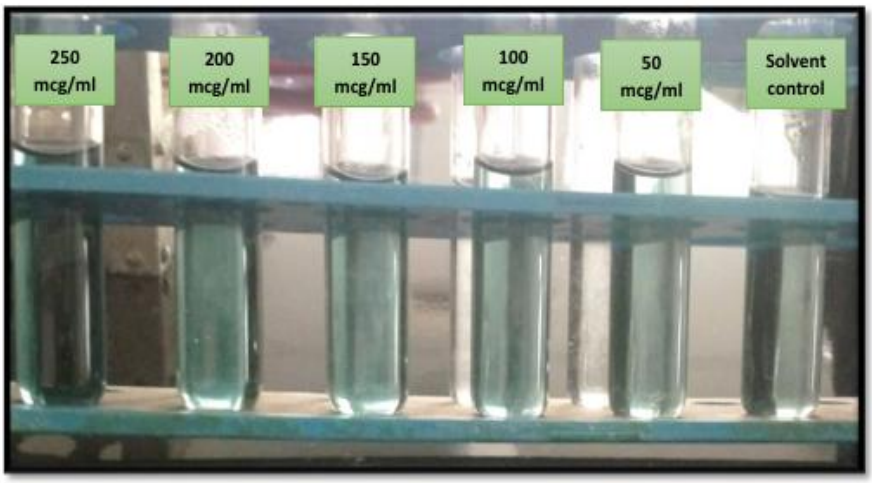
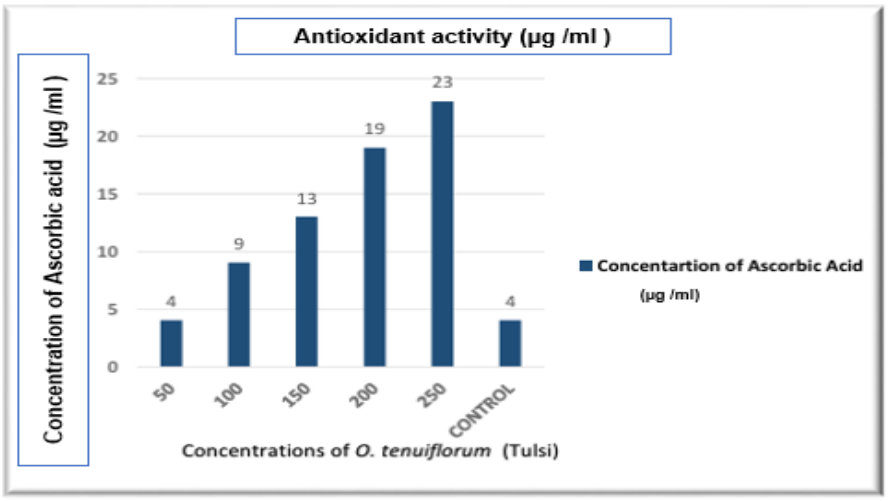


Figure 4. Antioxidant activity of *C. roseus.*

Table 2. Observations and Calculations for antioxidant activity of *O. tenuiflorum.*

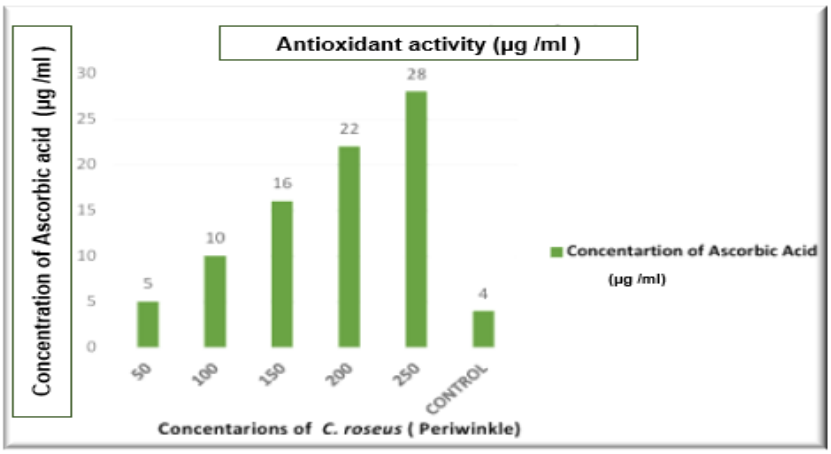
Concentration µg /ml used	Absorbance at 695 nm	Ascorbic acid equivalent (µg/ml)
50	0.03	4
100	0.07	9
150	0.10	13
200	0.14	19
250	0.17	23
Solvent control (Methanol)	0.03	4



Graph 1. Anti- oxidant activity (concentrations of *O. tenuiflorum* vs concentration of ascorbic acid in µg/ml).

Table 3. Observation and Calculations for antioxidant activity of *C. roseus.*

Concentration µg /ml used	Absorbance at 695 nm	Ascorbic acid equivalent (µg/ml)
50	0.06	5
100	0.08	10
150	0.12	16
200	0.16	22
250	0.21	28
Solvent control (Methanol)	0.03	4



Graph 2. Anti- oxidant activity (Concentrations of *C. roseus* v/s concentration of ascorbic acid in µg /ml).

The antioxidant activity of the plant extracts was assessed and compared based on their ability to reduce Mo (VI) to Mo (V), forming a phosphomolybdenum complex. The absorbance readings for both extracts increased with concentration, indicating dose-dependent antioxidant activity. At the highest tested concentration (250 µg/ml), *C. roseus* showed a higher absorbance value (0.21) compared to *O. tenuiflorum* (0.17), corresponding to an estimated ascorbic acid equivalent of 28 µg/ml and 23 µg/ml respectively. This suggests that *C. roseus* possesses greater free radical scavenging activity than *O. tenuiflorum*. The superior antioxidant capacity of *C. roseus* could be attributed to the presence of bioactive compounds such as alkaloids, flavonoids, and saponins, which are known to contribute to radical scavenging. While both plants contain similar phytoconstituents, the variation in concentration or specific structure of these compounds may account for the observed differences in antioxidant potential. This result supports the traditional medicinal use of *C. roseus* in managing oxidative stress-related conditions and suggests its potential as a natural antioxidant source in therapeutic applications.

The anti-mitotic activity of methanolic extracts of *O. tenuiflorum* and *C. roseus* was assessed using the *Allium cepa* root tip assay. Concentrations ranging from 50 to 250 µg/ml were tested. Methanol served as the solvent control, Cyclophosphamide as the positive control, and distilled water as the negative control. Observations were made after 24 hours (Day 1) and 48 hours (Day 2) of exposure, and the mitotic index (MI) was calculated accordingly. The mitotic index is a measure of the percentage of dividing cells in the root meristem and is indicative of the cytotoxic and anti-proliferative effects of the test substances.

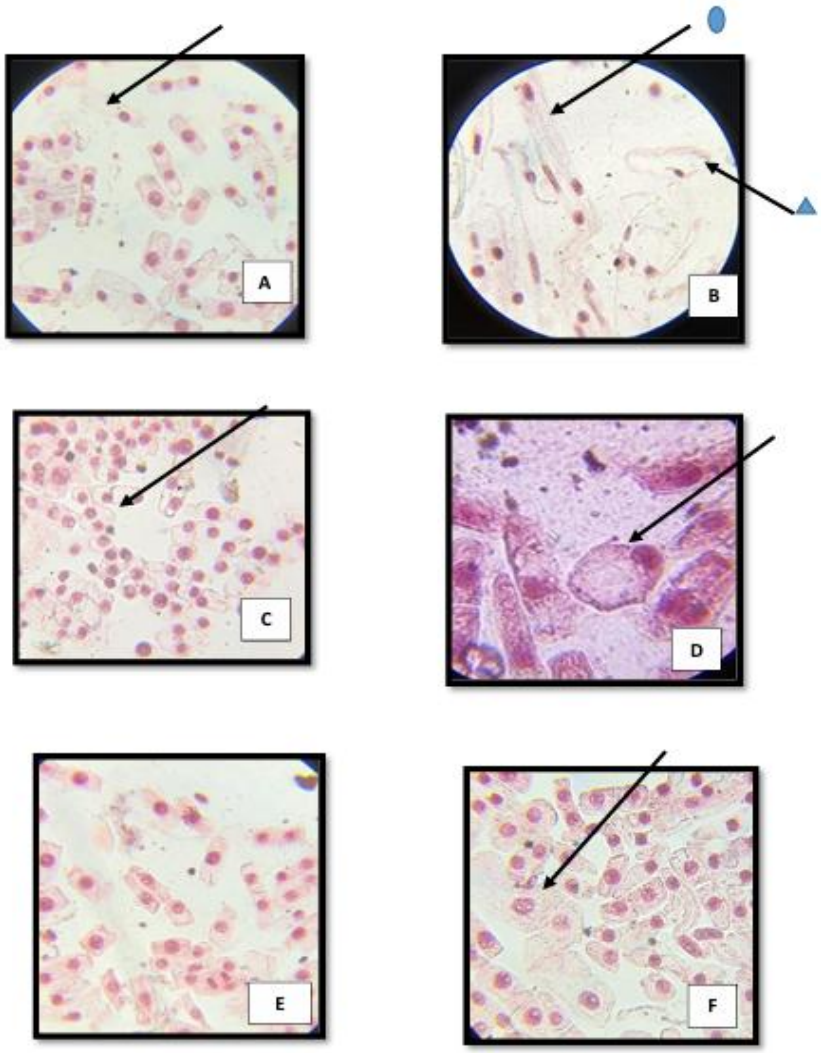


Figure 5. Mitotic abnormalities in *O. tenuiflorum*-treated *Allium cepa* root tips at 24 hours showing necrosis and cell fragmentation. A- Extended cell (50 µg/ml), B- Cell fragmentation and receding of cell death (50 µg/ml), Extended nucleus, C- Acentric nucleus (100µg/ml), D- Necrotic cell (150µg/ml), E- Negative Control, F- Solvent Control (Methanol) – Bulging of the cytoplasm.

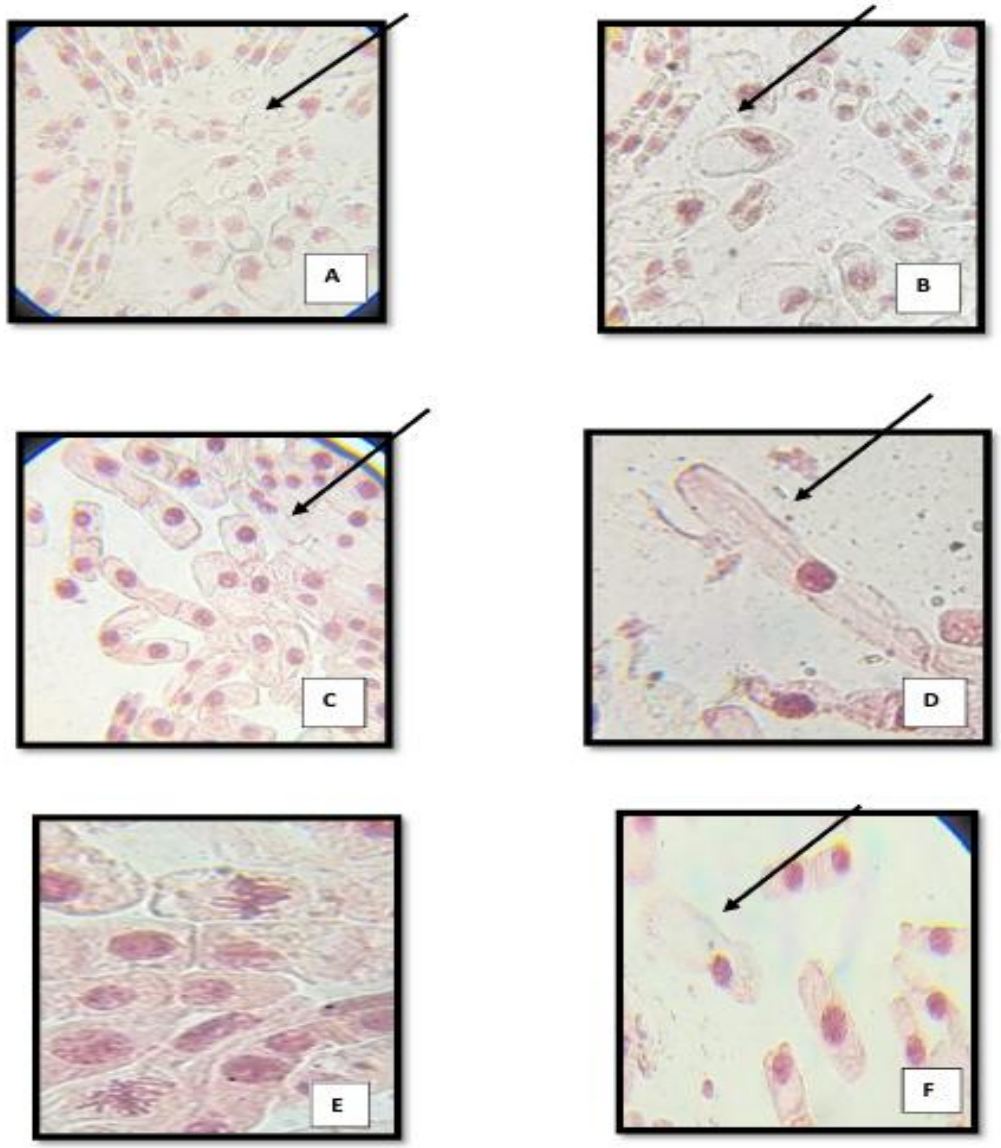


Figure 6. Mitotic abnormalities in *O. tenuiflorum*-treated *Allium cepa* root tips at 48 hours showing necrosis and cell fragmentation. A- Necrotic cell (50µg/ml), B- Necrotic cell along with vacuolization (100µg/ml), C- Extended cell (150µg/ml), D- Cell fragmentation and receding of cell death (200 µg/ml), E- Negative control, F- Solvent control (Methanol)- Cell fragmentation.

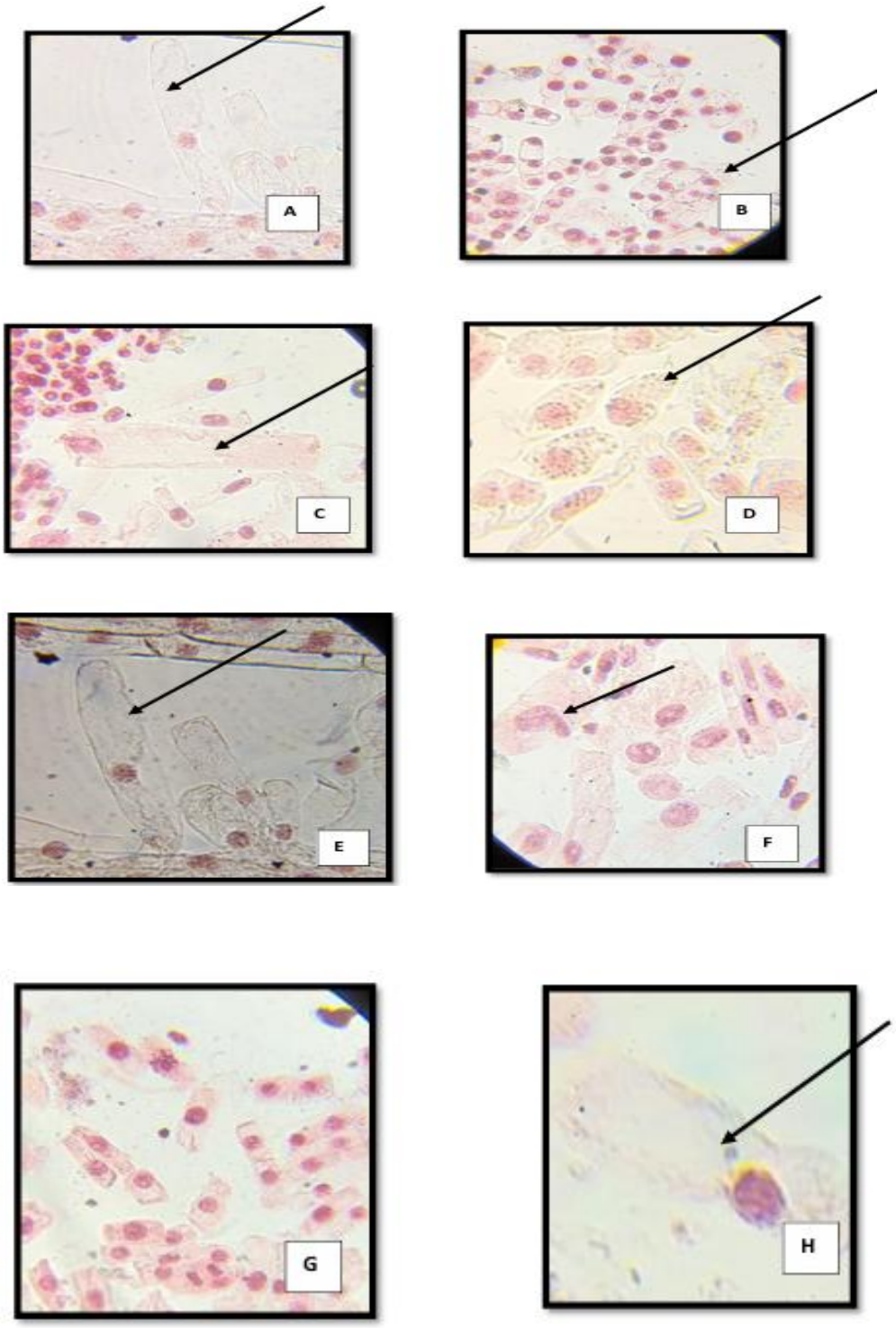


Figure 7. Mitotic abnormalities in *C.roseus* -treated *Allium cepa* root tips at 24 hours showing necrosis and cell fragmentation. A- Extended cell, disturbed spindle and cytoplasm destruction (50µg/ml), B- Acentric nucleus (150 µg/ml), C- Acentric nucleus along with cell fragmentation (200 µg/ml), D- Necrotic cell(200µg/ml), E- Cell fragmentation (200µg/ml), F- Presence of nuclear bud (250µg/ml), G- Negative control, H- Solvent control (Methanol)- Extended cell with cell fragmentation (250µg/ml).

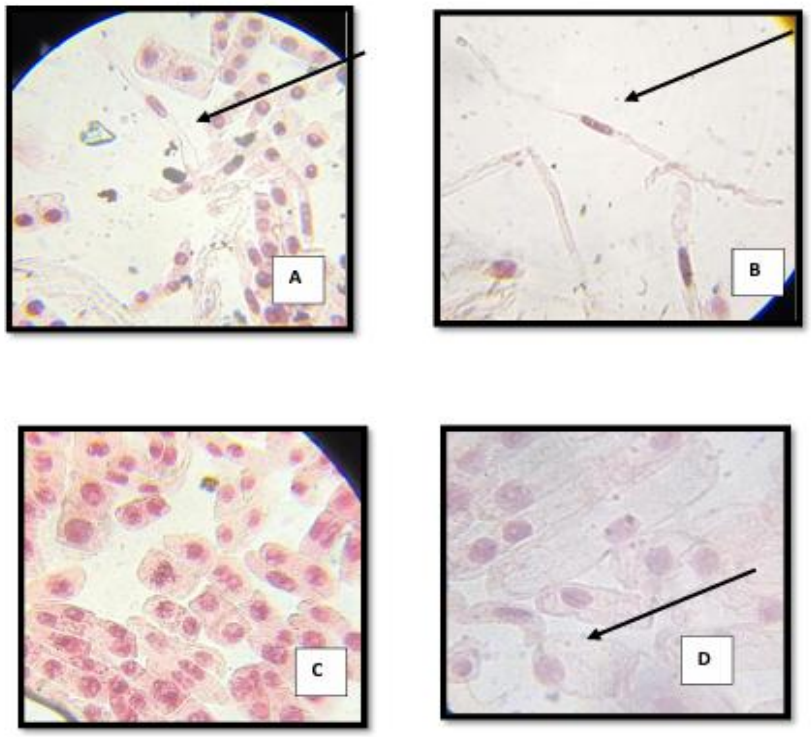
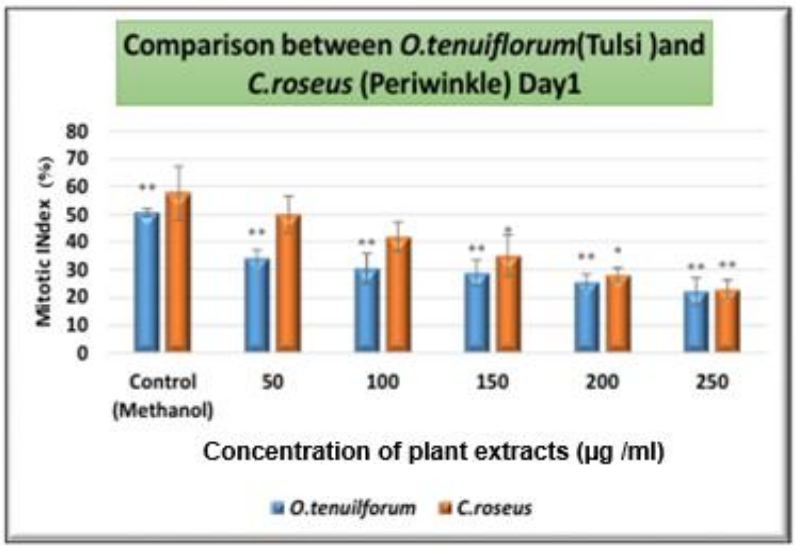
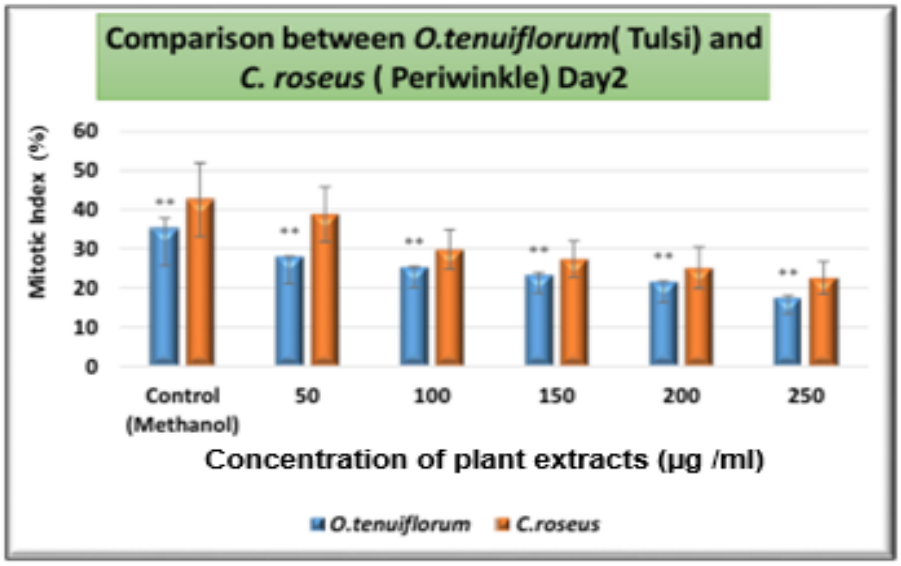


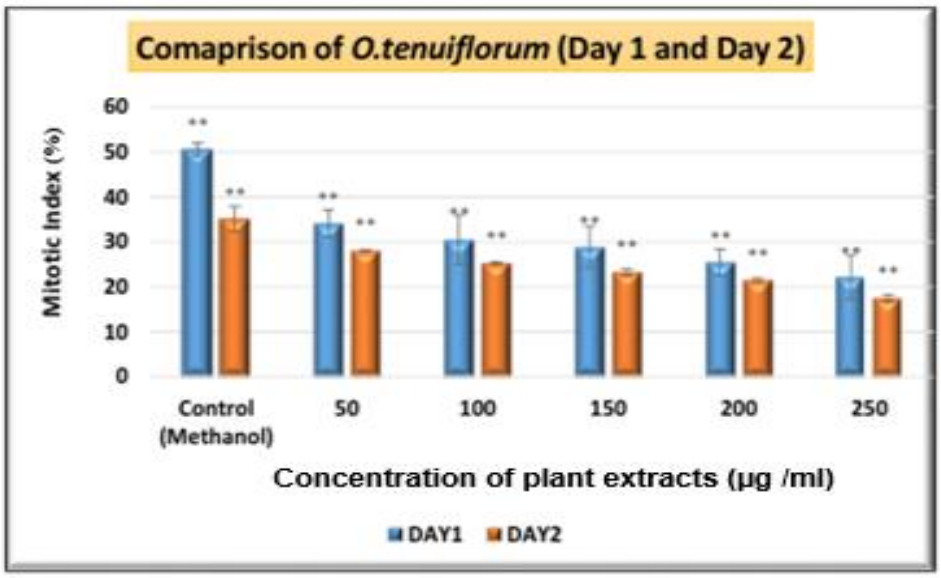
Figure 8. Mitotic abnormalities in *C. roseus*-treated *Allium cepa* root tips at 48 hours showing necrosis and cell fragmentation. A- Cell fragmentation and receding of cell contents (50µg/ml), B- Extended cell (200 µg/ml), C- Negative control, D- Solvent control (Methanol)- Necrotic cell.



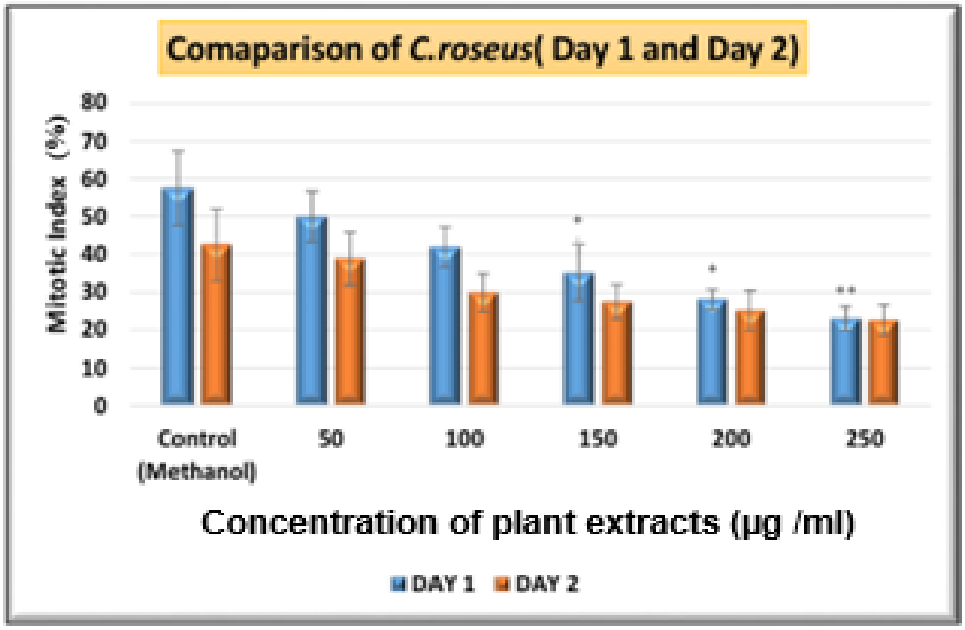
Graph 3. Mitotic Index in Onion (*Allium cepa*) root tip cells exposed to different concentrations of the plant extract of *O.tenuiflorum* and *C.roseus* on Day 1. Data are expressed as mean ± SD (n= 3) * denotes significant difference between control and treatment (*$p < 0.05$, **$p < 0.01$).



Graph 4. Mitotic Index in Onion (*Allium cepa*) root tip cells exposed to different concentrations of the plant extracts of *O.tenuiflorum* and *C.roseus* on Day 2. Data are expressed as mean ± SD (n= 3) * denotes significant difference between control and treatment (*p < 0.05, **p < 0.01)



Graph 5. Mitotic Index in Onion (*Allium cepa*) root tip cells exposed to different concentrations of the plant extract of *O.tenuiflorum* on Day1 and Day 2. Data are expressed as mean ± SD (n= 3) * denotes significant difference between control and treatment (**p < 0.01).



Graph 6. Mitotic Index in Onion (*Allium cepa*) root tip cells exposed to different concentrations of the plant extracts of *C.roseus* on Day1 and Day 2. Data are expressed as mean ± SD (n= 3) * denotes significant difference between control and treatment *p < 0.05, **p < 0.01

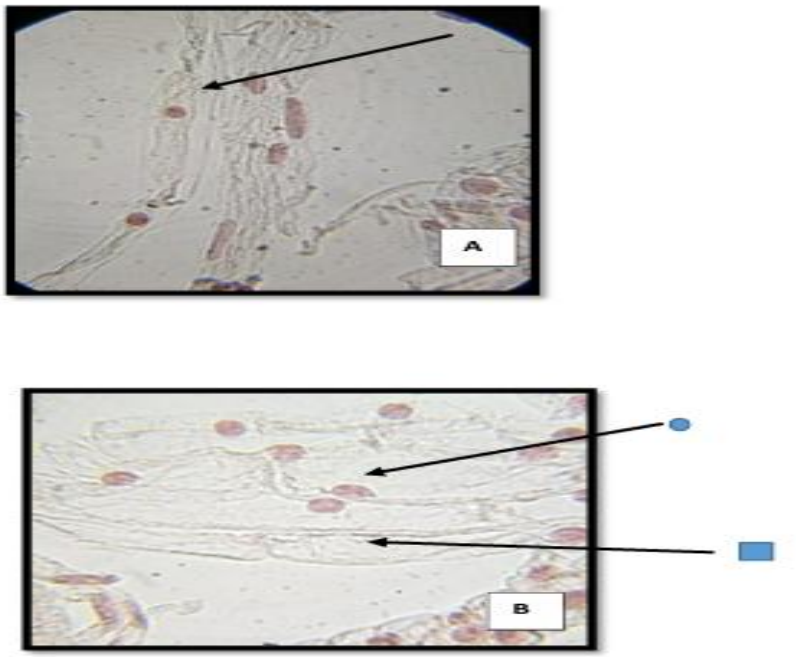


Figure 9. Mitotic abnormalities in Cyclophosphamide-treated *Allium cepa* root tips at 24 hours showing necrosis and cell fragmentation. A- Cell fragmentation, B- Acentric nucleus, Absence of nucleus

The *Allium cepa* root tip assay is a well-established cytogenetic method to evaluate genotoxicity and anti-mitotic potential of plant-derived compounds. The reduction in the mitotic index following treatment with both *O. tenuiflorum* and *C. roseus* extracts suggests their ability to inhibit cell division. The significantly stronger effect observed with *O. tenuiflorum* could be attributed to its broader spectrum of phytochemicals, including flavonoids, phenols, di- and tri-terpenes, and steroids. It can be hypothesized that these compounds interfere with

spindle formation or DNA synthesis, leading to cell-cycle arrest and triggering either programmed cell death (apoptosis) or necrosis.

The gradual increase in inhibition from Day 1 to Day 2 indicates a time-dependent cytotoxic effect, which is desirable in anti-cancer strategies targeting rapidly dividing cells. Mitotic aberrations such as chromosomal fragmentation and necrosis further confirm the cytostatic and cytotoxic effects of the plant extracts. Given these observations, the methanolic extracts of *O. tenuiflorum* and *C. roseus* show promising anti-mitotic properties and can be explored further for their potential role in cancer therapy and natural anti-proliferative agents in pharmaceutical applications. In a preliminary study, the cytotoxic effect of the plant extracts (*O. tenuiflorum* and *C. roseus*) on cancerous cells (rat derived astrocytoma C6 cells) using concentrations (5g/ 100ml and 250µg/ml) was determined 24 hrs post treatment. Both *O. tenuiflorum* and *C.roseus* plant extracts were tested on rat glioma (C6) cancer cells. At concentrations of 5 g/100 ml and 250 µg/ml, both extracts showed induced cytotoxic effects in glioma cells as evidenced by morphological studies and reduced viability after 24 hours. In comparison, the untreated cancer cells showed no killing and continued to grow normally.

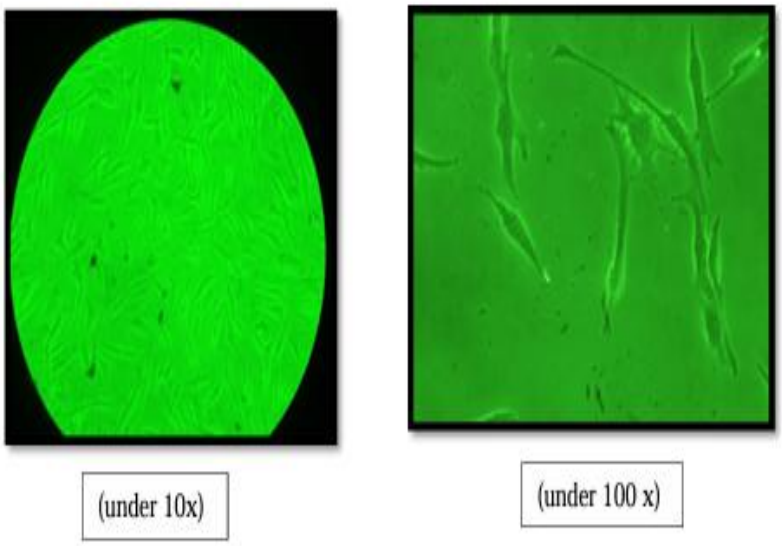


Figure 10. Cancer cells before the treatment with the plant extracts.

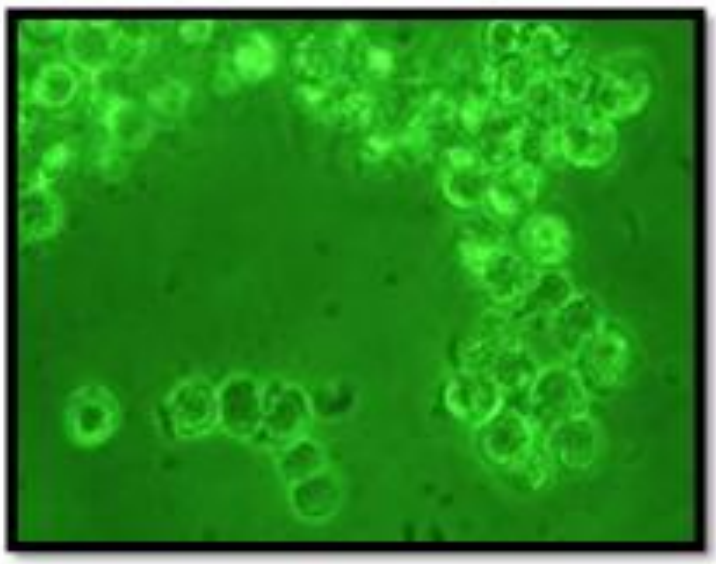


Figure 11. Cancer cells after treatment with the plant extracts.

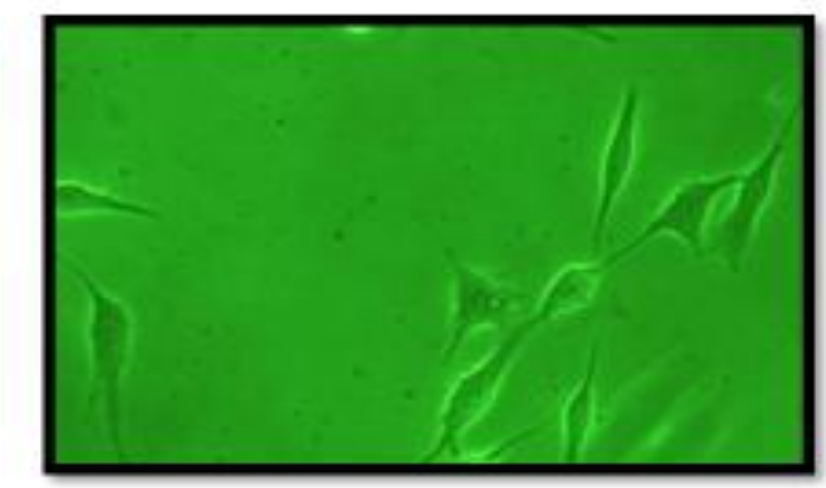


Figure 12. Cancer cells without treatment with the plant extracts (Control).

These results suggest that the plant extracts have cytotoxic effects on glioma cells. The cell death observed indicates that compounds present in *O. tenuiflorum* and *C. roseus* may help stop the growth of cancerous cells. This highlights their potential use in developing anti-cancer treatments, though further detailed studies are needed to confirm these findings. The viability of white blood cells (WBCs) after treatment with plant extracts was assessed using the Trypan Blue dye exclusion method. The following results were observed after 30 minutes of incubation at room temperature: (untreated cells): 100.00%. Cyclophosphamide (positive control): 17.00%. *C. roseus* extract: 34.00%. *O.tenuiflorum* extract: 52.25%. These results indicate that both plant extracts showed cytotoxic effects on WBCs, with *C. roseus* being more toxic than *O. tenuiflorum*, as evidenced by a lower percentage of viable cells compared to *O. tenuiflorum*. Cyclophosphamide showed the highest level of cell killing, as expected.

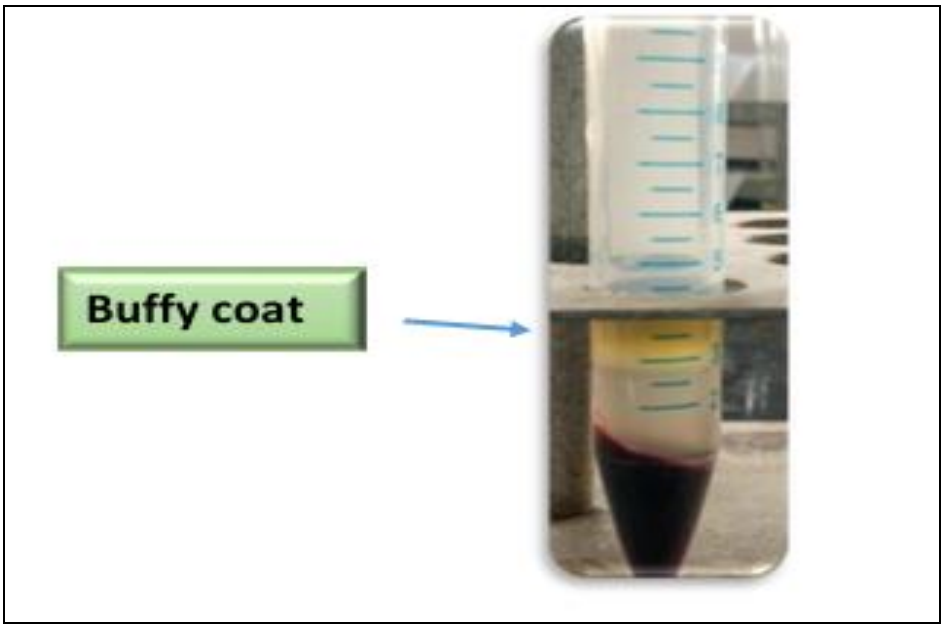


Figure 13. Buffy coat obtained by Ficoll- Hypaque Density Gradient centrifugation.

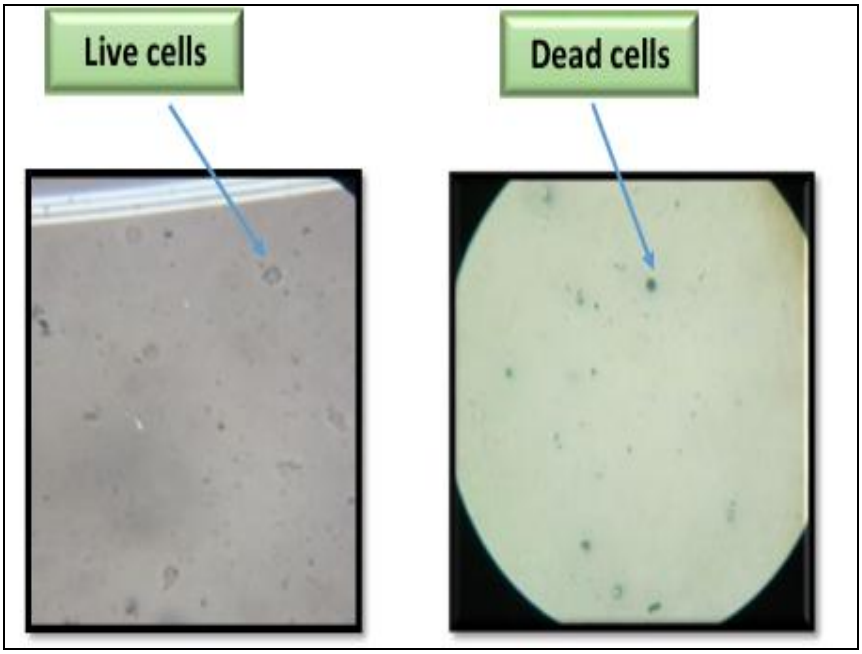


Figure 14. Live and Dead WBCs after treatment with the plant extracts (under 40x).

The % viability was calculated using the following formula:

$$\% \textbf{ Viability} = \frac{\text{Total Viable cells}}{\text{Total cells}} \times 100$$

Table 4. Effect of Plant Extracts and Cyclophosphamide on Cell Viability.

Sample Used	% Viability
C. roseus	34.00
O. tenuiflorum	52.25
Cyclophosphamide	17.00
Control	100.00

The dye exclusion assay revealed that the plant extracts possess cytotoxic properties, although less severe than the chemotherapeutic drug cyclophosphamide. Among the two plant extracts, *C. roseus* demonstrated stronger cytotoxic activity, likely due to the presence of active alkaloids known for their anticancer potential. In contrast, *O.tenuiflorum* was comparatively less toxic, suggesting its safer profile for further therapeutic exploration. These observations are consistent with previous studies indicating the bioactive potential of these medicinal plants. While the cytotoxic effect on normal WBCs raises concerns about safety, the comparatively lower toxicity of *O. tenuiflorum* makes it a promising candidate for further study in targeted therapies. Additional experiments are needed to test selective toxicity on cancer cells and to determine the appropriate therapeutic window.

Table 5. Comparative Analysis of *O. tenuiflorum* and *C.roseus* extracts.

Parameter	*O. tenuiflorum*	*C. roseus*
Antioxidant Activity (at 250 µg/ml)	23 µg/ml ascorbic acid equivalent	28 µg/ml ascorbic acid equivalent
Antimitotic Activity (*Allium cepa* assay)	Strong inhibition; necrosis, fragmentation, acentric nuclei	Moderate inhibition; nuclear buds, cytoplasm destruction
Mitotic Index Reduction (48 hrs, 250 µg/ml)	Greater reduction in MI	Moderate reduction in MI
Cytotoxicity on Glioma (C6) Cells	Significant cell death after 24 hrs	Significant cell death after 24 hrs
Effect on WBC Viability (Trypan Blue assay)	52.25% viability (less toxic)	34% viability (more toxic)
Phytochemicals Present	Alkaloids, flavonoids, saponins, diterpenes, triterpenes, phenols, steroids	Alkaloids, flavonoids, saponins, diterpenes
Extract Recovery Yield	60% w/v	93.6% w/v

CONCLUSION

India has a long-standing tradition in the use of medicinal plants, which are valuable sources of bioactive compounds with therapeutic potential. In this study, the medicinal plants *C. roseus* (Periwinkle) and *O. tenuiflorum* (Krishna Tulsi) were evaluated for their antioxidant, cytotoxic, and anticancer properties. Both extracts exhibited notable antioxidant and anticancer activity and were found to contain key phytochemicals that may contribute to their biological effects. Importantly, the cytotoxicity of these extracts was also assessed on normal white blood cells (WBCs) using a Trypan Blue exclusion assay. Compared to the chemotherapeutic drug cyclophosphamide, both plant extracts demonstrated reduced toxicity towards WBCs. Among the two, *O. tenuiflorum* showed greater protective activity, indicating its potential role in reducing side effects during cancer treatment. These findings suggest that *O. tenuiflorum* and *C. roseus* possess promising anticancer properties with comparatively lower toxicity to normal cells. *O. tenuiflorum*, in particular, may be explored further for its potential use in combination therapy, potentially enhancing the efficacy of treatment while minimizing damage to healthy immune cells. This approach offers a new direction for the development of safer, plant-based cancer therapies.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Zoology, Sophia College for Women (Empowered Autonomous), Mumbai for the facilities provided to carry out this research work.

CONFLICT OF INTERESTS

The authors declare no conflict of interests

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., & Rollinger, J. M. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances, 33*(8), 1582–1614.

Zhang, J., Onakpoya, I., Posadzki, P., & Eddouks, M. (2015). The safety of herbal medicine: From prejudice to evidence. *Evidence-Based Complementary and Alternative Medicine, 2015*, 1–3.

Petrovska, B. B. (2012). Historical review of medicinal plants usage. *Pharmacognosy Reviews, 6*(11), 1–5.

Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules, 21*(5), 559.

Sathisha, A. D., Lingaraju, H. B., & Prasad, K. S. (2015). Evaluation of antioxidant activity of medicinal plant extracts produced for commercial purpose. *Journal of Chemistry, 8*(2), 882–886.

Suresh, S., Srinivasan, A., Scott, J., Rao, S., Chidambaram, B., & Chandrasekar, S. (2017). Profile and outcome of pediatric brain tumors – Experience from a tertiary care

pediatric oncology unit in South India. *Journal of Pediatric Neurosciences, 12*(3), 237–244.

Rajesh, H., Rao, S. N., Prathima, K. S., Megha, R. N., Rejeesh, E. P., & Lovelyn, J. (2013). Phytochemical analysis of aqueous extract of *Ocimum sanctum* Linn. *International Journal of Universal Pharmaceutical and Biological Sciences, 2*(2), 462–468.

Cohen, M. M. (2014). Tulsi-*Ocimum sanctum*: A herb for all reasons. *Journal of Ayurveda and Integrative Medicine, 5*(4), 251–256.

Kabesh, K., Senthilkumar, P., Ragunathan, R., & Kumar, R. R. (2015). Phytochemical analysis of *Catharanthus roseus* plant extract and its antimicrobial activity. *International Journal of Pure and Applied Bioscience, 3*(2), 162–172.

Thenmozhi, A., & Mahadevarao, U. S. (2011). Evaluation of antimitotic activity of *Solanum torvum* using *Allium cepa* root meristematic cells and anticancer activity using MCF-7 human mammary gland breast adenocarcinoma cell lines. *Drug Invention Today, 3*(12), 290–296.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry, 269*(2), 337–341.

Sehgal, R., Roy, S., & Kumar, V. L. (2006). Evaluation of cytotoxic potential of latex of *Calotropis procera* and podophyllotoxin in *Allium cepa* root model. *Biocell, 30*(1), 9–13.