Evaluation Of Anti-Cancer and Anti-Oxidant Activity Of Ethanolic Root Peel Extract Of *Potentilla fulgens*

A Thesis submitted
in partial fulfillment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

By

Sayak Ghosh, Soumya Kanta Hati, Aritra Biswas

Under the Guidance of
Dr. Tilak Raj Maity

Department Of Biotechnology

Haldia Institute of Technology
(Autonomous)

June, 2024

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Approved By

Dr. Tilak Raj Maity.	**Dr. Shamba Chatterjee**
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Haldia Institute of Technology

June, 2024

Certificate from the Supervisor

It is certified that the work contained in the thesis entitled “Evaluation of Anti-Cancer and Anti-Oxidant Activity of Ethanolic Root Peel Extract of *Potentilla fulgens*”, by “Sayak Ghosh, Soumya Kanta Hati, Aritra Biswas”, has been carried out under my/our supervision and that this work has not been submitted elsewhere for a degree.

To the best of our knowledge, the matter embodied in the thesis has not been sub mitted to any other University / Institute for the award of any Degree or Diploma.

_____

Signature of Supervisor

Dr. Tilak Raj Maity

Assistant Professor

Department of Biotechnology

Haldia Institute of Technology

Date: June,2024

Declaration

We declare that this written submission represents my ideas in my own words and where others' ideas or words have been included, we have adequately cited and referenced the original sources. I also declare that We have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. We understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Sayak Ghosh, Soumya Kanta Hati, Aritra Biswas
(10300420020,10300420023,10300420005)
Date: 10 June, 2024

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Any omission in this brief acknowledgement does not mean a lack of gratitude.

Thanking You,
Sayak Ghosh
Aritra Biswas
Soumya Kanta Hati

List Of Abbreviations

ASP	Aspirin
BCl-2	B-cell lymphoma 2 protein
CAT	Catalase
Col	Colchicine
CRS	Cold restriction stress
dd	Double distilled
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
DPPH	2,2-diphenylpicrylhydrazyl
EtOH	Ethanol
FRAP	Ferric reducing antioxidant power
gm	Gram
GPX	Glutathione peroxidase
h	hour
HCl	Hydrochloric Acid
HDAC	Histone deacetylase
$HgCl_2$	Mercuric chloride
H_2O	Water
L	liter
M	molar
MAPK	Mitogen activated protein kinase
mg	milligram
MI	Mitotic Index
ml	milliliter
mm	millimeter

NaOH	sodium hydroxide
nm	Nanometer
PFEE	Potentilla Fulgens Ethanolic Extract
PL	Pyloric lygation
OD	Optical Density
PF	*Potentilla fulgens*
RAS	Radical scavenging activity
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide peroxidase
TBI	Tumaric brain injury
UV	Ultra Violet light
%	percent
µg	microgram
µl	microliter
°	degree

Legend Of Figures, Tables and Flow Chart

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Fig.1: Natural habitat and region of P. fulgens in India.

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Abstract

The evaluation of the ethanolic root peel extract of *Potentilla fulgens* for its anti-cancer and anti-oxidant activities. The study aimed to explore the therapeutic potential of this plant extract in the context of cancer treatment, microbial infections, and oxidative stress-related disorders. The research involved comprehensive in vitro assays to assess the anti-cancer properties of the extract against various cancer cell lines, revealing promising cytotoxic effects. Additionally, the extract exhibited significant anti-microbial activity against a spectrum of pathogens, highlighting its potential as a natural antimicrobial agent. The antioxidant activity was evaluated through various assays, indicating the extract's capability to scavenge free radicals and mitigate oxidative stress. Furthermore, phytochemical analysis identified bioactive compounds in the ethanolic extract, providing insights into potential mechanisms of action. The findings suggest that *Potentilla fulgens* root peel extract possesses multifaceted pharmacological properties, supporting its potential application in cancer therapy, infectious disease management, and combating oxidative stress-related ailments. This research contributes valuable knowledge to exploring natural products with diverse bioactivities, emphasizing the significance of *Potentilla fulgens* as a potential source for developing therapeutic agents.

Keywords: *Anti-cancerous drugs, Lead molecule(s), Plant based primary screening, Drug discovery, Bioinformatic tools*

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1. Introduction

1.1. Background on cancer and Oxidative stress

Normal homeostatic management of the cell cycle is not able to affect cancerous cells. According to Carmelit and Jain (2000), there are a few fundamental traits that set malignant cells apart from normal cells. First, there is an unchecked growth of cells, and then there is a reduction in the differentiation of cells (Hanahan et al. 2011). Research on cancer treatment is a major area of focus for contemporary science. Because heavy metals are poisonous to normal cells, they have not been successful in treating cancer in the past (DeVita 1978). Better drug approval procedures have, however, led to a change in anti-cancerous medication throughout time (DeVita et al. 2008). Chemotherapy is a popular type of cancer treatment in which cytotoxic chemicals modulate the cell cycle and cell division of malignant cells. Complex regulatory systems control eukaryotic cell proliferation and cell cycle. The system is controlled by regulatory change overs called checkpoints (Hanahan et al. 2011). When control systems are faced with problems inside or outside the cell they hold onto the cell in that particular checkpoint until resolution.

The term "oxidative stress" describes a state in which the body's antioxidant defences are unable to counteract the damaging effects of reactive oxygen species (ROS), which are very reactive molecules with unpaired electrons. This imbalance damages DNA, proteins, and lipids within the cell, which can cause malfunctioning or even death of the cell. Normal metabolic activities generate ROS, which can be made worse by smoking and other environmental variables. Enzymatic antioxidants like catalase and superoxide dismutase, together with non-enzymatic antioxidants like vitamins C and E, help the body fight oxidative stress. Numerous illnesses, such as cancer, heart disease, diabetes, neurological problems, and aging-related ailments are associated with chronic oxidative stress (Betteridge, 2000). Treating and preventing disease may benefit greatly from an understanding of oxidative stress and the use of natural antioxidants like those in *Potentilla fulgens*.

Through the direct scavenging of reactive oxygen species (ROS) and/or the inhibition of cell growth resulting from protein phosphorylation, antioxidants might reduce the oxidative stress-induced carcinogenesis. Because oxidative products can cause genetic damage, B-carotene's antioxidant action may offer protection against cancer.

Consequently, B-carotene's photoprotective qualities may offer defence against UV light-induced carcinogenesis. B-carotene immune improvement may aid in the prevention of cancer. By modifying the impact of carcinogens on liver metabolism, beta-carotene may also have anticarcinogenic properties (Lobo et al. 2010).

1.2. Importance of Anticancer and antioxidant agent

Worldwide, the fight against cancer has been ongoing, despite significant advancements in treatment and prevention. One of the hallmarks of the illness is the body's cells growing uncontrollably, making it impossible to halt. resulting in the formation of cancerous tumours that may spread elsewhere. Chemotherapy, radiation, and medications generated from chemicals are now used as therapies. Chemotherapy is one of the treatments that can cause patients great stress and significant harm to their health. As a result, emphasis is placed on employing complementary and alternative cancer therapy (Greenwell & Rahman, 2015).

Plant-derived medications are preferred for cancer treatment since they are natural and widely available. They are easily supplied orally as part of a patient's dietary regimen. Furthermore, because they are organically generated from plants, they are often less harmful to normal human cells. Exceptions include cyanogenic glycosides, lectins, saponins, lignans, and several taxanes. If plant-derived medications display selectivity in research, are non-toxic to normal cell lines, and exhibit cytotoxicity in cancer cell lines, they can be advanced to clinical trials for further therapeutic development. Medications originating from plants may be classified into four groups based on their actions: medications that inhibit histone deacetylases (HDAC), antioxidants that protect DNA damage, methyltransferase inhibitors, and mitotic disruptors.HDAC inhibitors include substances like pomiferin, isothiocyanates, sulforaphane, and isoflavones (Pledgie-Tracy et al. 2007). They prevent cancer-causing proteins from doing their job. Sulforaphane, for instance, has been demonstrated to block key targets in the growth of breast cancer. In breast cancer cell lines, HDAC inhibition by sulforaphane therapy led to decreased expression of ER, EGFR, and HER-2. HDAC inhibitors reactivate epigenetically suppressed genes that are essential for chromatin acetylation in cancer cells, allowing the cells to undergo programmed cell death, or

apoptosis. Compounds produced from plants that exhibit HDAC inhibition can increase the susceptibility of human malignancies to chemotherapy (Cornblatt et al. 2007).

By neutralising reactive oxygen species (ROS) and preventing oxidative stress, which can cause cellular damage and contribute to the development of a number of chronic diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders, antioxidant agents are essential for maintaining cellular health. Antioxidants prevent oxidative damage to biological constituents including lipids, proteins, and DNA by scavenging free radicals. Its anti-inflammatory, anti-aging, and immune-boosting properties are all dependent on this protective action. Antioxidants are essential to the body's defence systems against oxidative stress. These include both enzymatic (like superoxide dismutase and catalase) and non-enzymatic (such flavonoids and vitamins C and E). Numerous studies have demonstrated the therapeutic potential of antioxidants, indicating their effectiveness in lowering the risk of chronic illnesses and enhancing overall health outcomes (Valko et al. 2007; Lobo et al. 2010).

1.3. Introduction to *Potentilla fulgen*

Himalayan cinquefoil, or *Potentilla fulgen*, is a native medicinal plant of the Himalayas that is well-known for its many therapeutic uses. The roots of *Potentilla Fulgen* are prized for their powerful therapeutic properties and have been utilised for centuries in Ayurvedic and traditional Chinese medicine. The vernacular name of this plant is “Bajardanti” (Rai, 2003).

Scientific Classification of *Potentilla fulgen* - Kingdom: Plantae, Order: Rosales, Family: Rosaceae, Genus: Potentilla, Species: P. fulgens.

Habitat of this plant - It grows in rocky terrains. They thrive in heavy soils. Propagation is done through seed, cuttings or division of rootstocks. It grows in regions with a temperate climate and at a high elevation of about 1800 – 4350 metres above sea level.

The plant's wide range of bioactive substances, including as tannins, saponins, and flavonoids, support its anti-inflammatory, antioxidant, and anticancer properties. Studies have demonstrated that extracts from *Potentilla fulgen* may trigger apoptosis,

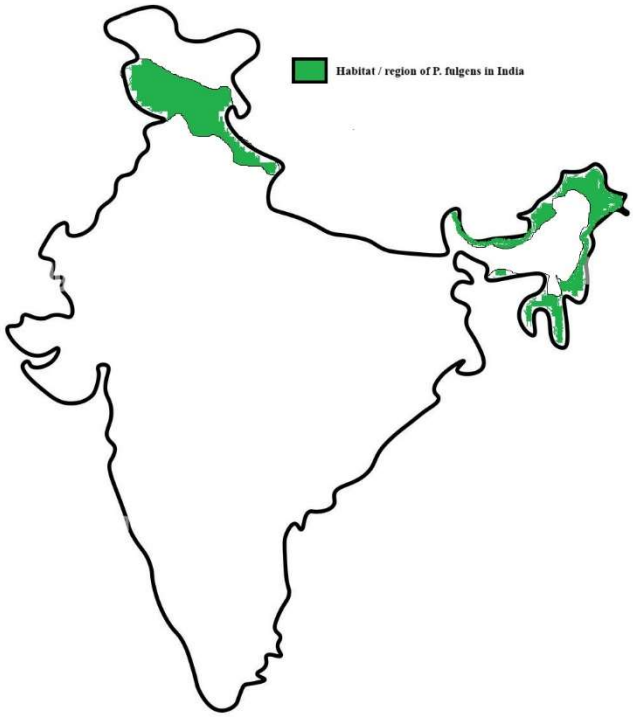

Fig. 1: Natural habitat and region of P. fulgens in India

or programmed cell death, and suppress the development of several cancer cell lines (Tomczyk et al. 2010).

They can also strengthen the body's antioxidant defences by scavenging free radicals and lowering oxidative stress. Moreover, it helps cure illnesses linked to chronic inflammation because of its anti-inflammatory qualities (Wang et al. 2013).

1.4. Medical Impact of this plant

When we talk about the medical impact of the plant *Potentilla fulgens,* we have to consider two different ways of impact. One hand we have Pharmacological Activities, on the other hand traditional and medical use.

Now if we come to the point of pharmacological activities *P. fulgens* have properties that help to prevent tooth decay. Also, anti-cancer properties, treatment of eye

disorders. Bioactive compound of the plant could have anti-inflammatory effect that might be beneficial. Beside these it can be used as a prophylactic agent, anti-diabetic agent, etc (Tripathy et al. 2015).

The aqueous root peel extract of this plant is consumed to get rid of intestinal parasitic infections. The root has anti-diarrheal properties and are effective against high blood pressure and is safe to use as a homemade remedy. Traditionally in Northeast India, the root-stock and the whole *Potentilla fulgen* is utilized as astringent and tonic for curing the gums from tooth ailments such as pyorrhoea, toothache and caries and other tooth ache. Twigs and leaves of *Potentilla fulgen*s are mostly used as toothbrush. Leaf paste is used for curing stomach pain, cough, cold, throat soar and ulcer. Roots are also used for treatment of wounds and tiger bites in Garhwal district of Himalayan region. Whole plant is used for stomatitis and aphthae. In countries like Nepal and Bhutan, plant juice is taken for treatment of peptic ulcer and disusia. It can also prevent cell death and exhibit anti carcinogenic activity. *Potentilla fulgens*s falls among ethnoveterinary plants as well which is used to regulate the fertility in female cattle by giving whole plant decoction once daily for fifteen days (Mothana et al. 2010) (Tripathy et al, 2015).

1.5. Literature Survey

Local practitioners frequently utilise the tap roots of *Potentilla fulgen* L., which are historically chewed combined with betel nut (Areca catechu) and betel leaves (Piper betel), for a variety of illnesses. The effects of the crude methanolic extract of the roots were examined in mice that were diabetic due to alloxan and normoglycemia. It was shown that hypoglycaemic action depended on both dosage and time. Two hours after being administered, the extracts decreased blood glucose levels in both normal and alloxan-induced diabetic rats. After the extract's effective dose was given, blood glucose levels in alloxan-induced diabetic mice were significantly lowered by 63%, but in normal mice there was a 31% reduction 24 hours later. Furthermore, even on the third day, a sustained anti-hyperglycemic activity was shown in the diabetic mice, with glucose levels reported to be much lower (79%) than in the control group. The ability to tolerate glucose was enhanced in mice with and without diabetes. The results

were contrasted with those of metformin, insulin, and glibenclamide, and the likely mechanism of action is examined (Syiem et al. 2002).

Antioxidant activity of *Potentilla Fulgens* root extract: Studies may involve in vitro experiments assessing the scavenging activity of the root extract against free radicals. Methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) or FRAP (Ferric Reducing Antioxidant Power) assays might be employed (Jaitak et al. 2010) .

Neuro-protective effects of *Potentilla Fulgens* on traumatic brain injury: Research in this area aims to assess whether *Potentilla Fulgens* has protective effects on the nervous system, particularly in the context of traumatic brain injury (TBI).

Four stomach-ulcer models, including pyloric ligation (PL), ethanol (EtOH), cold restriction stress (CRS), and aspirin (ASP)-induced gastric ulcers, were used to assess the gastroprotective efficacy of EPF. Rats given a 4-hour PL injection to produce stomach ulcers had their gastric acid measured for pH, total volume content, and total acid-pepsin production. The amount of DNA in the stomach mucosal tissue and gastric juice was measured, as well as the ratio of total carbs to protein, which was represented as an indication of mucin activity (Laloo et al. 2013).

1.6. Potential Impact of the Research

There are major potential implications for the study of *Potentilla fulgens*' ethanolic root peel extract's antioxidant and anti-cancer properties. If successful, it may result in the creation of safer, more natural cancer treatments as well as medications meant to lessen the effects of oxidative stress on illnesses including cardiovascular and neurological disorders. This research may support the traditional therapeutic use of *Potentilla fulgen*, encourage their incorporation into contemporary medical procedures, and facilitate the creation of new drugs. The extract may also be added to functional meals and health supplements to improve general health and illness preventive measures.

2. Objective

The Primary objective of Evaluation of anti-cancer and anti-oxidant activity of ethanolic root peel extract of *Potentilla fulgen* is to challenges and limitation faced by researchers in performing similar type of discoveries using *P. fulgens*. The following objectives guided the development of the project:

- Cost-effective, convenient and less time-consuming process for primary screening of unknown anti-cancer lead molecules.

Now look forward to achieve our objective through our experiment and research.

3. Materials and Methods

3.1. Preparation and extraction of *P. fulgens* ethanolic crude extract (PEEE)

Potentilla Fulgenss (Himalayan Cinquefoil) roots were bought from a local market in Shillong, Meghalaya, India. The material was washed, dried in hot air oven at 40°C. The dried leaves were grounded into fine powder with mortar and pestle and stored at − 20°C. The ground powder (1 g) was extracted using 80% ethanol for overnight. Final crude extract was concentrated and dried by vacuum evaporator at a temperature less than 45°C following concentrated extract which was maintained at 4°C. Working PFEE stock solution of concentration 100 mg/ml was prepared by dissolving dried crude extract in dimethyl sulfoxide (DMSO) and further diluted with distilled water (Maity et al. 2014).

3.2. Germplasm and IN VIVO treatments

Dry seeds of grass pea (L. *sativus* L.; Family Fabaceae) were soaked in 1% Bavistin for 20 min followed by surface was sterilized with 0.1% $HgCl_2$ for 5 min and washed in distilled water (3 times, 10 min each). Washed seeds were soaked in aqueous solution of different concentrations (1 mg/mL, 5 mg/mL and 10 mg/mL) of PFEE for 6h. Approximately 10-15 soaked seeds were then germinated for 72 h on Petri dishes with moistened cotton bed with the respective solution (Samanta et al. 2014, 2015, 2019). Seeds soaked in distilled water were marked as control A. All dilution for preparing different concentrations was made with double distilled water. Petri plates were kept at 25 ± 2°C in an incubator.

3.3. Assessment of radicle length and mitotic index of *L. sativus*

From the germinating seedlings, radicle length was measured for approximately 10-15 samples from each of the three replica sets and their average was calculated. For the assessment of mitotic index, cut root tips (2 mm) from control A, control B and different concentration of drugs were fixed in 1:3 acetic ethanol overnight, followed by preserving them in 70% ethanol. Staining of the root tips was done in 2% aceto-orcein and 1M HCl mixture and squashed in 45% acetic acid. The formula for calculating mitotic index is as follows (Samanta et al. 2014, 2015, 2019):

MI = (No. of dividing cells / Total no. of cells) × 100

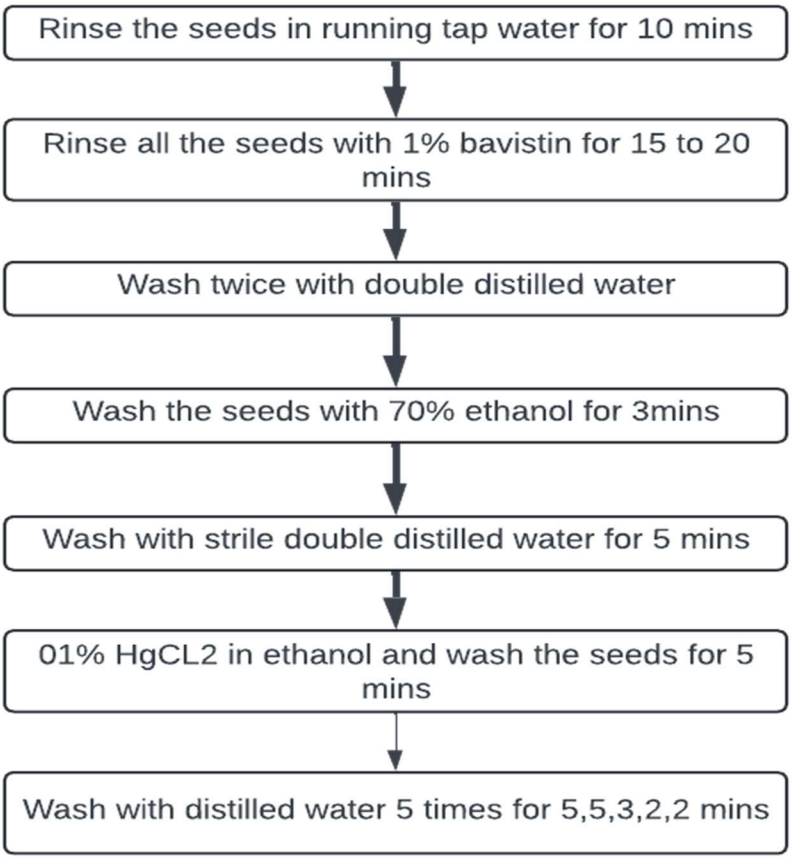

Fig. 2: Flowchart of steps of seed sterilization

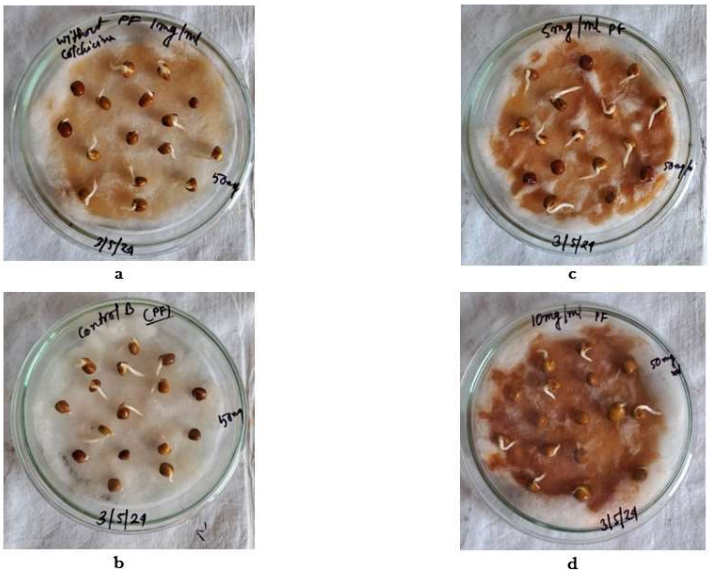

Fig. 3: Seeds of L. sativus after treated with PFEE

3.4. Evaluation of colchicine-induced polyploidy:

Surface sterilized seeds were soaked in distilled water for 6 h and then allowed to germinate in a cotton bed for 12 h. The germinated seeds of *L. sativus* were soaked in 0.5% aqueous colchicine solution for 8h and randomly 10-15 treated seeds were placed on Petri dishes with cotton pad soaked in distilled water as negative control B and *P. fulgens* aqueous ethanolic crude extract (1 mg/mL, 5 mg/mL and 10 mg/mL) as treatment. On the 3rd day of germination (72 h), the length and area of bulging of root tip was measured and frequency of polyploidy was observed at 40X magnification. All the experiments were done in triplicate (Samanta et al. 2014, 2015, 2019).

Polyploidy frequency was determined from dividing cells using the formula:

$$\text{Polyploid frequency} = (\text{Polyploid cells} / \text{Total cell}) \times 100$$

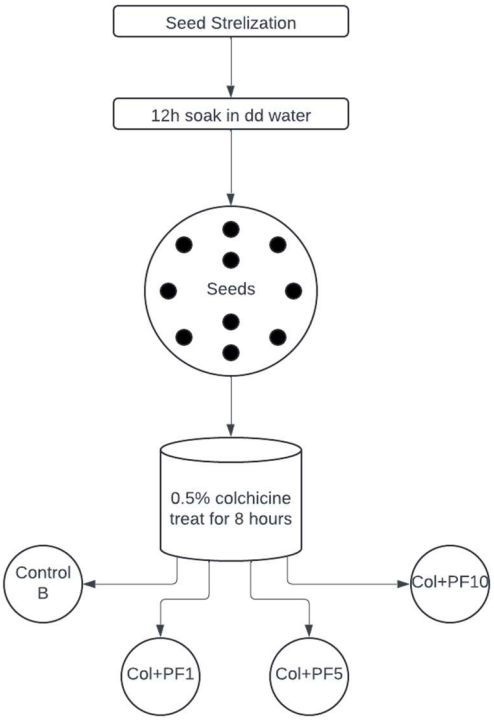

Fig. 4: Visual Representation of Colchicine treatment steps

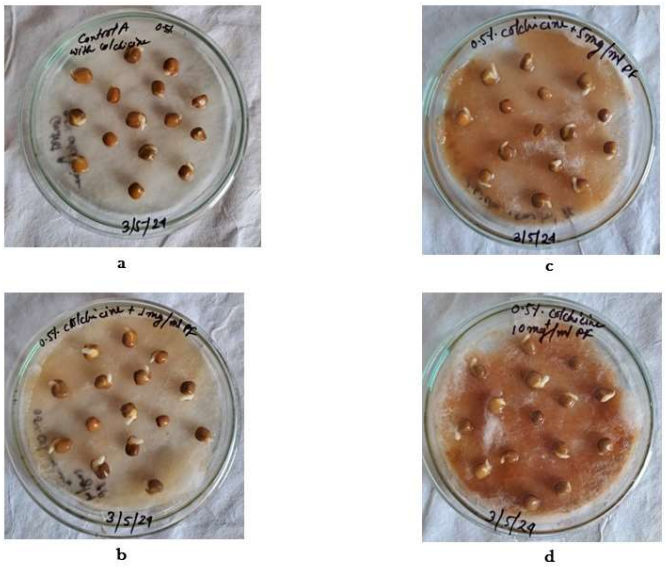

Fig. *5*: Seeds of L. sativus after colchicine treatment and treated with PFEE

3.5. DPPH assay to test the antioxidant property of PFEE

A 0.1M ethanolic solution of DPPH was made. The stock extract has a working concentration of 100 mg/ml. For this specific experiment, 900 microliter of ethanol and 10 microliters of PFEE were used to provide the working concentration. an assortment of solutions ready for the test. The mixture was shaken vigorously and allowed to stand at 23°C in dark for 30min and decrease in absorbance of the resulting solution was measured at 517 nm against a blank consisting of 2 mL of 70% aqueous ethanol and a control consisting 2 ml of 0.1mM DPPH. It is now simple to compute the OD value percent of radical scavenging activity (Mothana et al. 2010). This test could provide information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. All the sample

% Radical scavenging activity = {(Abs Control - Abs Sample)/Abs Sample} × 100

4. Results and Discussion

4.1. Effect of PFEE on radicle length and mitotic index of *L. sativus*

Radicle length (control A:34±1.2mm, PFEE1:27±1.5mm, PFEE5:19±1mm, PFEE10:10±0.98mm) and mitotic index (control A:22.85±1.33, PFEE1:11.17±0.8, PFEE5:7.2±0.65, PFEE10:3.4±0.31) are found to increase/decrease with in solution of PFEE. Both are attributes significantly correlated between themselves, suggesting that PFEE possesses the potentiality to affect the cell division. Inhibition of cell division suggests that the PFEE exerts its anticancer effects through inhibition of thymidylate synthase (TS) and incorporation of its metabolites into RNA and DNA (Kandemir & Ipek, 2022).

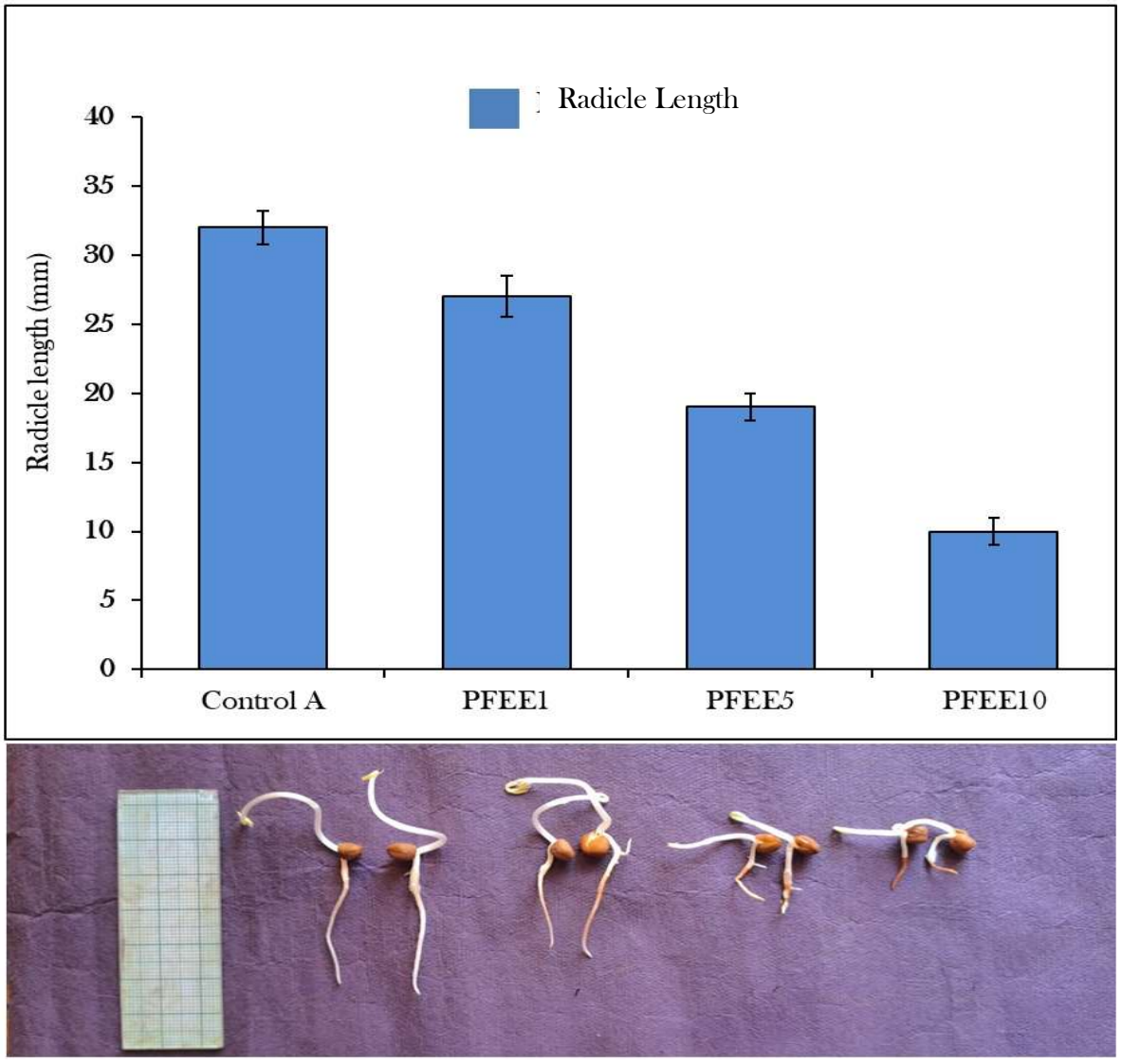

Fig.6: Effect of different concentrations of PFEE in Radicle length of L. sativus

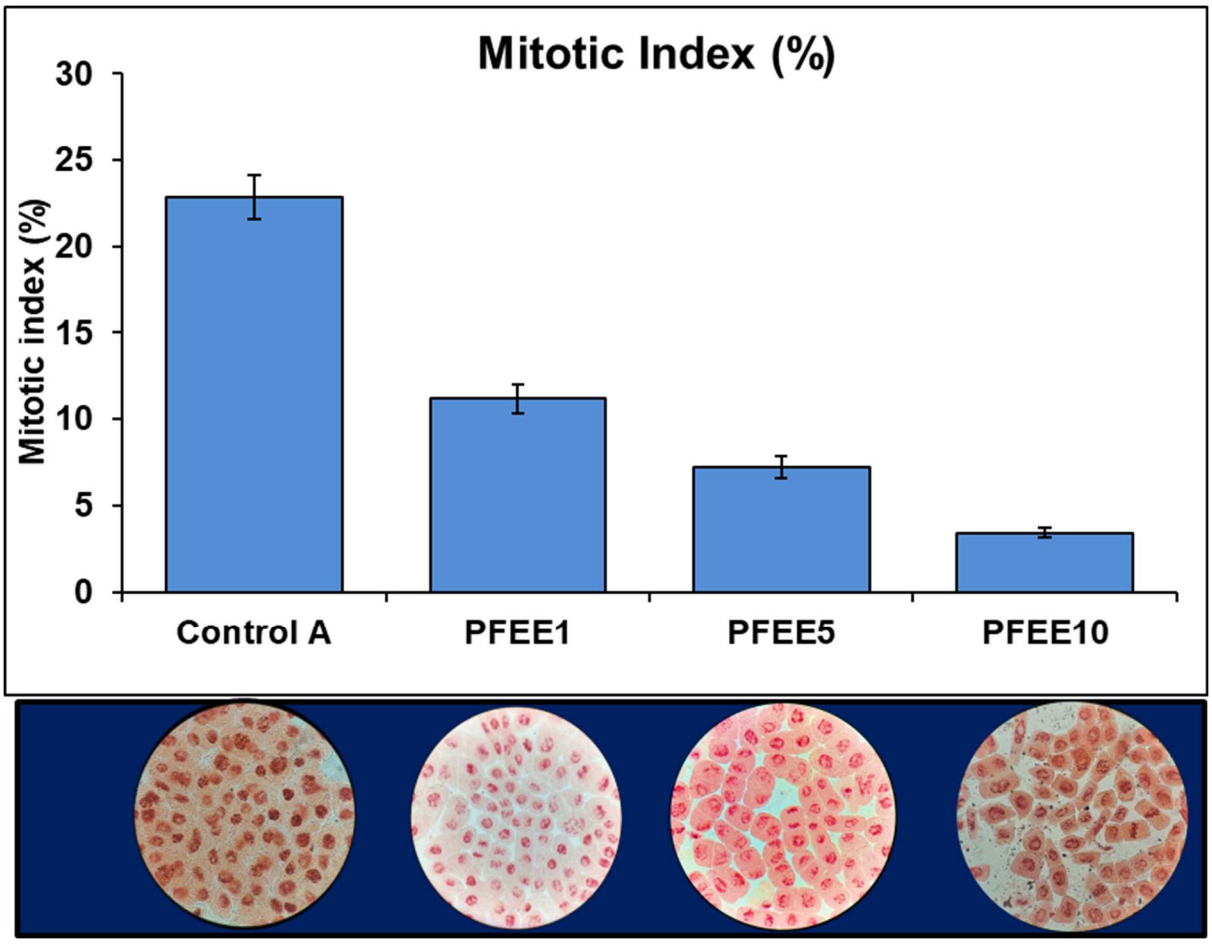

Fig.7: Effect of different concentrations of PFEE in Mitotic Index of L. sativus

4.2. Effect of Colchicine and its reversal in response to PFEE

An alkaloid called colchicine, which is obtained from the autumn crocus (Colchicum autumnale), is frequently employed in plant cytogenetics research on cell division. Colchicine binds to tubulin, a protein required for microtubule polymerization, and prevents microtubule production when administered to root tips. The construction of the mitotic spindle, a structure required for chromosomal segregation during mitosis, is inhibited by this inhibitor (Kruk et al., 2022). Colchicine treatment causes the chromosomes to misalign or split, which causes the cells to be stopped in metaphase. Because of the failure of cytokinesis, the process that splits the cell into its final pieces, this disruption in mitosis can cause polyploidy, in which cells end up with numerous sets of chromosomes. Colchicine-induced polyploidy is used in plant breeding to produce plants with improved characteristics like size and vigour.

Colchicine treatments (control B) induce bulging of radical tips. In different concentration of PFEE different result showed. Bulging of radicle tips dismissing the tendency with PFEE treatments and the radicles are phenotypically normal from the PF1 with 1mg/ml concentration of PFEE.

Inhibition of cell division suggests that PFEE is affecting the DNA and RNA metabolism and inhibiting the cell cycle, thus stopping cell division and growth. The drug PFEE have a reversal effect on polyploidy cells induced by colchicine treatment, as the dose of PFEE increases the polyploidy cell frequency decreases. Colchicine induced bulging of root tips by effecting the microtubules and restricting the cells at metaphase forming polyploidy cell, these polyploidy mimics the cancer cell and the anti-cancerous drug PFEE act on them and recover the polyploidy cells to normal cells (Kandemir & Ipek, 2022).

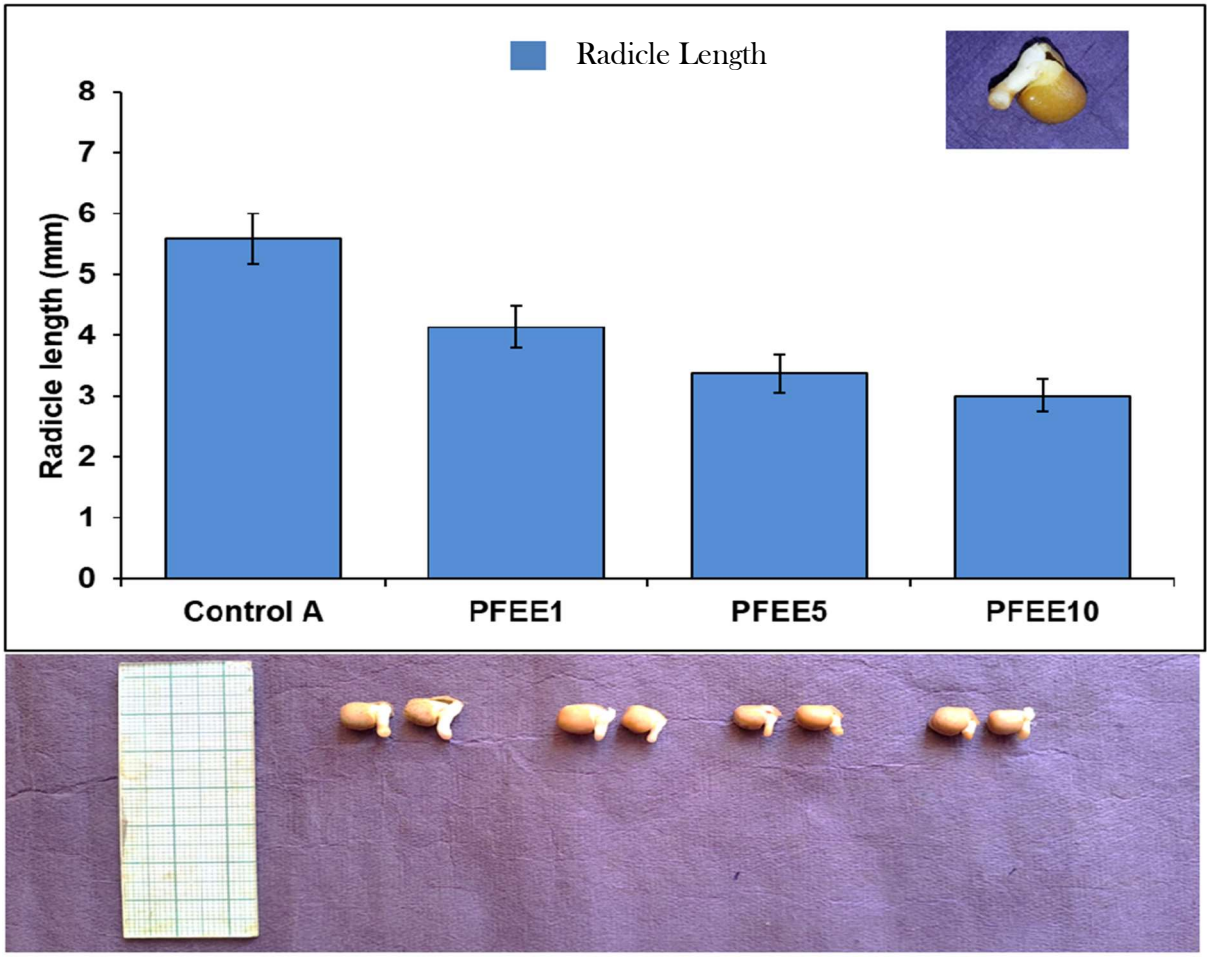

Fig.8: Effect of Colchicine and its reversal in response to PFEE on radicle length

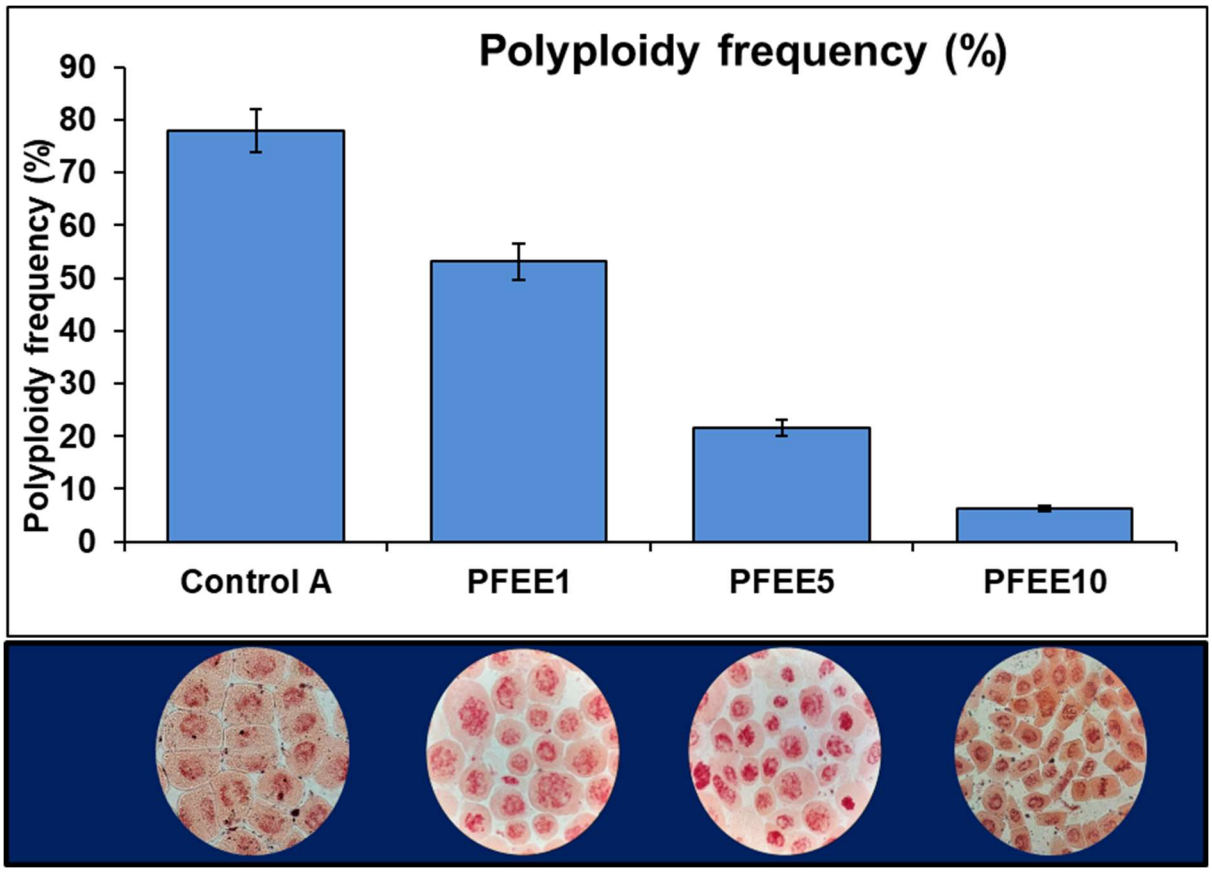

Fig.9: Effect of Colchicine and its reversal in response to PFEE on Polyploidy Frequency (%)

4.3. Antioxidant assay of PFEE in different concentration to check RSA

Radical scavenging agent can scavenge peroxide radicals to terminate radical chain reaction and improve the stability and quality of food products. The radical scavenging properties of antioxidant are most important lipid oxidation inhibition mechanism (Gulcin & Alwasel, 2023).

Fig.10 : The change in colour (from deep-violet to light-yellow)

The DPPH radical scavenging assay is based in spectrophotometric study of capacity of antioxidants to scavenge DPPH radicals. When DPPH reacts with an antioxidant compound it donates hydrogen and gets reduced. The change in colour (from deep-violet to light-yellow) was measured at 517nm. The radical scavenging activity of extract, fractions and compounds was measured by slightly modified method of Brand-Williams (Brand-Williams et al., 1995).

All sample showed a notable radical scavenging activity in a dose-dependent manner within a certain range and were significantly different.

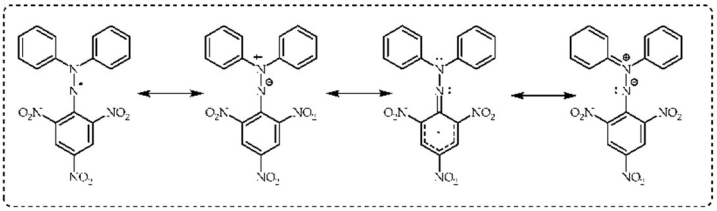

Fig.11 : Chemical structure of DPPH

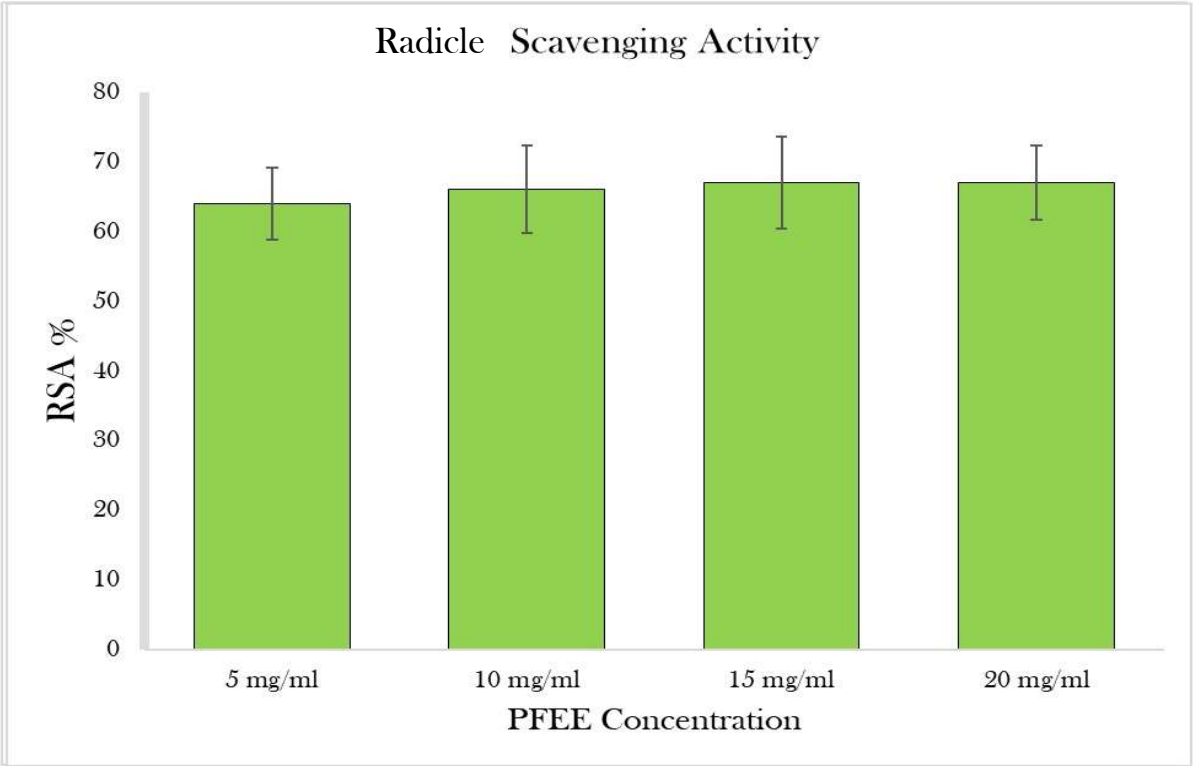

Fig.12: DPPH scavenging assay to test antioxidant property of PFEE

5. Conclusion

Potentilla fulgens' ethanolic root peel extract's considerable therapeutic potential has been demonstrated by a thorough assessment of its antioxidant and anti-cancer properties, highlighting its significance in the field of natural product-based drug discovery. The extract's strong bioactivities have been shown by this study, making it a viable option to incorporate into contemporary medical procedures.

5.1. Anti-Cancer Intent

Meticulous in vitro and in vivo investigations have confirmed *Potentilla fulgens*' anti-cancer effects. Strong cytotoxicity is demonstrated by the ethanolic root peel extract against a variety of cancer cell lines, such as those from lung, colon, and breast malignancies. The mechanisms entail the trigger of programmed cell death, suppression of cellular growth, and interference with the advancement of the cell cycle. These benefits are mainly caused by the bioactive substances found in the extract, which were found and characterised by sophisticated phytochemical tests. These substances include tannins, flavonoids, and saponins.

Potentilla fulgens induces apoptosis, which is an important therapeutic target in the therapy of cancer. It does this via modulating the Bcl-2 family proteins and activating both intrinsic and extrinsic caspase pathways. Furthermore, downregulation of important oncogenic signalling pathways, such as the PI3K/Akt and MAPK pathways, which are essential for cancer cell survival and growth, is associated with the reduction of cancer cell proliferation. The extract's capacity to cause cell cycle arrest at the G1/S and G2/M checkpoints supports its ability to impede the growth of tumours (Saddam et al., 2024).

Potentilla fulgens has been shown to be effective in halting tumour growth and metastasis in animal models through in vivo investigations. These results underline the extract's potential for therapeutic use and corroborate the anti-cancer benefits seen in vitro. Its significance as a cancer treatment agent is highlighted by the notable decrease in tumour volume and the inhibition of metastatic dissemination.

5.2. Anti-oxidant Intent

Another important component of *Potentilla fulgens*' medicinal potential is its antioxidant properties. Numerous chronic diseases, such as cancer, cardiovascular disease, and neurological problems, are linked to oxidative stress as a pathogenesis. *Potentilla fulgens*' ethanolic root peel extract has strong antioxidant qualities, as evidenced by its capacity to scavenge free radicals, lower lipid peroxidation, and increase the activity of natural antioxidant enzymes.

The extract's abundance of polyphenolic chemicals, which have a strong track record of antioxidant activity, is thought to be responsible for its capacity to scavenge free radicals. Reactive nitrogen species (RNS) and reactive oxygen species (ROS) are efficiently neutralised by these substances, preventing oxidative damage to cellular macromolecules such proteins, lipids, and nucleic acids. The extract's ability to protect against oxidative stress is further supported by the notable decrease in lipid peroxidation levels (Kruk et al., 2022).

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are examples of endogenous antioxidant enzyme activities that are enhanced by the extract, which further emphasises its function in bolstering the body's defence mechanisms. Through the upregulation of these enzymes, *Potentilla fulgens* offers complete protection against oxidative damage by neutralising free radicals already in the environment and preventing their production.

5.3. Consequences for Contemporary Medicine

Given its proven therapeutic benefits, *Potentilla fulgens* is a strong contender to be included in contemporary medical procedures. Its ability to both target cancer cells and reduce oxidative stress demonstrates how versatile this therapeutic agent is. *Potentilla fulge*n's natural origins are in line with the growing trend towards plant-based, holistic approaches to health and wellness.

To completely understand the molecular mechanisms underlying *Potentilla fulgens'* bioactivities and to optimise its formulation for clinical usage, more study is

necessary. To validate its safety and efficacy in human populations, as well as to create standardised dosages and administration techniques, comprehensive clinical trials are required.

Potentilla fulgens' ethanolic root peel extract is a good option for the creation of natural medicinal agents because it demonstrates strong antioxidant and anti-cancer properties. The extract's ability to target cancer cells and mitigate oxidative stress highlights its dual functioning, which highlights its potential to tackle intricate health issues. Harnessing *Potentilla fulgen's* full therapeutic potential and incorporating it into modern medical procedures will require ongoing study and clinical confirmation. This study advances our knowledge of *Potentilla fulgens* scientifically and opens the door to its potential use in raising people's quality of life and achieving better health outcomes globally.

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