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Evaluating the Proliferative and Inhibitory Effects of Selected Indian Spices and Herbs on Vigna Radiata Cell Growth

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ABSTRACT

Scientists have always been on the hunt for a therapeutic chemical with the potential to treat deadly diseases. There is a growing interest in using natural compounds derived from plants as a natural cancer cell treatment. Herbs and spices such as turmeric, garlic, cinnamon, clove, and tulsi are rich in bioactive compounds and have long been studied for their medicinal value in humans. However, their potential role in modulating cancer cell proliferation is underutilized. By exploring the proliferative and inhibitory effects of these traditional Indian herbs on Vigna radiata, this study contributes new knowledge to medical science and phytochemistry. It also opens new avenues for applying culturally significant, easily accessible, sustainable and inexpensive natural resources in modern cancer treatments. This study focuses on finding the specific herb extracts which are potent inhibitors of cell proliferation, in turn reducing the cancerous cell growth, leading to an invaluable impact on cancer treatment worldwide. Furthermore, this research aligns with global goals for sustainable development, particularly those related to accessible, low-cost and sustainable healthcare.

Keywords: *Vigna radiata, Cell Proliferation, Spices, Extracts, Eugenol, Inhibitory Effects, Seed Germination, Radicle and Plumule Growth, Qualitative Tests.*

AIM

This study aims to assess the proliferative and inhibitory consequences of aqueous extracts of the selected spices and herbs on *Vigna radiata* seed germination and early stage cell proliferation.

OBJECTIVES

i. To prepare aqueous extracts of turmeric, clove, cinnamon, garlic, tulsi and gooseberry.
ii. To assess the impact of above mentioned extracts on rate of seed germination, radicle length, and plumule length of *Vigna radiata.*
iii. To compare the proliferative and inhibitory effects of different herbs and spices extracts on early-stage plant development.
iv. To analyze patterns of variation in growth responses based on type of extracts and concentration of extracts.
v. To interpret the collected results in context of the phytochemical properties of the tested herbs and their potential applications in cancer cell treatment.

RESEARCH QUESTION

What are the proliferative or inhibitory effects of selected Indian herbs and spices (turmeric, clove, cinnamon, garlic, tulsi) on the cell proliferation of *Vigna radiata*?

PROBLEM STATEMENT

Despite extensive evidence of their medicinal value, the effects of Indian herbs and spices such as gooseberry, turmeric, garlic, cinnamon, clove, and tulsi on plant cell proliferation, particularly in *Vigna radiata*, remain underexplored, limiting their potential application as sustainable and safer alternatives for cancer cell treatment.

RELEVANCE

Scientists have always been on the hunt for a therapeutic chemical with the potential to treat deadly diseases. There is a growing interest in using natural compounds derived from plants as natural cancer cell treatment. Herbs and spices such as turmeric, garlic, cinnamon, clove, and tulsi are rich in bioactive compounds and have long been studied for their medicinal value in humans. However, their potential role in modulating cancer cell proliferation is underutilized.

By exploring the proliferative and inhibitory effects of these traditional Indian herbs on *Vigna radiata*, this study contributes new knowledge to medical science and phytochemistry. It also opens new avenues for applying culturally significant, easily accessible, sustainable and inexpensive natural resources in modern cancer treatments.

This study focuses on finding the specific herb extracts which are potent inhibitors of cell proliferation, in turn reducing the cancerous cell growth, leading to an invaluable impact on cancer treatment worldwide.

Furthermore, this research aligns with global goals for sustainable development, particularly those related to accessible, low-cost and sustainable healthcare.

BACKGROUND RESEARCH

Cell proliferation is a pivotal biological process responsible for the repair, growth, and reproduction of all living organisms. In plants, cell proliferation plays a pivotal role in seed germination, plumule elongation, radicle elongation, and the overall development of the plant. (Verma et al., 2024).

Achieving an understanding of the factors which enhance or inhibit cell proliferation in plants offers an invaluable correlation between plant cell proliferation and cancer cell proliferation due to their consistent mitotic cell division, opening up avenues for alternative cancer cell proliferation.

The Indian subcontinent is known for its exceptional biodiversity and ancient medicinal systems, particularly Ayurveda, which has long utilized herbs and spices for their therapeutic potential. Many of these fauna are abundant in bioactive phytochemicals, for instance flavonoids, phenolics, terpenes, and organosulfur compounds, that exhibit antioxidant, antimicrobial,anti-inflammatory, and cytotoxic attributes. (Aggarwal et al., 2011; Butt & Sultan, 2011).

Among the vast array of Indian herb species, our study focuses on 6 well-known spices from the Indian subcontinent: Turmeric (*Curcuma longa*), Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum verum*), Garlic (*Allium sativum*), Tulsi (*Ocimum sanctum*) and Gooseberry (*Phyllanthus emblica*).

These spices and herbs were selected based on both their traditional Ayurvedic and scientific studies supporting their bioactivity. Turmeric contains curcumin, a compound which is known to modulate gene expression and inhibit irregular proliferation of cells, including within cancer cell strains. (Gupta et al., 2013)

Garlic contains allicin and other sulfur-containing compounds shown to arrest the cell-division cycle and induce programmed cell death *(Hosseini et al., 2015)*.

Clove and cinnamon are rich in eugenol and cinnamaldehyde, respectively compounds that have demonstrated strong antioxidant and anti-proliferative properties. (Ranasinghe et al., 2013)

Tulsi, commonly termed the "Elixir of life," exhibits an extensive scale of bioactive effects including adaptogenic and growth-regulating properties. (Mondal et al., 2009)

Although these effects have been well-documented in animal and microbial systems, limited research has been conducted on their influence on plant cell growth, particularly in legumes. *Vigna radiata* (commonly known as moong or mung bean) is a widely cultivated pulse crop in India and serves as an ideal model organism due to its rapid germination, uniform growth, and agricultural significance (Singh et al., 2016).

The reason we approached this topic as our research. All these spices and herbs are used in Indian recipes for cooking a variety of dishes. A herbal drink termed "kadha" is consumed regularly, which is a concoction of spices and herbs boiled in water. Kadha is mentioned widely in Indian scriptures like Ayurveda. It is said to have antimicrobial properties and is consumed to boost immunity. Its usage skyrocketed in Indian households during the Covid pandemic (Maurya and Sharma, 2020).

We children are coaxed by our elders to consume it regularly to prevent falling ill, especially during rainy and winter seasons. We were intrigued by this practice and hence wanted to do research on the potential usage of Indian herbs and spices for combating deadly diseases like cancer.

HYPOTHESIS

The application of certain spice extracts will inhibit the germination and cellular growth of *Vigna radiata* due to their known antimicrobial and allelopathic compounds, whereas others may have a neutral or stimulatory effect.

VARIABLES

Table 1: Variables

Independent Variable	1. Type of Herbs/Spices in Extract: • Cinnamon • Clove • Garlic • Gooseberry • Holy Basil • Turmeric • Control (Distilled Water) 2. Concentration of Extract: • ~25 g/dm³	1. Solutions were chosen due to their Indian heritage and mention in Ayurveda. A control was taken to compare our results, so that we can reach a conclusion. 2. To mitigate the error caused while preparing the extracts in Trial.
Dependent Variable	*Vigna radiata:* • Number of Germinated Seeds • Radicle & Plumule Length *Allium cepa*: • Number of New Roots	1. To calculate the rate of germination for each extract and compare with the control. 2. Alternate model organ to check consistency in results.

Control Variable	1. Volume of Extract Added Per Day 2. Number of Seeds 3. Concentration of Extract Per Test 4. Amount of Sunlight 5. Humidity 6. Carbon dioxide Concentration in Air 7. Size of Beaker/Petri Dish 8. Equipment Company (Borosil)	1. Ensures that the volume doesn't affect the results. 2. Same sample size for each extract. 3. Each batch made had the same concentration of the corresponding extract. 4, 5, 6. All samples were kept beside one another to ensure these factors did not limit growth. 7.Surface area does not affect growth. 8.
Uncontrolled Variable	1. Bending of Radicle 2. Growth of Mold 3. Atmosphere 4. Seed Quality 5. Humidity	1. Lead to inconsistent measurements, which could affect accuracy of results. 2. Discard samples and shorten the term of the experiment. 3. All experiments were conducted in the same room, to make it as consistent as possible. 4. All seeds were chosen from the same packet, but some differences can exist. 5. All tests were conducted in a closed room, but any change to the humidity levels weren't controlled.

APPARATUS

Table 2: Apparatus Used

Sr. No.	Apparatus	Quantity	Purpose
1	Petri Dishes	7	Germination of seeds
2	Syringes	7	Accurately add extracts
3	Rulers	4	Measure lengths of radicle and plumule
4	Beakers (500 cm^3)	6	Storing extracts
5	Beakers (250 cm^3)	2	Storing reagents for tests
6	Measuring Jar (1000 cm^3)	1	For distilled water
7	Clingfilm	2 Rolls	Acts as a lid
8	Labels	2 Sheets	Label everything
9	Mortar & Pestle	1 pair	Crush spices/herbs to make extract
10	Stopwatch	2	Record timings
11	Glove	1 box	Safe handling of extracts and chemicals
12	Lab Coat	4	Precautionary measure
13	Forcep	2	Handle samples
14	Clean Glass Slide	14	Study the root tip
15	Microscope	1	Observation of cell division
16	Top Pan Balance	1	Measuring mass

CHEMICALS

Table 3: Chemicals Used

Sr. No.	Chemical	Purpose
1	DCPIP solution (1 g/dm^3)	Test for Vitamin C
2	Salkowski Reagent	Test for presence of auxin
3	Acetocarbine Stain	To study mitosis in root tip cells
4	Distilled Water	Preparation of extracts and control setup
5	Absolute Ethanol	For Salkowski test
6	Biuret Reagent	For Protein test

Table 4: Volume of Water and Mass of Spices Used for 25.7 g/dm^3 Concentration of Extracts

Spice / Herb	Volume of Distilled Water (cm^3)	Mass of Spice/Herb (g)	Concentration of Extract (g/dm^3)
Gooseberry	350.00	9.02	25.77
Cinnamon	350.00	9.02	25.77
Clove	350.00	8.98	25.66
Garlic	350.00	9.02	25.77
Tulsi	350.00	9.00	25.71
Turmeric	350.00	9.02	25.77

METHOD

- Set-Up
  - i. Accumulate the following 6 spices/herbs:
    - a. Gooseberry (*Phyllanthus emblica*)
    - b. Cinnamon (*Cinnamomum verum*)
    - c. Clove (*Syzygium aromaticum*)
    - d. Garlic (*Allium sativum*)
    - e. Tulsi (*Ocimum tenuiflorum*)

f. Turmeric (*Curcuma longa*)

ii. Take 7 batches of 50 seeds (1 for each spice/herb and 1 control).
iii. Label each of the containers with the spices' names.
iv. Prepare the extracts as mentioned in table 1.
v. Add the extracts to the containers.

- Extract Preparation
  i. Crush the spices/herbs using a mortar and pestle.
  ii. Measure the mass of the herbs and spices using top pan balance.
  iii. The mass of all the herbs and spices were kept 9.02 g
  iv. Take 350 cm^3 distilled water in 6 different beakers, add the measured quantity of herbs and spices.
  v. Boil each solution for a period of 10 minutes.
  vi. Allow solutions to cool down at 30^0C, and use it for the experiment.
- Data Collection
  i. Observe and record the number of seeds germinated.
  ii. Measure the radicle length of 15 random germinated seeds from each extract.
  iii. Record values in the table.
  iv. Once seeds begin to display radicle/plumule growth, count the number of seeds displaying both radicle and plumule growth and measure the lengths.
  v. Repeat step 4 for a total period of 5 days.
  vi. Study the root tip of 1 seed from each extract under a microscope.
  vii. Perform the following qualitative tests on seeds from each extract:
    - Salkowski Test to check for the concentration of auxin for each extract.
    - DCPIP test for analysing the antioxidant activity
    - Biuret Test for checking presence and concentration of protein.
- Salkowski Test Procedure
  i. Measure 1 g of seeds in a beaker on a top-pan balance.
  ii. In a mortar, using a syringe, add 5 cm^3 of absolute ethanol.
  iii. Add the 1 g seed sample and then crush using a mortar. The ratio of mass of seeds used to volume of absolute ethanol should be 1 : 5.
  iv. Crush with regular hand motion for 20 to 30 seconds.
  v. Pour it in the corresponding labelled test tube and leave it to stand so that the supernatant can be obtained.
  vi. Take 1 cm^3 of the supernatant using a measuring cylinder. Add it to a labelled test tube.
  vii. Repeat the above for the seeds from the other extracts.
  viii. Add 2 cm^3 of Salkowski reagent to each test tube, keeping the ratio of the volumes of the supernatant to the reagent 1 : 2.
  ix. Swirl test tube and cover with a cling film.
  x. Place the test tube rack in a dark shelf and wait for 25 minutes.
  xi. Observe the colour change and derive conclusions based on the next step of the procedure.
  xii. A pink, reddish-pink, or reddish-brown colour develops, indicating the presence of auxins. The intensity of the color can vary depending on the auxin concentration. If the solution remains colorless or pale yellow, which is the original color of the Salkowski reagent. This indicates that auxin is not present in the sample or is in concentrations too low to be detected by the test.
- DCPIP Test Procedure
  i. Measure 2 g of every spice using a top-pan balance and put it labelled beakers.
  ii. Pour 8 cm^3 of absolute ethanol in a mortar and add 2 g of spice to it.
  iii. To prepare ethanol based extract - Crush the spice in the ethanol using a pestle with regular hand motion for 20 to 30 seconds. The ratio of mass of spice to volume of ethanol should be 1:4.
  iv. Pour the extract into labelled beakers.
  v. In labelled test-tubes, add 0.5cm^3 of DCPIP of 0.1% (w/v) concentration using a syringe.
  vi. Take out 3 cm^3 of extract using a syringe and add it to a labelled test tube.
  vii. Swirl the test tube.
  viii. Record colour change.
  ix. If the solution turns pale or colourless, antioxidants are present. The paler the solution, the higher the concentration of the antioxidants. Its solution remains blue or purple, no or very less antioxidants are present.
  x. The DCPIP test was performed using a water-based extract as well. Extracts used for it were the same prepared in the 4th step of the setup.
- Biuret Test Procedure
  i. Pick out 5 to 6 germinated seeds from each beaker.
  ii. Measure 1 gram of seeds on top-pan balance for each of the 7 samples.

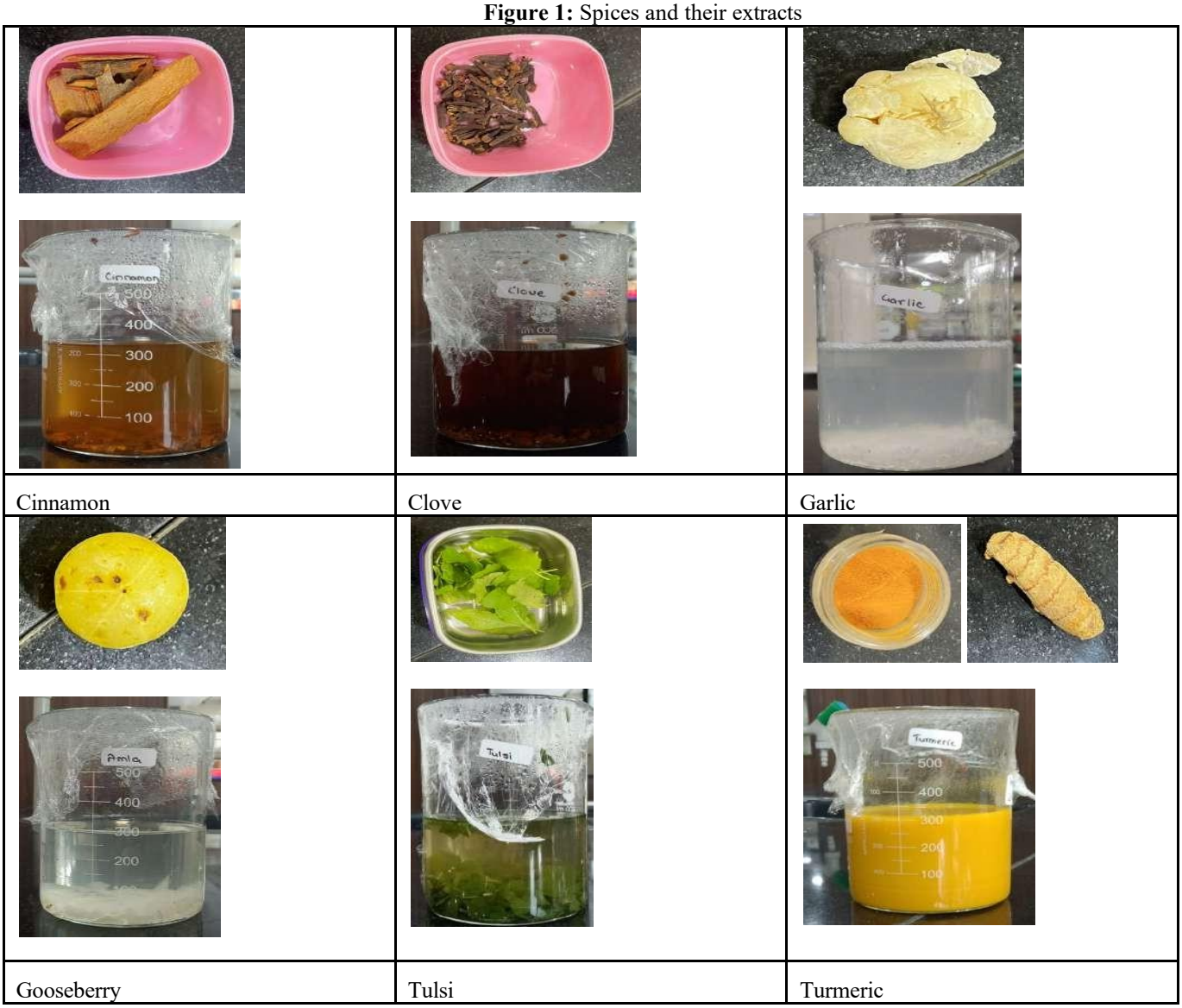
iii. Prepare water-based extracts of the seeds - Use a mortar and pestle to crush the 1 gram of seeds (for 20 seconds with regular hand motion) with 5 cm^3 of distilled water. The ratio of mass of seeds used to volume of distilled water used should be 1:5.
iv. Pour individual seed extracts in test-tubes held in a test-tube rack and let the extracts stand for 10 minutes.
v. Using a measuring cylinder, measure 1.5 cm^3 of supernatants in separate labelled test tubes.
vi. In the same test-tube, add 1.5 cm^3 of Biuret solution drop-by-drop using a syringe. The ratio of Biuret solution to seed extract should be 1:1.
vii. Swirl solution in the test tube regularly.
viii. Observe the colour change and derive a conclusion based on step 9.
ix. If the colour of the biuret reagent changes from blue to purple, it indicates the presence of proteins.A darker purple colour would indicate higher concentration of proteins. If the colour remains blue it indicates negative test results.

SAFETY AND ETHICAL CONSIDERATION

i. Sterilize all the equipment and apparatus before using autoclave or using solutions such as 70% ethanol.
ii. Disinfectants such as ethanol are highly flammable. It's critical to be cautious when using it near a flame.
iii. Wear safety gear such as gloves and labcoat while working with chemicals in the laboratory.
iv. Wash your hands with soap before and after conducting any experiment to avoid contaminations of the seeds.
v. The contaminated seed samples need to be discarded with utmost precaution considering them as a biohazard.
vi. In case of any spillage or breakage inform the lab attendant and teacher supervisor at the earliest so that it can be taken care of properly.
vii. Use tongs to handle hot beakers to avoid burns.
viii. Mortar and pestle need to be kept on a hard and firm surface while grinding the specimens.
ix. Use a clean white tile and a sharp knife angled away from the body while cutting the fresh herbs.
x. Do not eat anything while working in the laboratory as consuming anything from the laboratory can lead to accidental ingestion of hazardous chemicals or harmful microorganisms.

OBSERVATIONS

Figure 1: Spices and their extracts

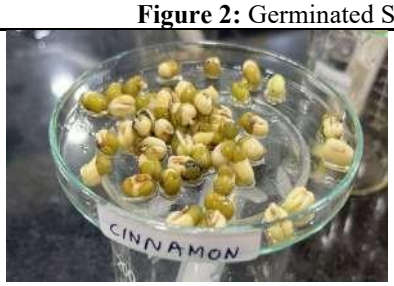


Cinnamon	Clove	Garlic
Gooseberry	Tulsi	Turmeric

RADICLE AND PLUMULE LENGTH RECORDING

Figure 2: Germinated Seeds

CONTROL



Cinnamon

Clove

Garlic

Gooseberry

Tulsi


Turmeric

ROOT GROWTH OF ONIONS KEPT IN EXTRACTS

Figure 3: Growth of Onion Roots (25th August)

		
CONTROL	Cinnamon	Clove
		
Garlic	Gooseberry	Tulsi
		
Turmeric		

Figure 4: Growth of Onion Roots (12th September)

CONTROL

Cinnamon

Clove

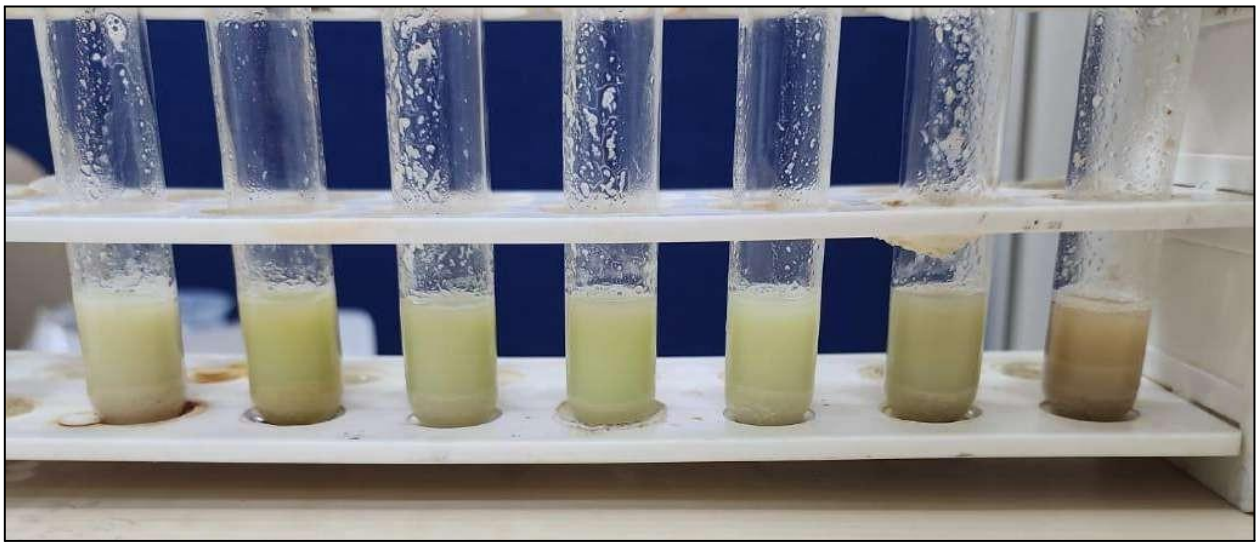
Garlic

Gooseberry

Tulsi

Turmeric

Figure 5: Extracts used for the Biuret test



(In image from left to right) Cinnamon, Turmeric, Tulsi, Control, Garlic, Gooseberry, Clove

Figure 6: Biuret Test

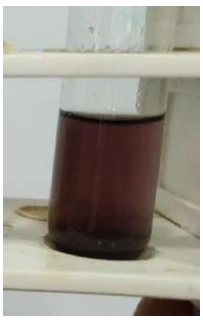
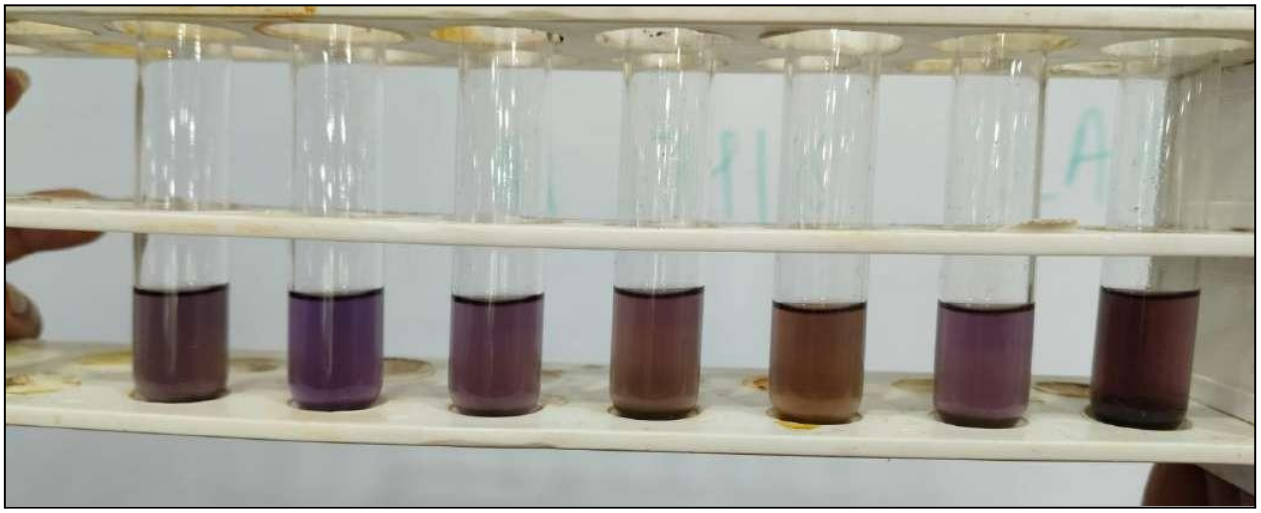
		
CONTROL	Cinnamon	Clove
		
Garlic	Gooseberry	Tulsi
		
Turmeric		

Figure 7: Results of Biuret Test (Reference image of figure 6)



(In image: Left to Right) Gooseberry, Garlic, Control, Tulsi, Turmeric, Cinnamon, Clove DCPIP TEST (Replicate 1)

Figure 8: 10 cm³ of spice extracts without DCPIP

Figure 9: 10 cm³ of Spice Extracts with 1 cm³ of 0.1% (w/v) DCPIP aqueous solution

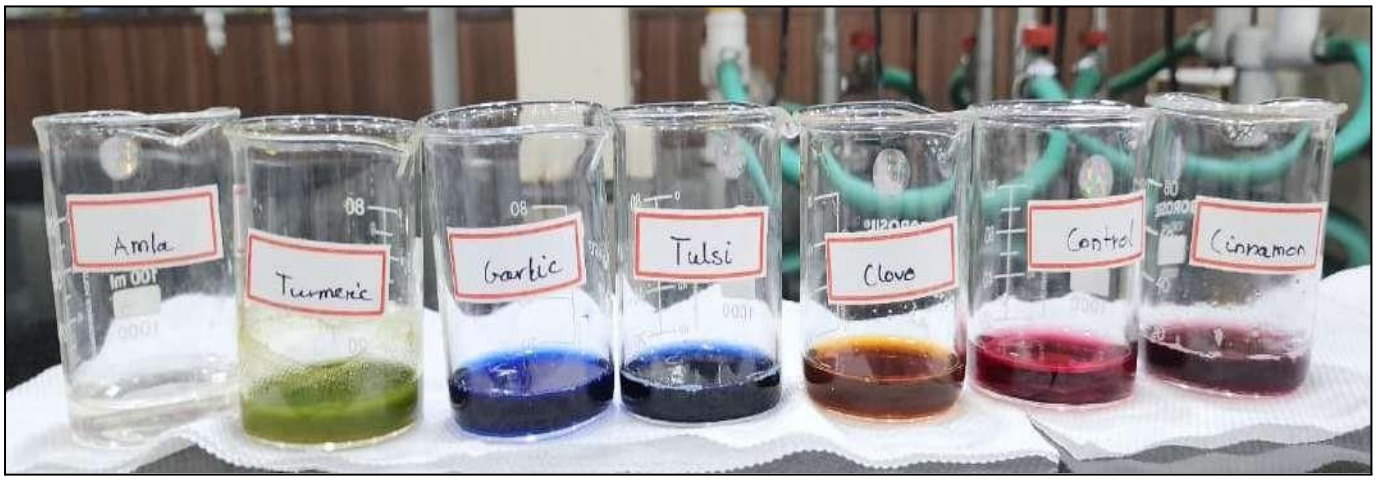
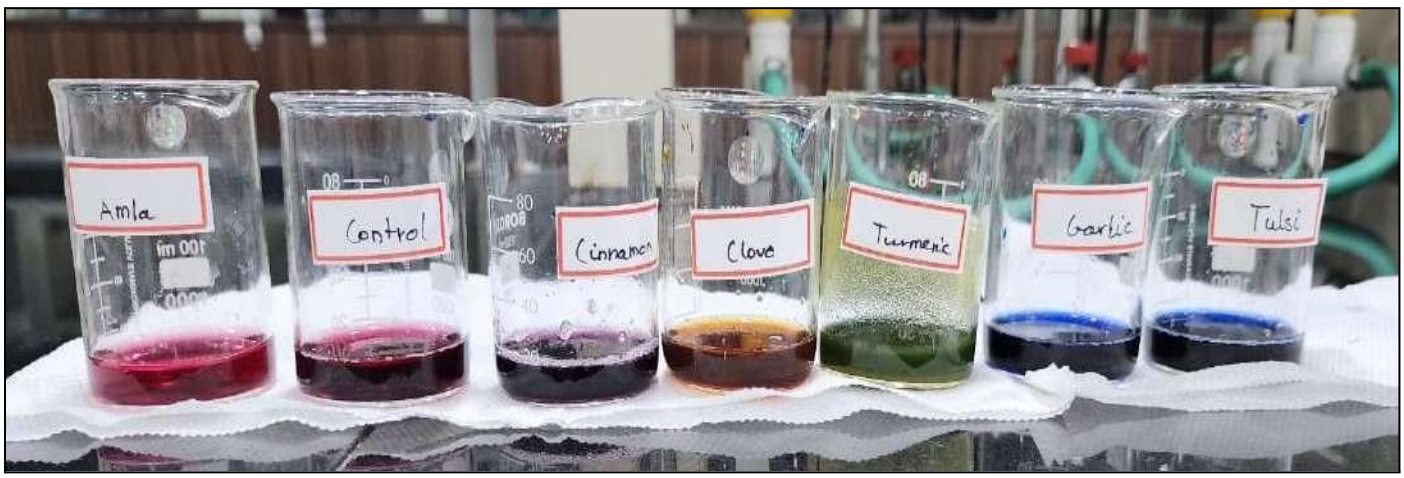


Figure 10: 10 cm³ of Spice Extracts with 2 cm³ of 0.1% (w/v) DCPIP aqueous solution



DCPIP TEST (Replicate 2)

Figure 11: Results of Final Replicate of DCPIP Test

		
CONTROL	Cinnamon	Clove
		
Garlic	Gooseberry	Tulsi
		
Turmeric		

Figure 12: Samples Of DCPIP test (Replicate 2) (Reference image for figure 11)

Figure 13: Ethanol Based Spice/Herbs Extracts for DCPIP Test replicate

Figure 14: Results of Salkowski test- Replicate 1

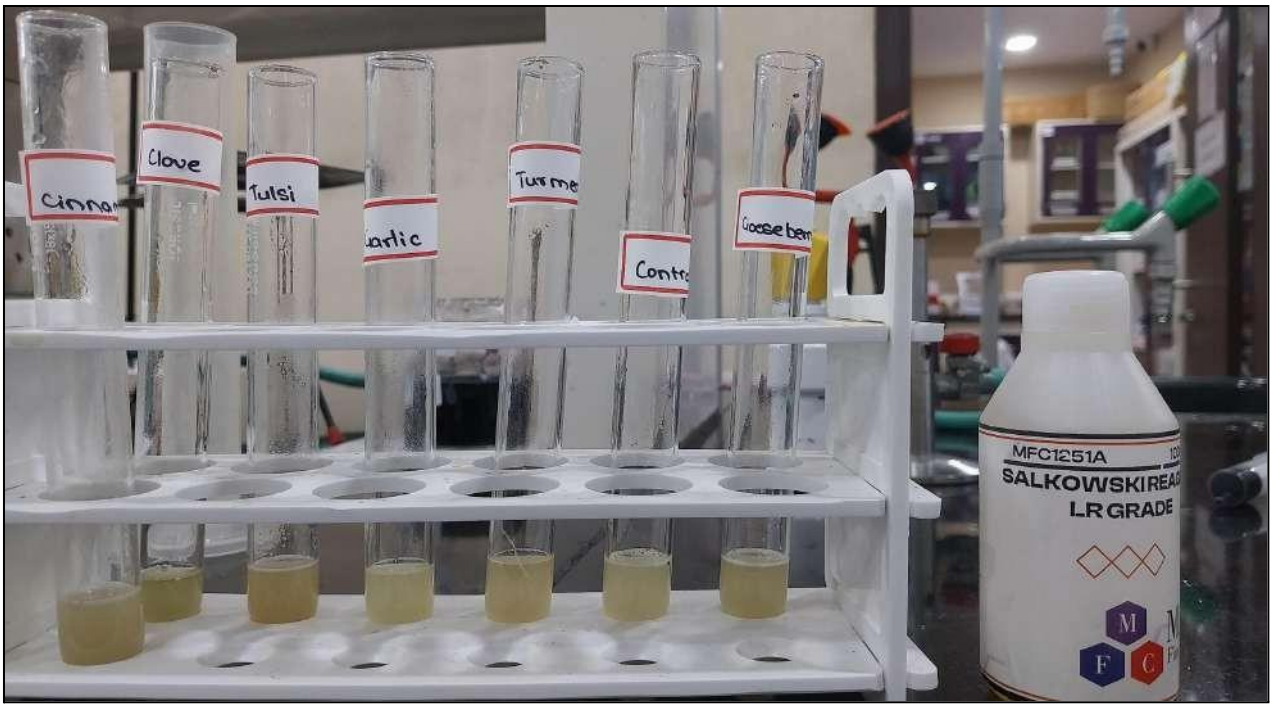


Figure 15: Results of of Salkowski Test - Replicate 2

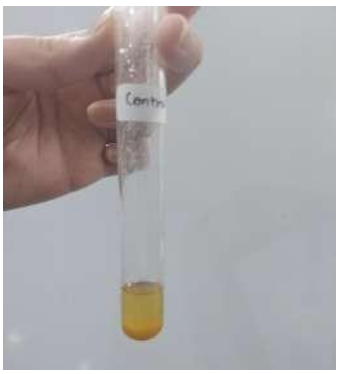
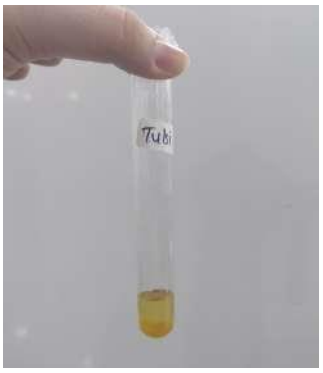
		
CONTROL	Cinnamon	Clove
		
Garlic	Gooseberry	Tulsi
		
Turmeric		

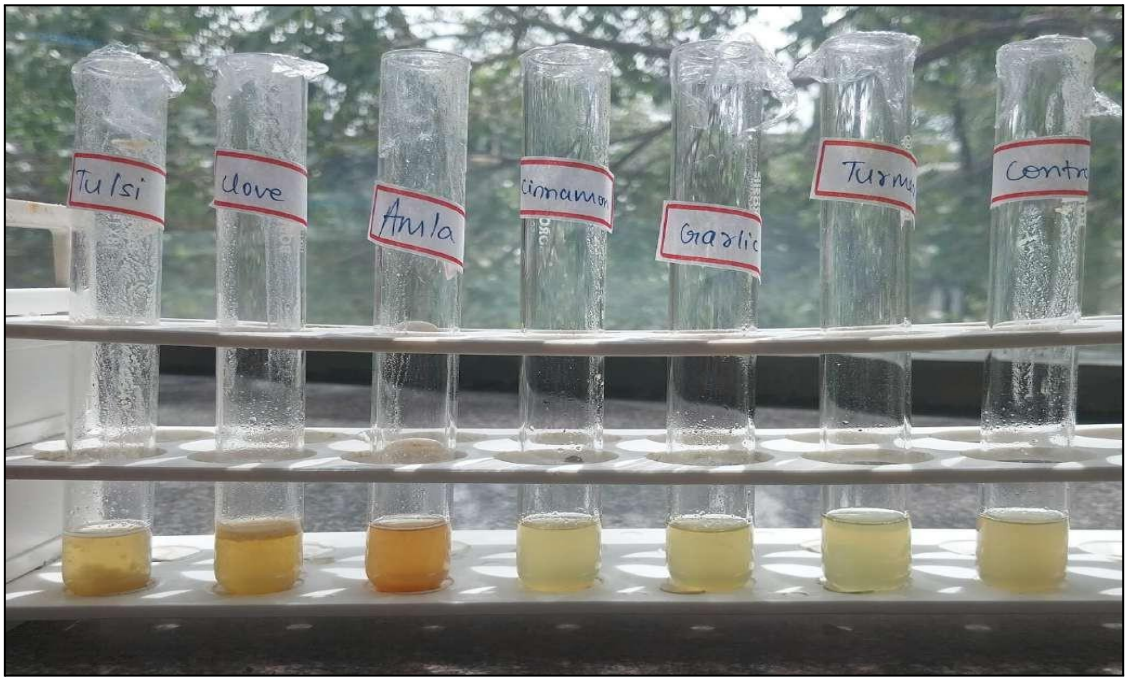
Figure 16: Salkowski Test-Replicate 2 (Reference image for figure 15)

In image from (Left to Right): Turmeric, Gooseberry, Garlic, Control, Clove, Tulsi, Cinnamon

Figure 17: Results of Salkowski Test- 3rd Replicate

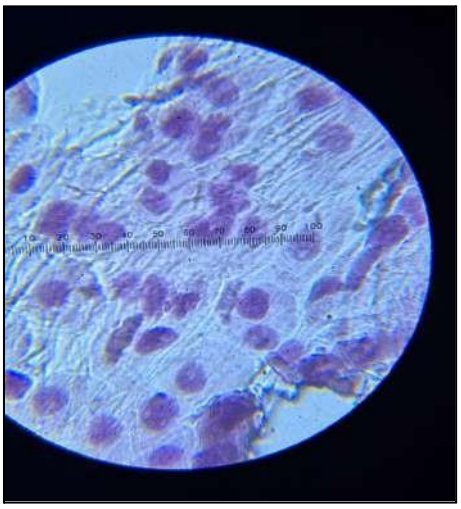
		
CONTROL	Cinnamon	Clove
		
Garlic	Gooseberry	Tulsi
		
Turmeric		

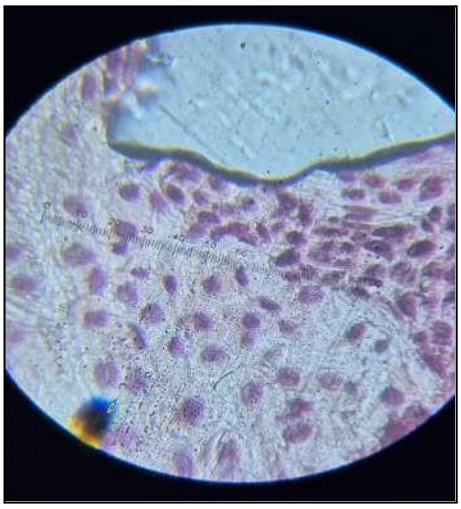
Figure 18: Result of Salkowski Test - 3rd Replicate (Reference image for figure 17)



(In image from left to right): Tulsi, Clove, Gooseberry, Cinnamon, Garlic, Turmeric, Control

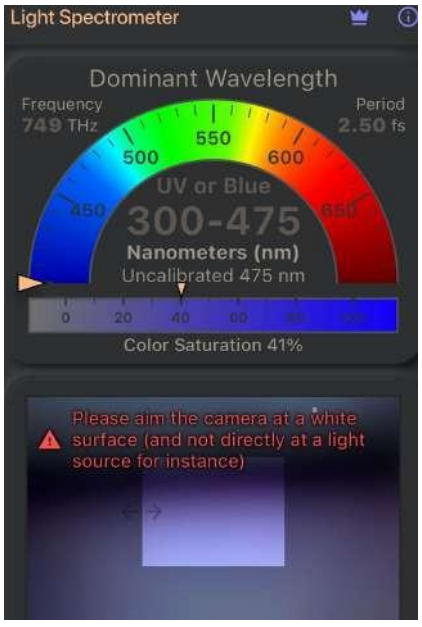
Figure 19: Photomicrograph of root tips of *Vigna radiata* seeds grown in distilled water(control)



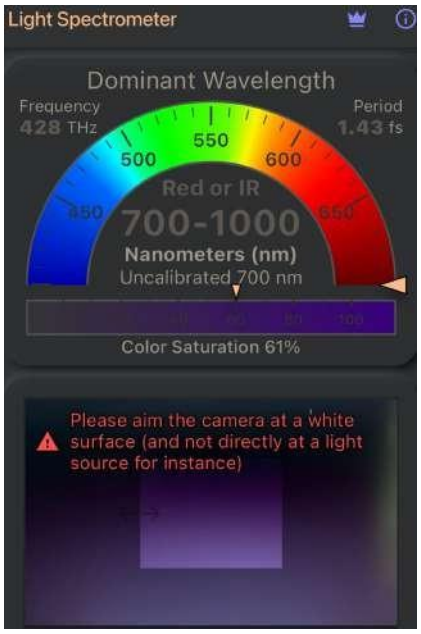


SPECTROMETER APP IMAGES

Fig 20: Spectrometer images - Biuret test



CONTROL

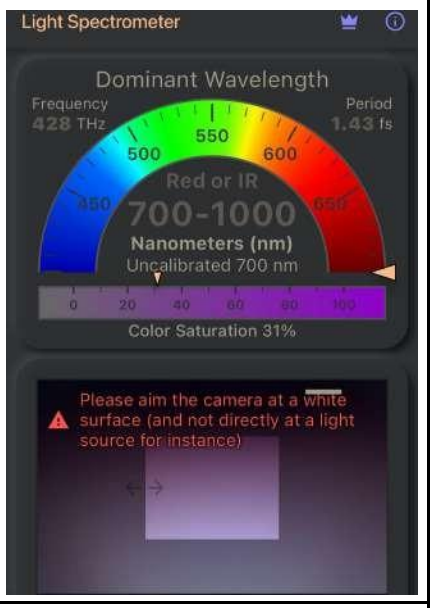


Cinnamon

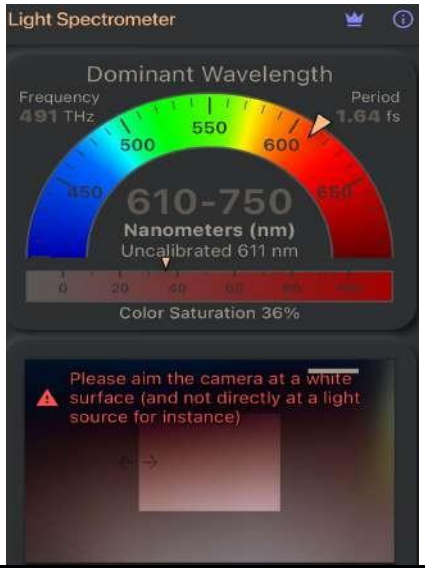
Clove

Garlic

Gooseberry



Tulsi

	
Turmeric	

TABLES

PILOT STUDY DATA

Table 1: Length of Radicle (6th August - Pilot study Day 1)

	RADICLE LENGTH in different extracts / mm						
Seed Number	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
1.	23	9	7	17	6	13	7
2.	15	6	7	18	14	14	4
3.	13	9	3	17	6	15	7
4.	15	10	4	13	14	12	5
5.	13	5	5	18	2	7	5
6.	18	4	4	14	5	9	8
7.	17	10	7	10	10	7	6
8.	13	8	5	6	16	11	3
9.	11	10	3	16	13	15	4
10.	20	12	4	13	5	7	3
11.	18	12	5	18	17	6	3
12.	12	12	3	9	12	5	4
13.	18	4	4	14	12	16	5
14.	10	7	4	14	12	9	17
15.	15	10	5	18	13	13	3
AVERAGE	15.4	8.53	4.67	14.3	10.5	10.6	5.6

Table 2: Standard Deviation Values for Radicle Length (Pilot study - Day 1 - 6th august)

Condition (Spice/Herb)	**Standard deviation values for radicle length**
Gooseberry	3.009144
Cinnamon	2.774029
Clove	1.397276
Garlic	3.696846
Tulsi	4.549202
Turmeric	3.680062
Control	3.54159

Table 3: Length of Radicle and Plumule In Different Herbs/Spices Extracts (Pilot Study - Day 2 - 8th August)

Key: RL → Radicle Length in mm
PL → Plumule Length in mm

	Gooseberry		Cinnamon	Clove	Garlic		Tulsi		Turmeric		Control	
Seed Number	RL	PL	RL	RL	RL	PL	RL	PL	RL	PL	RL	PL
1.	31	8	11	8	17	2	59	10	27	3	14	5
2.	24	3	14	7	21	7	46	10	24	6	5	1
3.	33	2	10	5	25	9	43	8	20	2	10	7
4.	34	12	7	6	35	9	29	11	25	4	7	1
5.	28	5	8	4	17	3	21	15	28	5	4	6
6.	32	25	9	8	33	7	56	10	35	6	9	2
7.	38	12	13	7	13	1	55	10	50	11	5	2
8.	40	7	5	9	22	3	49	7	26	8	7	-
9.	50	13	7	6	35	5	34	1	41	11	9	-
10.	46	10	8	3	39	7	40	8	30	6	19	-
11.	33	6	10	6	8	2	31	7	31	5	2	-
12.	51	10	9	13	27	6	37	7	17	9	4	-
13.	43	10	6	5	27	5	39	5	50	10	3	-
14.	33	10	6	2	17	2	35	5	16	3	4	-
15.	33	9	10	2	18	2	38	7	20	7	7	-
AVERAGE	36.6	9.5	8.67	5.47	23.6	4.7	40.8	8.1	29.3	6.4	7.6	1.6

Table 4: Standard Deviation Values (Radicle and Plumule lengths) (Pilot Study - Day 2 - 8th August)

Extract	Standard deviation values for radicle length	Standard deviation values for plumule length
Gooseberry	7.899367063	5.570724103
Cinnamon	2.559761894	-
Clove	2.865226659	-
Garlic	9.014274394	2.716790824
Tulsi	10.67841615	3.217511609
Turmeric	10.67484805	2.898275349
Control	4.511361319	2.507132682

EXPERIMENTAL DATA

Table 5: Radicle Length (Replicate 1 - Day 1 - 13th August)

	RADICLE LENGTH in Different Spice Extracts / mm						
Seed Number	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
1.	26	10	-	45	52	20	30
2.	29	6	-	28	32	28	38
3.	12	4	-	15	32	21	23
4.	13	5	-	36	36	20	42
5.	12	8	-	33	33	14	43
6.	27	7	-	31	31	8	16
7.	16	10	-	32	30	20	41
8.	9	7	-	38	33	24	34
9.	13	10	-	26	38	21	31
10.	19	5	-	35	35	24	35
11.	18	9	-	32	38	21	7
12.	14	5	-	12	37	10	35

13.	12	5	-	39	28	6	37
14.	9	7	-	40	33	24	41
15.	6	8	-	35	38	20	18
AVERAGE	15.7	7.1	-	31.8	35.1	18.7	31.4
Standard deviation	6.9144431	2.0517124 09	-	8.84146 0771	5.61206 184	6.3635868 89	10.769003 14

Table 6: Number of Germinated Seeds- Replicate 2 - 24th August (DAY 1)

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
No. of Germinated Seeds	50	26	10	40	48	47	49
No. of Non-Germinated seeds	0	24	40	10	2	3	1
Percentage of Germinated Seeds	100	52	20	80	96	94	98

Table 7: Radicle Length Replicate 2- (Day 1)

	RADICLE LENGTH / mm						
Seed Number	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
1.	10	3	9	9	17	4	15
2.	9	3	2	9	7	5	18
3.	3	2	2	5	11	8	18
4.	13	2	2	4	10	12	15
5.	9	3	2	2	16	5	12
6.	11	2	2	11	3	5	15
7.	14	6	1	5	2	17	15
8.	3	3	8	4	21	7	11
9.	10	2	2	17	21	14	3
10.	7	3	1	8	6	20	2
11.	13	9	1	7	2	15	4
12.	3	5	0	9	5	7	15
13.	4	6	0	10	6	4	15
14.	12	3	0	18	2	5	22
15.	9	1	0	7	8	20	4
AVERAGE	8.7	3.5	2.1	8.3	9.1	9.9	12.3

Table 8: Standard Deviation Values - Radicle Length - Day 1

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
Standard deviation	3.84831441 1	2.099886618	2.72204 405	4.498677 054	6.6961 2539	5.9023804 72	6.181385 266

Table 9: Number of Germinated Seeds (Replicate 2 - 25th August - Day 2)

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
No. of Germinated Seeds	50	50	21	49	49	50	49
No. of Non-Germinated seeds	0	0	29	1	1	0	1
Percentage of Germinated Seeds	100	100	42	98	98	100	98

Table 10: Radicle Length - Replicate 2 - (Day 2)

	RADICLE LENGTH / mm						
Seed Number	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
Germinated	50	50	21	49	49	50	49
1.	18	12	2	15	19	29	31
2.	19	9	4	19	12	20	5
3.	28	5	4	20	28	37	30
4.	21	9	5	24	25	15	22
5.	20	14	7	18	19	6	26
6.	25	7	1	20	34	6	35
7.	25	12	7	20	29	22	11
8.	20	15	4	17	26	10	5
9.	19	15	4	15	15	16	18
10.	11	12	3	14	18	13	15
11.	22	15	2	19	12	3	22
12.	12	10	3	11	18	30	15
13.	20	5	2	16	24	18	15
14.	23	9	3	17	26	14	22
15.	23	6	5	17	24	28	28
AVERAGE	22.2	13.7	5.1	20.7	25.2	21.1	23.3

Table 11: Standard Deviation Values (Radicle Length - Day 2)

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
Standard deviation	4.516635916	3.57903950 9	1.75119 0072	3.11371 7729	6.41946 4449	9.915356 056	9.133924 208

Table 12: Number of Germinated Seeds (Replicate 2-26th August - Day 3)

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
No. of Germinated Seeds	50	50	27	49	49	50	49
No. of Non-Germinated seeds	0	0	23	0	0	0	0
Percentage of Germinated Seeds	100	100	54	100	100	100	100

Table 13: Number of Seeds Showing Radicle / Plumule Growth(26th August -DAY 3**)**

	Gooseberry	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**
Number Of Seeds With Radicle	50	50	27	50	50	50	50
Number Of Seeds With Plumule	17	10	0	14	12	13	26

Table 14: Length of Radicle and Plumule (26th August-Day 3)
Key: RL → Radicle Length in mm
PL → Plumule Length in mm

	RADICLE AND PLUMULE LENGTH OF SEEDS /mm												
	Gooseberry		Cinnamon		Clove	Garlic		Tulsi		Turmeric		Control	
Seed Number	RL	PL	RL	PL	RL	RL	PL	RL	PL	RL	PL	RL	PL
1.	42	5	21	2	15	20	2	27	4	49	3	31	5
2.	38	8	21	2	7	30	4	35	4	4	2	18	3
3.	35	7	18	3	3	35	5	32	8	41	4	16	4
4.	34	6	25	2	6	37	2	8	3	36	1	32	5
5.	21	5	20	1	13	9	3	35	4	43	1	26	8
6.	23	6	28	3	3	40	2	34	5	31	5	27	5
7.	28	5	18	3	2	24	2	40	6	50	6	59	5
8.	33	7	19	2	5	36	2	31	3	29	1	30	5
9.	30	5	19	3	17	33	3	26	3	28	1	21	2
10.	32	2	25	3	5	36	5	18	2	34	2	37	2
11.	35	1	21	-	5	28	2	25	3	44	3	28	3
12.	30	5	11	-	10	27	4	35	5	36	3	16	5
13.	25	1	18	-	2	35	3	36	-	13	2	20	6
14.	16	2	21	-	4	23	2	11	-	31	-	16	6
15.	21	3	10	-	6	11	-	10	-	45	-	27	7
AVERAGE	29.5	4.5	19.7	1.6	6.47	28.3	2.7	26.7	3.3	34.3	2.3	26.9	4.7

Table 15: Standard Deviation Values (Radicle and Plumule Length - Day 3)

Extract	Standard deviation values for radicle length	Standard deviation values for plumule length
Gooseberry	7.170044	2.231805
Cinnamon	4.715728	0.699206
Clove	4.73387	-
Garlic	9.391993	1.141139
Tulsi	10.43255	1.642245
Turmeric	12.70246	1.609268
Control	11.03544	1.709915

TEST ON ONIONS

Table 16: Number of New Roots on Onion After soaking in Spice Extracts for 48 Hours

Extract name	Number of new growths observed in onion roots (White growths) (Taken on 26th August)
Gooseberry	1
Cinnamon	1
Clove	0
Control	3
Garlic	3
Tulsi	39
Turmeric	26

Table 17: Mass of Seeds (Replicate-1-Day 1- 11th August)

Extract name	Mass of seeds before soaking/ g
Gooseberry	3.18
Cinnamon	3.23
Clove	3.29
Control	3.29
Garlic	3.11
Tulsi	3.20
Turmeric	3.24

Table 18: Mass of Germinated Seeds (Replicate-1-Day 2- 12th August)

Extract name	Mass of germinated seeds/ g
Gooseberry	16.520
Cinnamon	15.680
Clove	15.440
Control	6.240
Garlic	13.650
Tulsi	6.920
Turmeric	13.080

Table 19: Dry Mass of 3 Seeds (Replicate - 1 - Day 3 – 13th August)

Extract Name	Average Dry mass of 3 seeds/ g
Gooseberry	0.260
Cinnamon	0.210
Clove	0.210
Control	0.230
Garlic	0.220
Tulsi	0.290
Turmeric	0.250

OBSERVATION OF ONION ROOT GROWTH IN SPICE EXTRACTS

The onions placed in spice extracts showed substantial root growth. Control and Tulsi showed the most significant root growth, followed by Turmeric, Cinnamon and Gooseberry. The least growth was observed in Garlic and Clove. According to our observations, Tulsi boosted root growth in Onion, while Clove inhibited growth. This supports the trend of other tests where Clove inhibited cell proliferation and growth. (**Figure 3**)

BIURET TEST

Extracts	Colour Observed	Protein Content
Gooseberry	Purple	Moderate
Garlic	Dark Purple	High
Control	Brownish Purple	Moderate
Tulsi	Brownish Purple	Moderate
Turmeric	Yellowish Brown (Slightly Blue)	Low or None
Cinnamon	Violet	Moderate
Clove	Brownish Purple	Moderate

DCPIP Test (Replicate 1)

DCPIP test with 1 cm³ of 0.1% w/v DCPIP:

Extract	Colour Observed	Concentration of Antioxidants
Gooseberry	Colourless	Highest
Turmeric	Green	High
Garlic	Blue	None
Tulsi	Dark Blue	None
Clove	Brown	High
Control	Magenta	None
Cinnamon	Maroon	Moderate

DCPIP Test (Replicate 2)

DCPIP test with 2 cm³ of 0.1% w/v DCPIP:

Similar results as that for the DCPIP test with 1cm³ of DCPIP. However, the colours are darker and Gooseberry Solution also turned pink.

DCPIP TEST with 2 cm^3 of 0.1 % w/v DCPIP in Ethanol based extracts (REPLICATE 2)

Extract	Colour Observed	Concentration Of Antioxidants
Gooseberry	Bright yellow	Highest
Garlic	Clear, light purple	Low
Control	Blue	Lowest
Tulsi	Deep maroon	Moderate
Turmeric	Maroon	Moderate
Cinnamon	Pinkish-red shade	Moderate
Clove	Light yellowish - brown	High

Salkowski Test (Replicate 1)

Extract	Colour Observed	Auxin Concentration
Tulsi, Clove, Gooseberry, Cinnamon, Garlic, Turmeric, Control	Pale brown shade	Likely negative or inconclusive result.

Salkowski Test (Replicate 2)

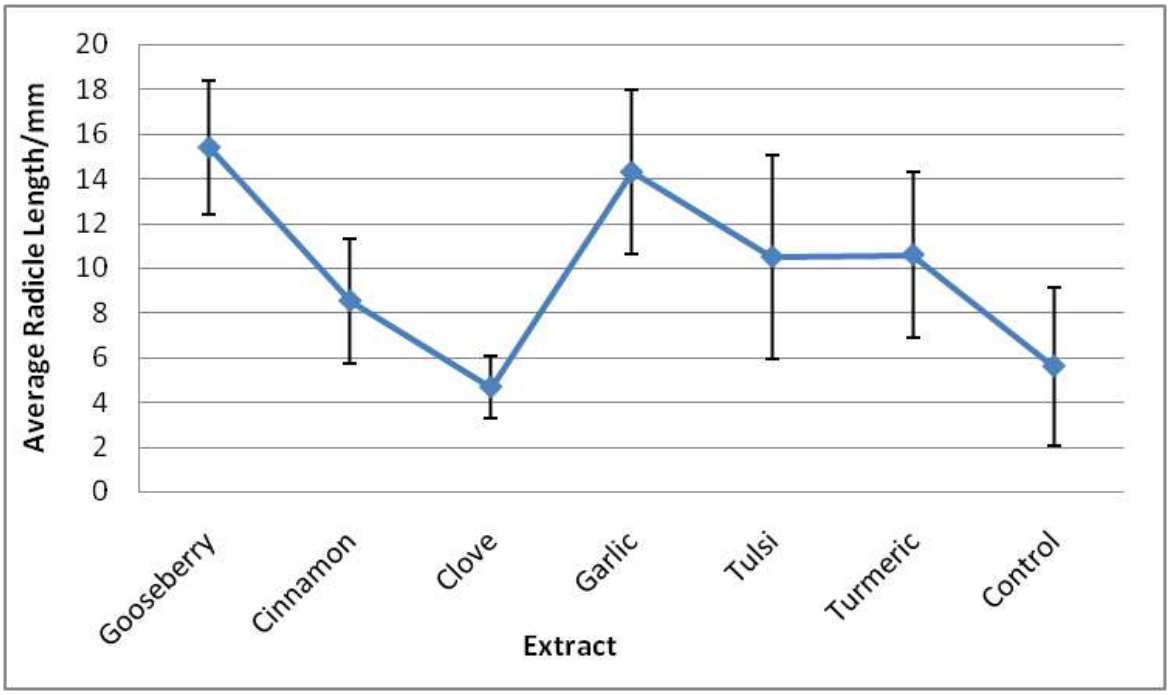
Extract	Colour Observed	Auxin Concentration
Tulsi, Control, Garlic	Yellowish-brow	Likely negative or inconclusive result.
Clove, Gooseberry, Turmeric	Darker yellowish-brown	Likely negative or inconclusive result.
Cinnamon	Yellow	Likely negative or inconclusive result.

Salkowski Test (Replicate 3)

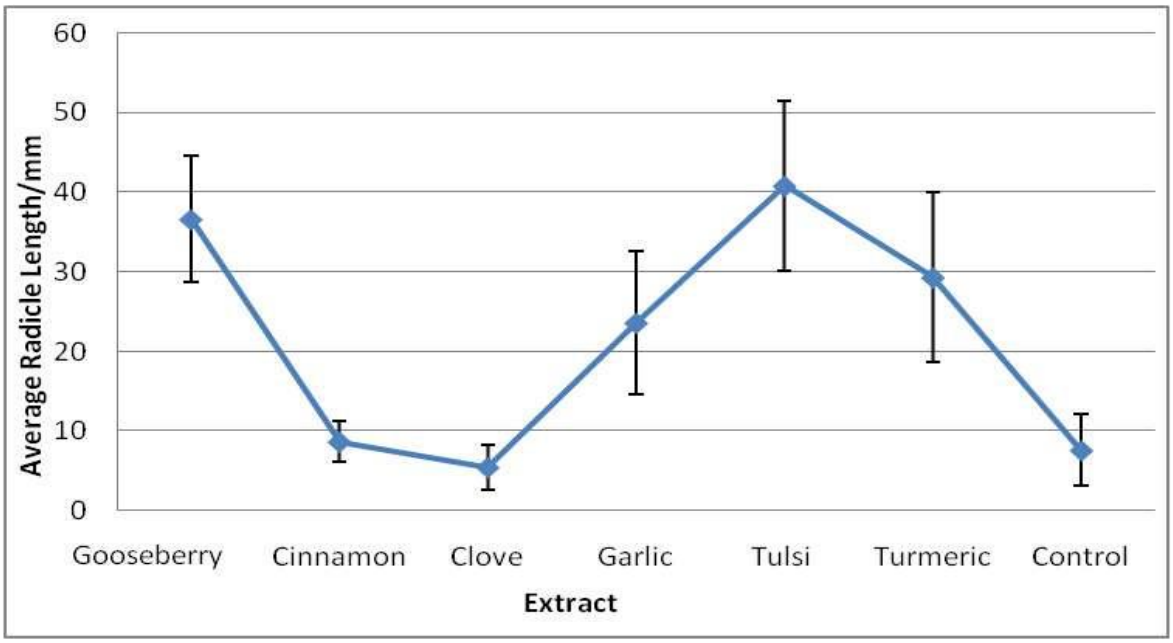
Extract	Colour Observed	Auxin Concentration
Tulsi	Light shade of amber	Likely negative or very low indole concentration
Clove	Amber	Likely negative or very low indole concentration
Gooseberry	Dark amber	Clear positive
Cinnamon	Pale Yellow	Negative
Garlic	Pale Yellow	Negative
Turmeric	Pale Yellow	Negative
Control	Pale Brownish-Yellow	Negative

GRAPHS

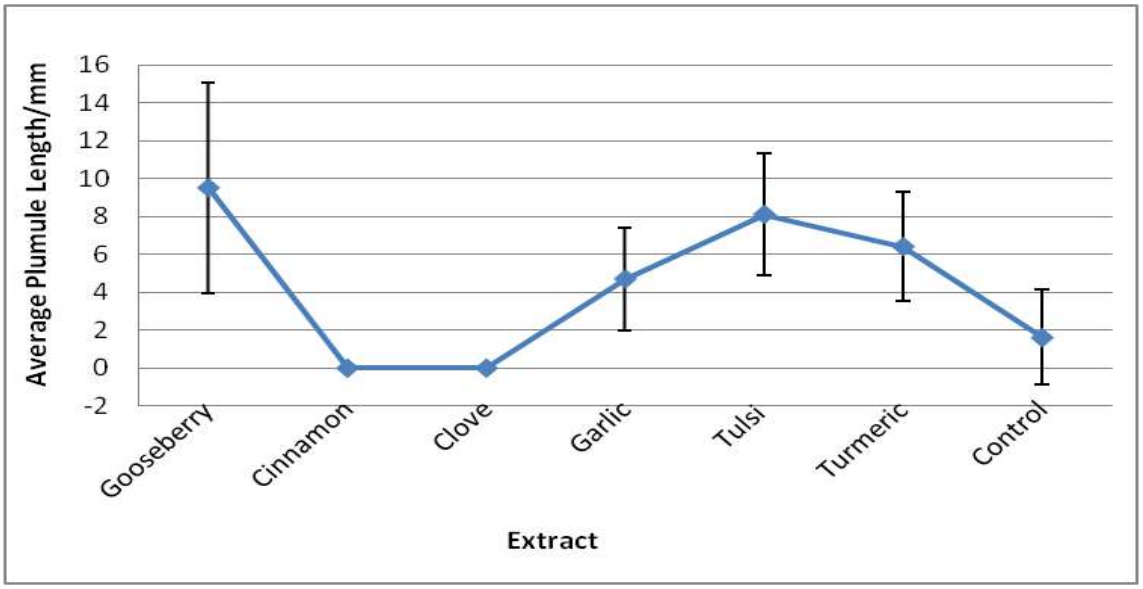
Graph 1: Average Length of Radicle (Pilot Study- Day 1-6th August)



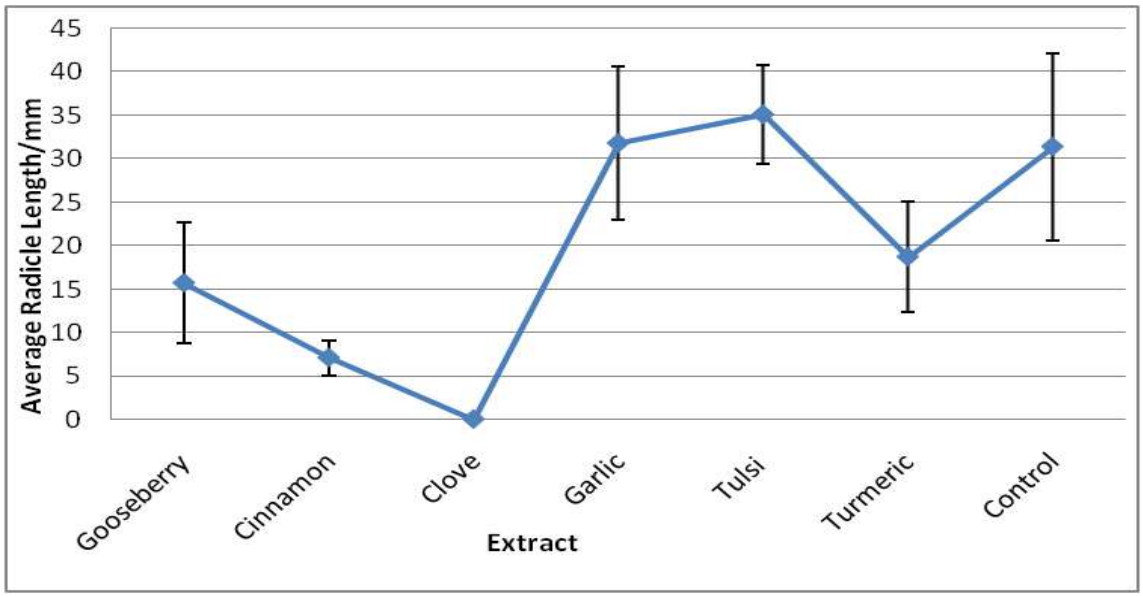
Graph 2: Average Length of Radicle (Pilot Study - Day 2-8th August)



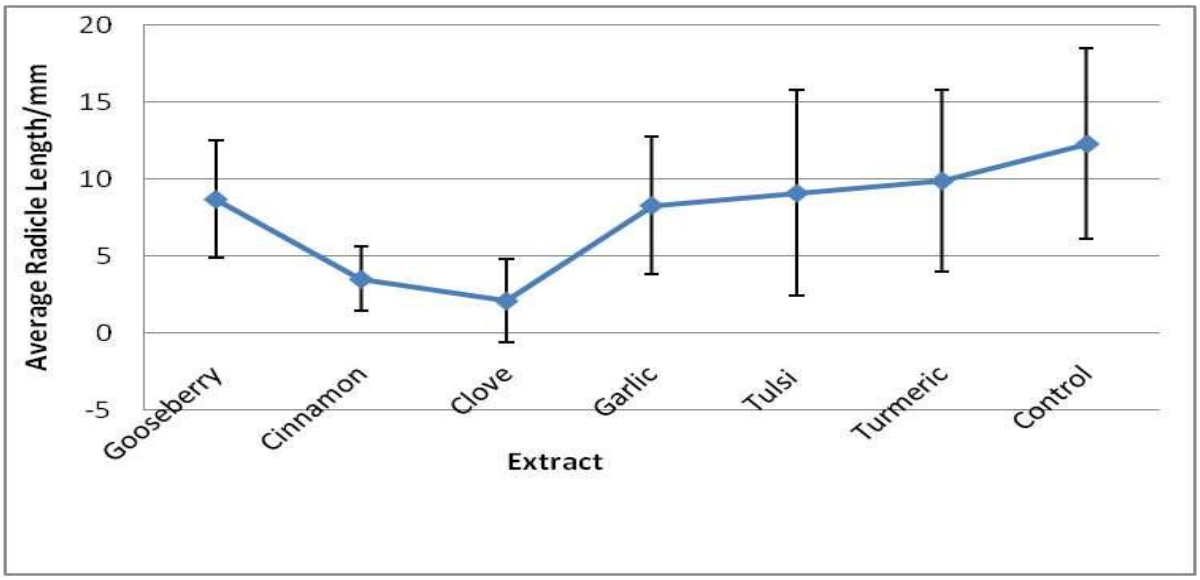
Graph 3: Average Length of Plumule (Pilot Study - Day 2)



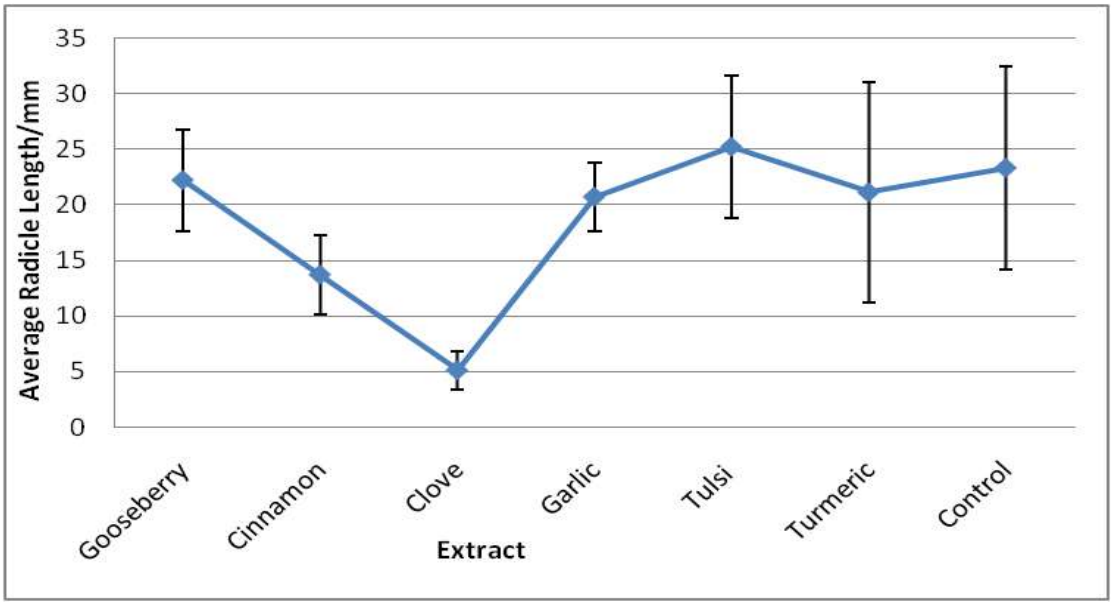
Graph 4: Average Length of Radicle (Replicate 1 - Day 3 -13th August)



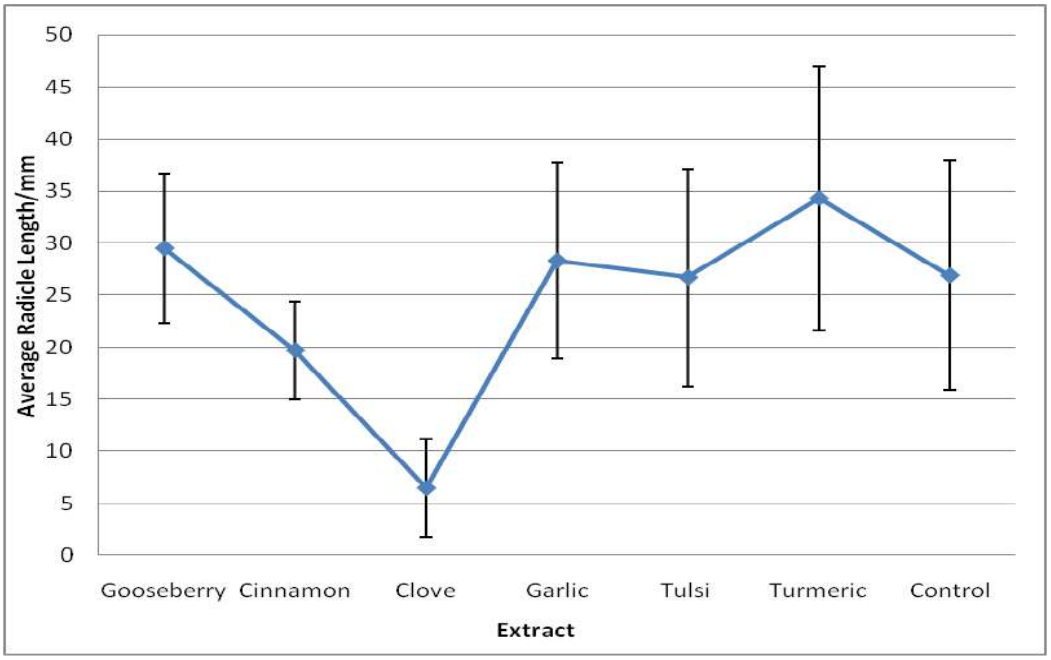
Graph 5: Average Length of Radicle (Replicate - 2 - Day 1 - 24th August)



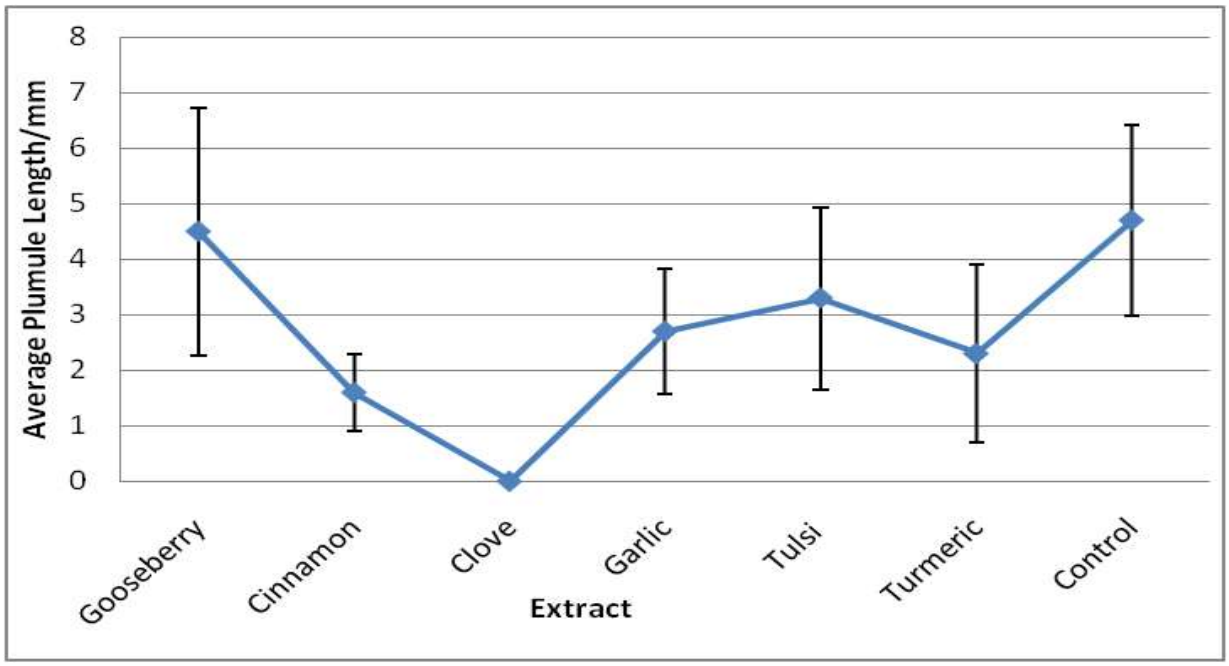
Graph 6 - Average Length of Radicle (Replicate 2- Day 2- 25th August)



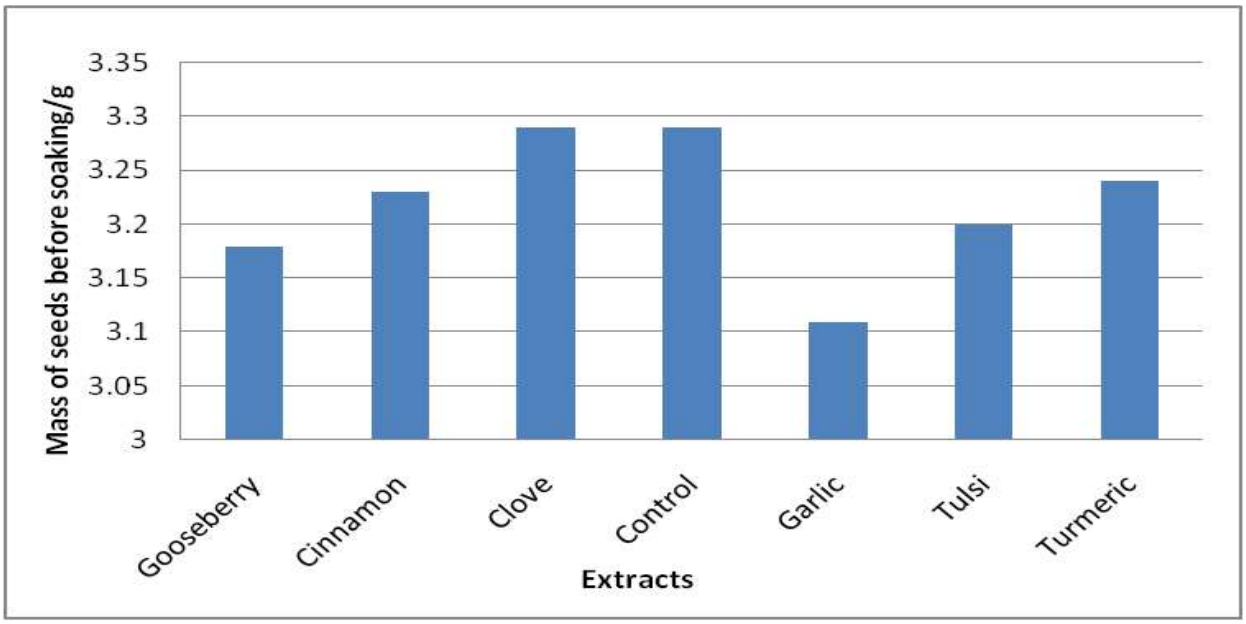
Graph 7: Average Length of Radicle (Replicate 2- Day 3- 26th August)



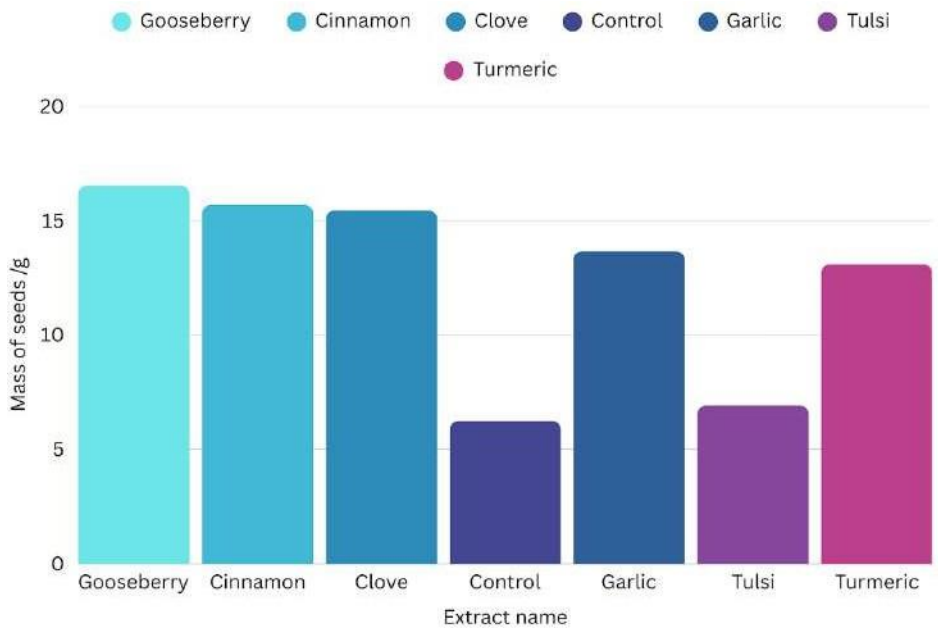
Graph 8: Average Length of Plumule (Replicate-2-Day 3-26th August)



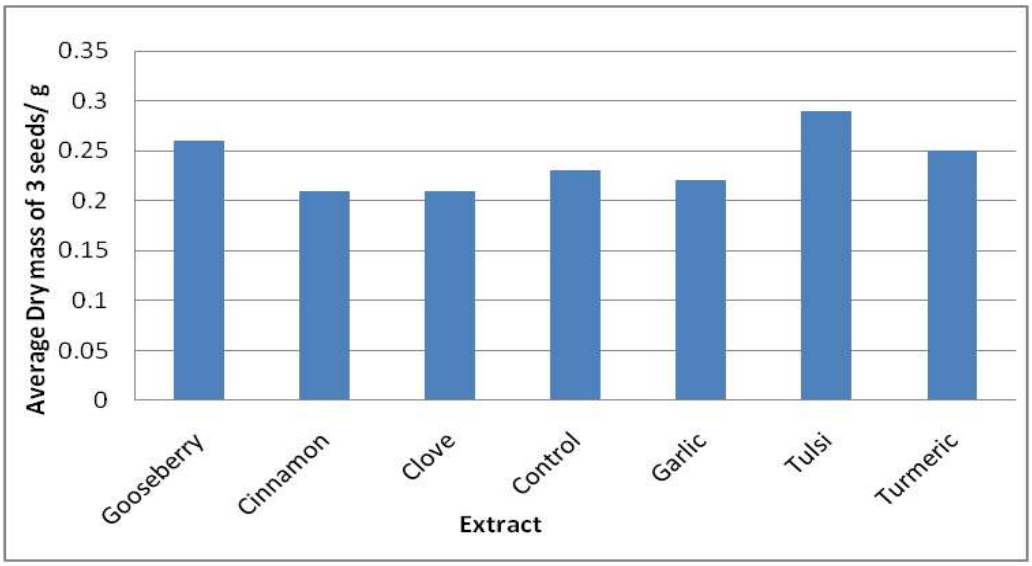
Graph 9: Mass of seeds before soaking 3 g of seeds per extract (Replicate 1 - 11th August)



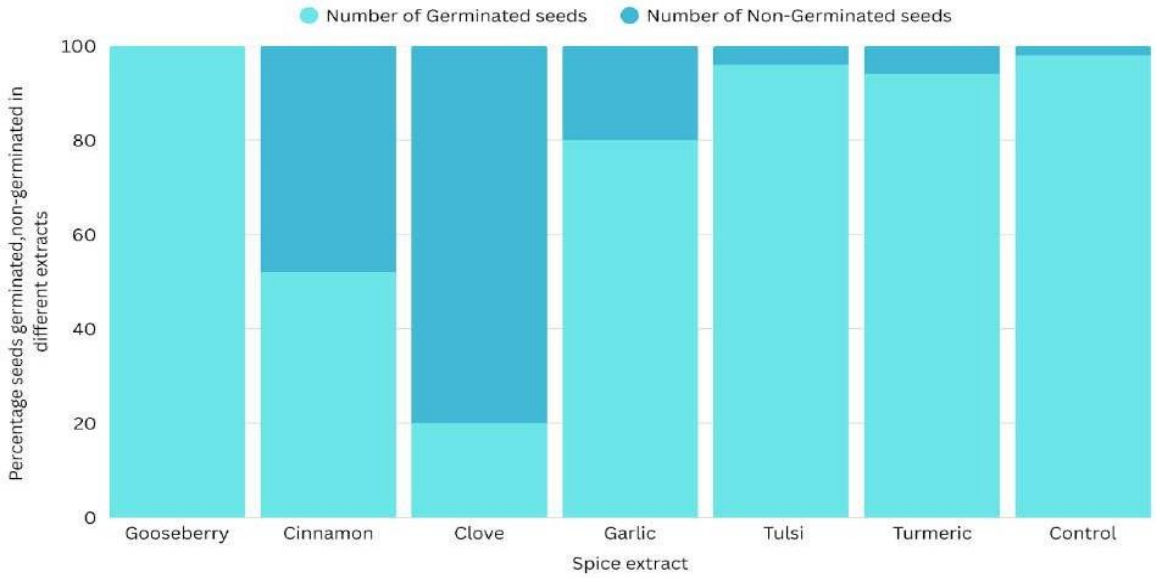
Graph 10: Mass of 50 Seeds After Germination (Replicate 1 -Day 2- 12th August)



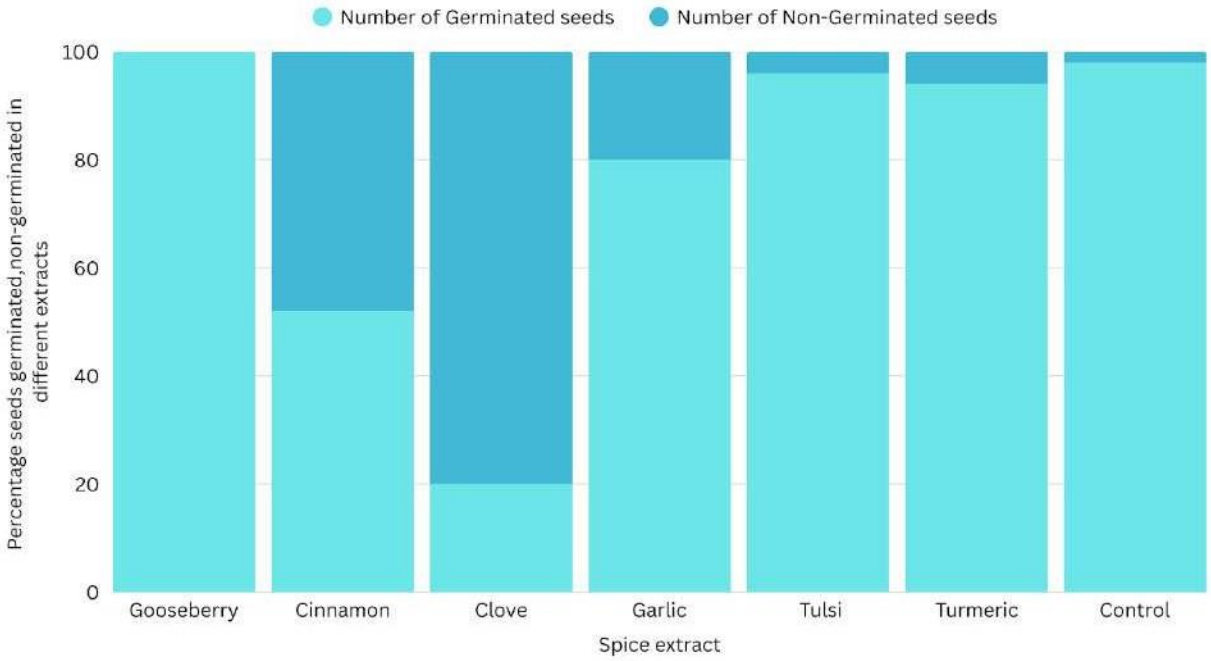
Graph 11: Dry Mass of 3 Seeds (Replicate 1 -Day 3-13th August



Graph 12: Percentage of Germinated, Non- Germinated Seeds (Replicate 2 -Day 1- 24th August)



Graph 13: Percentage of Germinated, Non-Germinated Seeds (Replicate 2 - Day 2- 25th August)



STATISTICAL TEST (ANOVA AND TUKEY'S HSD)

Method used for carrying ANOVA:

Single Factor test:

i. Using the Data Analysis Toolpak add-in in Excel, we conducted a single-factor ANOVA test on Microsoft Excel.
ii. Selected ANOVA: Single Factor (Single Factor was used as we had one independent and one dependent variable) from the Data Analysis menu to perform the statistical test.
iii. The output was then evaluated, which included the P-value.
iv. Statistical guidelines given below were used to interpret the results and determine the significance.

P-value	Significance level
≤ 0.01	Very strong
≤ 0.05	Strong
> 0.05	Weak or none

Evaluating P- value: **A p-value less than or equal to the significance level (typically ≤ 0.05) is statistically significant.** Meaning the observed data provide strong evidence. (Reference)

Method used for carrying out Tukey's HSD:

i. An online calculator was used to do the **Tukey's Honest Significant Difference (HSD) test** (Reference) to analyze which spice extract affected radicle and plumule length significantly.
ii. The data that was fed in included 15 individual measurements, each of **radicle length** and **plumule length** measured per day for every spice treatment.
iii. The factor '**k**' was set as **7**, representing the seven extracts added: Gooseberry, Cinnamon, Clove, Garlic, Tulsi, Turmeric, and Control.

Anova: Single Factor Test

Table 20: ANOVA Test Result for Radicle Length (Replicate 1-13th August)

Groups	Count	Sum	Average	Variance
Gooseberry	15	235	15.7	47.80952381
Cinnamon	15	106	7.1	4.20952381
Clove	15	0	0.0	0.0
Tulsi	15	477	31.8	78.17142857
Turmeric	15	526	35.1	31.4952381
Garlic	15	281	18.7	40.4952381
Control	15	471	31.4	115.9714286

Table 21: ANOVA Test Result for Radicle Length (Replicate 1-13th August)

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	16257.71429	6	2709.619048	59.61713465	1.5654E-30	2.192517789
Within Groups	4454.133333	98	45.45034014			
Total	20711.84762	104				

One-way ANOVA of your k=7 independent treatments:

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	16,257.7143	6	2,709.6190	59.6171	1.1102e-16
error	4,454.1333	98	45.4503		
total	20,711.8476	104			

Table 22: Tukey HSD results for Radicle Length (Replicate 1-13th august)

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Gooseberry vs Cinnamon	4.9406	0.0122981	* p<0.05
Gooseberry vs Clove	9.0002	0.0010053	** p<0.01
Gooseberry vs Garlic	9.2683	0.0010053	** p<0.01
Gooseberry vs Tulsi	11.1450	0.0010053	** p<0.01
Gooseberry vs Turmeric	1.7617	0.8643734	insignificant
Gooseberry vs Control	9.0385	0.0010053	** p<0.01
Cinnamon vs Clove	4.0597	0.0721838	insignificant
Cinnamon vs Garlic	14.2089	0.0010053	** p<0.01
Cinnamon vs Tulsi	16.0855	0.0010053	** p<0.01
Cinnamon vs Turmeric	6.7023	0.0010053	** p<0.01
Cinnamon vs Control	13.9791	0.0010053	** p<0.01
Clove vs Garlic	18.2686	0.0010053	** p<0.01
Clove vs Tulsi	20.1452	0.0010053	** p<0.01
Clove vs Turmeric	10.7620	0.0010053	** p<0.01
Clove vs Control	18.0388	0.0010053	** p<0.01
Garlic vs Tulsi	1.8766	0.8174453	insignificant
Garlic vs Turmeric	7.5066	0.0010053	** p<0.01
Garlic vs Control	0.2298	0.8999947	insignificant
Tulsi vs Turmeric	9.3832	0.0010053	** p<0.01
Tulsi vs Control	2.1064	0.7235945	insignificant
Turmeric vs Control	7.2768	0.0010053	** p<0.01

RADICLE LENGTH

Anova: Single Factor Test

Table 23: ANOVA Test Results For Radicle Length (Replicate 2-26th august)

Groups	Count	Sum	Average	Variance
Gooseberry	15	443	29.53333333	51.40952381
Cinnamon	15	295	19.66666667	22.23809524
Clove	15	103	6.866666667	22.40952381
Tulsi	15	424	28.26666667	88.20952381
Turmeric	15	403	26.86666667	108.8380952
Garlic	15	514	34.26666667	161.352381
Control	15	404	26.93333333	121.7809524

Table 24: ANOVA Test Result For Radicle Length (Replicate 2-26th august)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	10838.37	7	1548.338	20.77476	1.09E-17	2.092381
Within Groups	8347.333	112	74.52976			
Total	19185.7	119				

One-way ANOVA of your k=7 independent treatments:

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	7,209.1810	6	1,201.5302	14.5959	7.6779e-12
error	8,067.3333	98	82.3197		
total	15,276.5143	104			

KEY:

	A	B	C	D	E	F	G
Spice	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**

Table 25: Tukey HSD results for Radicle Length (Replicate 2-26th august)

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD Inferfence
A vs B	4.2118	0.0545126	insignificant
A vs C	9.6757	0.0010053	** p<0.01
A vs D	0.5407	0.8999947	insignificant
A vs E	1.1383	0.8999947	insignificant
A vs F	2.0205	0.7586898	insignificant
A vs G	1.1099	0.8999947	insignificant
B vs C	5.4639	0.0036734	** p<0.01
B vs D	3.6711	0.1383298	insignificant
B vs E	3.0735	0.3194271	insignificant
B vs F	6.2323	0.0010053	** p<0.01
B vs G	3.1019	0.3086697	insignificant
C vs D	9.1350	0.0010053	** p<0.01
C vs E	8.5374	0.0010053	** p<0.01
C vs F	11.6962	0.0010053	** p<0.01
C vs G	8.5658	0.0010053	** p<0.01
D vs E	0.5976	0.8999947	insignificant
D vs F	2.5612	0.5378506	insignificant
D vs G	0.5692	0.8999947	insignificant
E vs F	3.1588	0.2875930	insignificant
E vs G	0.0285	0.8999947	insignificant
F vs G	3.1304	0.2980543	insignificant

PLUMULE LENGTH:

Anova: Single Factor Test

Table 26: ANOVA Test Result For Plumule Length (Replicate 2-26th august)

Extracts	Count	Sum	Average	Variance
Gooseberry	15	68	4.53	4.98
Cinnamon	15	24	1.6	1.69
Clove	15	0	0	0
Tulsi	15	50	3.33	5.1
Turmeric	15	34	2.27	3.07
Garlic	15	41	2.73	1.78
Control	15	71	4.73	9.92

Table 27: ANOVA Test Results for Plumule Length (Replicate 2-26th august)

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	248.5904762	6	41.43174603	14.8475 5404	5.25135E-12	2.192517789
Within Groups	273.4667	98	2.790476			
Total	522.0571429	104				

One-way ANOVA of your k=7 independent treatments:

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	248.5905	6	41.4317	14.8476	5.2514e-12
error	273.4667	98	2.7905		
total	522.0571	104			

KEY:

	A	**B**	**C**	**D**	**E**	**F**	**G**
Spice	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**

Table 28 - Tukey HSD results for Plumule Length (Replicate 2-26th august)

treatments pair	**Tukey HSD Q statistic**	**Tukey HSD p-value**	**Tukey HSD inferfence**
A vs B	6.8009	0.0010053	** p<0.01
A vs C	10.5105	0.0010053	** p<0.01
A vs D	4.1733	0.0585978	insignificant
A vs E	2.7822	0.4440874	insignificant
A vs F	5.2553	0.0060265	** p<0.01
A vs G	0.4637	0.8999947	insignificant
B vs C	3.7096	0.1300097	insignificant
B vs D	2.6276	0.5107228	insignificant
B vs E	4.0187	0.0776635	insignificant
B vs F	1.5457	0.8999947	insignificant
B vs G	7.2646	0.0010053	** p<0.01
C vs D	6.3372	0.0010053	** p<0.01
C vs E	7.7283	0.0010053	** p<0.01
C vs F	5.2553	0.0060265	** p<0.01
C vs G	10.9742	0.0010053	** p<0.01
D vs E	1.3911	0.8999947	insignificant
D vs F	1.0820	0.8999947	insignificant
D vs G	4.6370	0.0235486	* p<0.05
E vs F	2.4731	0.5738520	insignificant
E vs G	3.2459	0.2568013	insignificant
F vs G	5.7190	0.0019685	** p<0.01

NUMBER OF SEEDS THAT HAVE GERMINATED OVER 3 DAYS: ANOVA TEST

Table 29: ANOVA Test Result For Number Of Germinated Seeds (Replicate 2)

SUMMARY			
Groups	**Count**	**Sum**	**Average**
Gooseberry	3	150	50
Cinnamon	3	126	42
Clove	3	58	19
Garlic	3	138	46
Tulsi	3	146	49
Turmeric	3	147	49
Control	3	147	49

Table 30: ANOVA Test Result For Number Of Germinated Seeds (Replicate 2)

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2165.81	6	360.9683	8.517228	0.000505	2.8477 26
Within Groups	593.3333	14	42.38095			
Total	2759.143	20				

One-way ANOVA of your k=7 independent treatments:

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	2,165.8095	6	360.9683	8.5172	0.0005
error	593.3333	14	42.3810		
total	2,759.1429	20			

KEY:

	A	**B**	**C**	**D**	**E**	**F**	**G**
Spice	**Gooseberry**	**Cinnamon**	**Clove**	**Garlic**	**Tulsi**	**Turmeric**	**Control**

Table 31: Tukey HSD results for Number Of Germinated Seeds

treatments pair	**Tukey HSD Q statistic**	**Tukey HSD p-value**	**Tukey HSD inferfence**
A vs B	2.1285	0.7159410	insignificant
A vs C	8.1591	0.0010053	** p<0.01
A vs D	1.0642	0.8999947	insignificant
A vs E	0.3547	0.8999947	insignificant
A vs F	0.2661	0.8999947	insignificant
A vs G	0.2661	0.8999947	insignificant
B vs C	6.0306	0.0107304	* p<0.05
B vs D	1.0642	0.8999947	insignificant
B vs E	1.7737	0.8469899	insignificant
B vs F	1.8624	0.8142293	insignificant
B vs G	1.8624	0.8142293	insignificant
C vs D	7.0949	0.0027610	** p<0.01
C vs E	7.8043	0.0011439	** p<0.01

C vs F	7.8930	0.0010276	** p<0.01
C vs G	7.8930	0.0010276	** p<0.01
D vs E	0.7095	0.8999947	insignificant
D vs F	0.7982	0.8999947	insignificant
D vs G	0.7982	0.8999947	insignificant
E vs F	0.0887	0.8999947	insignificant
E vs G	0.0887	0.8999947	insignificant
F vs G	0.0000	0.8999947	insignificant

EVALUATION

Our investigation successfully demonstrated measurable differences in the rate of germination and early growth patterns of *Vigna radiata* when grown in aqueous extracts of various Indian spices (Figure 2). By combining multiple measurable parameters such as the rate of germination (Graph 12), radicle and plumule growth (Table 14), auxin detection through Salkowski tests (Figure 17), protein detection and concentration through biuret test (Figure 6), and qualitative microscopic observations of the root tip along with the presence of antioxidants using DCPIP (Figure 11).

Our investigation provides a broad understanding of how different phytochemical profiles can influence cell proliferation, germination and seedling growth.

Multiple aspects of the design of our experiment strengthened the reliability of the results.

All *Vigna radiata* seeds were procured from a singular source to minimise the initial genetic variability. The spice extract concentrations were kept constant across applications of extract, and all petri dishes were placed in the same environment to reduce differences in our results due to environmental factors. The use of multiple biochemical colour-based assays (DCPIP, Salkowski, Biuret) added an extra parameter of physical growth measurements, allowing for relationships between the composition of extract and the observed growth of *Vigna radiata* seedlings to be inferred.

A number of factors have a probable chance of influencing the accuracy and consistency of our investigation's findings.

Although all the *Vigna radiata* seeds, as well as spices and herbs, were procured from the same brand and packaging, they were not identical in their genetic structure. The indistinct caused by these genetic variations may have led to differences in the overall health of the seed and germinating plant, natural reserves of nutrients, or the allele controlling the innate ability to germinate. This genetic variation could have led to variations in growth, affecting the results of our experiment.

Petri dishes (Figure 2) were used for the storage of the *Vigna radiata* seeds and were placed in close proximity to one another, yet there was no precise, explicit control over various factors such as temperature, humidity, or draught. These minor environmental shifts influence the rate of germination of the *Vigna radiata* seeds, and the development of the recently germinated seedlings. Windows of the room were closed throughout and room temperature was maintained, in spite of these, there may have been small fluctuations beyond our control.

We faced many difficulties while measuring radicle and plumule growth using available apparatus (ruler). Many radicles curved as they grew, which made it a challenge to line the ruler up against the radicle of *Vigna radiata* seedlings.These manual measurements introduced near negligible errors, with these small errors (+- 0.1 mm) compounding over many measurements, may have affected the reliability of readings. To eliminate anomaly 15 seeds were picked randomly from each extract sample and their radicle, plumule lengths were measured during the investigation.

The preparation of spice extracts also leads to another layer of variability and discrepancy. Each spice extract (Figure 1) was prepared by mixing fixed quantities of plant material with distilled water, but the chemical composition of these spices could not be homogenized.

Growth of fungus and mold was occasionally observed in petri dishes after a few days. This could have interfered with the development of *Vigna radiata* seeds and lead to a decrease in the number of reliable data points, observed in the fungal infested petri dishes. Profuse fungal growth was observed during pilot study and hence did not allow data collection beyond day 2. Petri dishes were covered during Replicates to take care of the error, hence data could be collected for up to 5 days. This could have been prevented throughout, by sterilizing all apparatus using Autoclave and surfaces with 70% absolute ethanol. Unavailability of Autoclave did not allow us to take care of this anomaly.

Our entire experiment was conducted over a short observation period. While the selected time frame was enough for the observation of early germination and growth, it did not allow for the detection of effects that may emerge, over a longer term and be displayed in the *Vigna radiata* seedlings over time. Extended monitoring over a longer period of time would have allowed for the appropriate classification of these effects.

The biochemical tests led to another layer of variability in our results. Colour based assays such as Salkowski (Auxin) and DCPIP (Vitamin C) tests were performed thrice however, Biuret test for proteins was performed only once due to our limited time frame.

Along with that, the naturally strong biological pigments in certain extracts, particularly turmeric and clove extracts, interfered with the resultant colour observations with these assays, making it difficult to interpret the results, with a high confidence rating.

Unavailability of scientific instruments for assay readings, such as colorimeters and spectrometers, in our school laboratory, did not allow reliable colour interpretation for qualitative tests. We tried to use online spectrophotometer applications (Spectrometer) (Figure 20) and took multiple images and checked through the above mentioned application,

However, the application detected a wide range of wavelengths for the same tests and the quality of the colours of the images captured by the app were not optimum, so we could not take accurate imaging or find an accurate value.

An improvement for this would be to prepare alternative solvent based extracts (ethanol) which masks the natural pigment of spices, allowing for greater accuracy of results as the resultant colours are no longer affected by these natural pigments.

The microscopic observations of the root tips of *Vigna radiata* seedlings were qualitative rather than quantitative. Because of limited knowledge of the usage of microscope and procedure of slide preparation, no mitotic indexes could be calculated, hence the effects at the cellular level of the extracts couldn't be studied in depth.Inspite of attempting to prepare a slide for onion tip of *Vigna radiata* seed, grown in control. (Figure 19)

Finally, our investigation had a specific focus on water-based extracts, with some key phytochemicals being more soluble in organic solvents, hence the experiment could not have captured the complete range of biological activity present in the spice extracts and its effects on cell proliferation or growth. We could have used other solvents such as ethanol to capture a wider range of phytochemicals released by spice extracts.

The masses of 50 ungerminated *Vigna radiata* seeds were measured as 3 grams. After germination, there was an increase in mass of seeds in all extracts . In our results (Figure 11), we observed that seeds grown in Gooseberry extracts had the most mass increase, followed by Cinnamon and Clove. Seeds in the Garlic and Turmeric extracts showed substantial increase in mass. However, seeds grown in Control and Tulsi samples had very little increase in mass. We notice how the results Cinnamon, Clove, Control and Tulsi contradicts the usual trend observed throughout the other tests. While Cinnamon and Clove should have shown the least mass increase and Tulsi and Control should have shown more, the results are the complete opposite as compared to other tests.

Furthermore, on measuring the dry mass of 3 germinated seeds from every sample, the values obtained were between the range of 0.2 g to 0.3 g. Since a 0.1 g difference in mass is usually negligible, the results obtained are not substantial or conclusive.

For the test of measuring mass of seeds to analyse another growth parameter, there were limitations which did not allow to get any conclusive results or derive a trend. For example, our sample size (3 seeds) was small and we did not conduct a substantial number of replicates. Since our focus lay on the other tests, the mass change factors were not well-considered, we were not successful to get the appropriate results to deduce a logical reasoning.

The Biuret assay detects the presence of peptide bonds, producing a violet or mauve colour when proteins react with copper (II) ions in an alkaline medium. The intensity of this purple shade correlates with the amount of protein present in the sample (Gornall et al., 1949). (Figure 6 and Figure 7)

The garlic sample displayed the most intense violet/mauve colouration, allowing us to infer that the seeds grown in this spice extract contain the relatively highest concentrations of proteins. This is then followed by cinnamon extracts a magnitude paler, allowing us to infer a medium concentration of protein in seeds.This is followed by tulsi, clove then turmeric, with the relatively least concentration of proteins inferred.

These observed differences in colour of biuret assays could be used to draw a correlation between how different phytochemical compositions affect seed metabolism. Essential oils and phenolics in clove and tulsi have been attributed to influencing plant physiological processes such as protein synthesis and nitrogen metabolism during germination (Chou, 1999). A contrast can then be inferred with the pale colour of tulsi and control seeds do not have the required compounds which stimulate the accumulation of proteins to the same extent as clove and tulsi. This allows us to infer that eugenol is responsible for inhibition of growth through the accumulation of proteins, reducing the seedlings actual utilization of accumulated proteins for growth. These results align with the previously published research on allelopathic and phytochemical effects on the physiological aspects of a seedling.

The DCPIP assay detects antioxidants as they act as a reducing agent, reducing the dye. When partially reduced, the solution turns pink. (Figure 9 and Figure 10). When fully reduced, the solution turns colourless or transparent. (Figure 9) The more the colour of the solution fades, the higher the antioxidant activity. The results of the DCPIP Assays in ethanol extracts are provided in this table (DCPIP TEST (REPLICATE 2)). The aqueous extracts are provided in this table (DCPIP TEST (REPLICATE 1)) Eugenol, present in clove extract, displays pro-oxidant and antioxidant activity depending on the concentration of the extract. (Figure 11) It requires 0.3 µmol/dm³ (Cortés-Rojas, de Souza and Oliveira, 2014) concentration to display pro-oxidant activity.

The eugenol concentration we have used being 2359.014 µmol/dm³, extracted from 9 g of cloves, is much greater hence it displays strong pro-oxidant activity, strongly inhibiting seed germination throughout the investigation (Graph 12, Graph 13) along with minimum radicle and plumule growth (Graph 7 and Graph 8). These findings were further checked through ANOVA and turkey HSD statistical tests which strongly support the significance of our results.

The DCPIP assays provided a qualitative comparison of antioxidant activity in different spices and herb extracts. Gooseberry displayed the quickest and most complete decolourization in aqueous extract indicating Gooseberry's strong reducing capacity with its high ascorbic acid content along with the results being backed up by previous research. (Pellegrini et al., 2000). Clove and turmeric also displayed remarkable activity of antioxidants, which is in alignment with the known curcuminoid and phenolic content of these compounds as well as the innate ability of these compounds to act as reducing agents (Lee and Shibamoto, 2001). Cinnamon displayed a very faint shade of blue, indicating several methodological limitations affect the reliability of the observations and results. Intrinsic extract pigments, especially from turmeric and clove, overlap with DCPIP absorbance and could mask the endpoints of the antioxidant observations if blanks and spectrophotometric corrections are not utilized during the testing procedure. (Prior, Wu and Schaich, 2005). Differences in the results between water and absolute ethanol extracts are also as expected (such as garlic), since the solvent significantly influences the organic compounds released by spice extracts and their effects on antioxidants, affecting the results of DCPIP test.

There was a significant difference in radicle, plumule lengths (Table 1 to Table 15 and Graph 1 to Graph 9), and germination percentage (Graph 12 and Graph 13) when *Vigna radiata* seeds were soaked in different spice extracts, and this was proved by the ANOVA statistical tests that we carried out. ANOVA results confirm that these differences were statistically significant for radicle length (Table 21 and Table 24), plumule length (Table 27) as well as germination percentage (Table 30), as for all these growth parameters, the P-values <0.01. Furthermore, Tukey HSD results for the plumule and radicle length showed a strong significant difference for all six extracts along with the control. For the rate of germination, clove shows the most statistically significant difference when compared to all other extracts and control. Gooseberry and Control had a moderate effect. Trends indicate that the phytochemical composition of each extract influenced seedling development. Certain compounds in Tulsi and Turmeric stimulated cell proliferation. Finally, all the results state that Clove and Cinnamon have the most inhibitory effects. The findings support our research aim of evaluating the proliferative and inhibitory effects of selected Indian spices on *Vigna radiata*.

The Salkowski test (Figure 14 to Figure 18) was the qualitative test we used to detect the amount of auxin present in extracts of the seeds, which provided us a clear visual to interpret. Certain limitations of the test also came to light during the procedure. The test demonstrated the highest positive test result for auxin in Gooseberry-treated seeds, consistent with existing research that states that Gooseberry supports a beneficial microbial community (endophytic fungi) (Waqas et al., 2012) which can enhance endogenous auxin production. (Salvi, N.D., Singh, S. and Kumar, S. (2020)).

However, since the method is qualitative, it could generate false positives due to its reactivity with a broad range of indolic and phenolic compounds(Guardado-Fierros et al., 2024) This characteristic is evidenced by the strong response of clove extract which seemed lighter than the Gooseberry-treated seeds, yet darker than the rest, since paradoxically, clove inhibited seed growth in other tests—a phenomenon attributed to the phytotoxic effects of eugenol that disrupts hormonal balance and cell integrity, rather than the promotion of auxin signaling. Other spices such as Turmeric, Garlic and Cinnamon showed moderate to low colour changes, which indicates a negative result, suggesting either lower auxin concentration or greater assay interference.

CONCLUSION

Our findings highlight clear growth modulating effects of spice extracts, particularly clove, which contains many bioactive compounds including eugenol. Past studies on the effect of clove indicate that clove oil could be used as a germination suppressant of potato tubers by affecting the oxidative degradation of lipids and enzyme activity such as Polyphenol Oxidase, Catalase, Glutathione-S-Transferase, Peroxidase along with Superoxide Dismutase and exhibits both antioxidant as well as pro-oxidant activities depending on its concentration (Kozhuharova et al., 2013). Future research scope could be focused on testing purified eugenol and separation of its specific effects from those of the crude extract, allowing us to link observed plant growth responses with the cancer cell proliferation and the utilization of chemical extracts from natural spices for the treatment of cancer.

Research (El-Saber Batiha et al.) mentions that dietary intake of clove as a nutritional supplement, in diabetic rats induced antioxidant enzymes. Furthermore, Cloves being added to regular diet reduced tissue damage in liver, lens and cardiac muscles in mice. Clove is also used in many herbal remedies along with its use to alleviate tooth ache in Indian households.

FDA has approved and confirmed safe dietary usage of clove, in smaller concentrations as higher concentration can cause cytotoxicity. (El-Saber Batiha et al.) Through our research, we clearly proved the role of clove in inhibition of cell proliferation which is backed by multiple scientific researches quoted throughout this report.

APPLICATIONS

Eugenol, a major bioactive compound in clove (*Syzygium aromaticum*), exhibits potent anticancer effects through a multitude of mechanisms. Some benefits are how it stops the cell cycle to inhibit proliferation, restricts angiogenesis essential for tumor growth and it suppresses metastasis by blocking cancer cell migration and invasion. Its anti-inflammatory and antioxidant properties mitigate oncogenesis by regulating immune responses and inflammatory cytokines, as observed in previously conducted research. (Haleem 2025; Begum et al. 2022, Happy Kurnia, Permatasari, et al. 2021). Multiple preclinical studies have demonstrated the anti-proliferatory effects of eugenol for cancers like lung, breast, gastric, prostate, colon, and cervical cancers (Zari, Zari and Hakeem, 2021).

Therefore, to maximize eugenol's potent anti-proliferatory effect, for real-life application, nanotechnology, particularly nanobots (Chavda et al., Khan et al.), could be used as a scalable and effective platform for delivering eugenol precisely to the position of tumors, thereby amplifying therapeutic efficacy while decreasing the toxicity in the system which is common in chemotherapy. The nanobots' unique ability to mold into specific shapes allows targeted drug release, which therefore reduces harmful side effects as compared to usual cancer treatment procedures like chemotherapy, due to eugenol's natural abundance and safety profile.(Abbasi et al.)

Since nanobots are a concept still under development, eugenol extracts could instead be added to monoclonal antibodies that are injected into the body. These monoclonal antibodies would attach to the tumor cells due to their complementary shapes to antigens on tumor cells, and hence would deliver the chemical eugenol directly to target cells. This would start effective action on preventing cancer cell proliferation.

Our experimental evidence, as shown in our tables and figures above, that include measuring radicle and plumule length of seeds, mass of seeds, DCPIP antioxidant tests, Biuret protein assays, and Salkowski auxin detection, confirms clove's superior cell growth inhibitory effects, supporting eugenol's application as a selective anticancer agent. Our experiment's positive results are supported by several published research papers, whose links are provided below in the references. (Begum et al. 2022, Debnath, Anirban, et al).

Furthermore, as mentioned in our evaluation, 9 g of cloves gave us a eugenol concentration of 2359.014 µmol/dm³. Eugenol concentration of 0.3 µmol/dm³ is enough to have pro-oxidant activity and cause cytotoxicity.

9 grams of clove cost Rs. 11 (£ 0.09). If 9 grams of clove can provide 7863 doses of 0.3 µmol/dm³ Eugenol, the cost per dose would be Rs 0.001 (£ 0.00001). This displays the cost-effectiveness of using eugenol from clove as a cell growth inhibitor.

Therefore, we can conclude that eugenol is a much inexpensive bio-product, which is a sustainable choice and a strong compound for potential drug production.

As a whole, eugenol proves to be the best cell proliferation inhibitor that could be used to stop tumor growth at the site by using nanobots or by adding eugenol compounds to monoclonal antibodies. In this manner, cancer treatment would be safer, more cost effective and more accessible.

REFERENCES

[1] Maurya, Dharmendra Kumar, and Deepak Sharma. "Evaluation of Traditional Ayurvedic Kadha for Prevention and Management of the Novel Coronavirus (SARS-CoV-2) Using in Silico Approach." Journal of Biomolecular Structure and Dynamics, vol. 40, no. 9, 30 Nov. 2020, pp. 3949–3964. https://doi.org/10.1080/07391102.2020.1852119

[2] Aggarwal, B.B., Sundaram, C., Malani, N. and Ichikawa, H., 2011. Curcumin: the Indian solid gold. In: The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease. Springer, pp.1–75. https://pubmed.ncbi.nlm.nih.gov/17569205

[3] Butt, M.S. and Sultan, M.T., 2011. Garlic: nature's protection against physiological threats. Critical Reviews in Food Science and Nutrition, 51(6), pp.583–595. https://pubmed.ncbi.nlm.nih.gov/19484634

[4] FAO, 2021. The state of food and agriculture 2021: Making agrifood systems more resilient to shocks and stresses. Rome: Food and Agriculture Organization of the United Nations. Available at: https://www.fao.org/publications/sofa/2021/en/ [Accessed 1 Aug. 2025].

[5] Gupta, S.C., Patchva, S. and Aggarwal, B.B., 2013. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS Journal, 15(1), pp.195–218. https://pubmed.ncbi.nlm.nih.gov/23143785

[6] Hosseini, A., Hosseinzadeh, H. and Najafi, H., 2015. The effect of garlic extract on cell proliferation and apoptosis of human cancer cell lines: a review. Iranian Journal of Basic Medical Sciences, 18(6), pp.524–530. https://pubmed.ncbi.nlm.nih.gov/10228605
[7] Mondal, S., Mirdha, B.R. and Mahapatra, S.C., 2009. The science behind sacredness of Tulsi (Ocimum sanctum Linn.). Indian Journal of Physiology and Pharmacology, 53(4), pp.291–306. https://pubmed.ncbi.nlm.nih.gov/20509321
[8] Ranasinghe, P., Pigera, S., Premakumara, G.A.S., Galappaththy, P., Constantine, G.R. and Katulanda, P., 2013. Medicinal properties of 'true' cinnamon (Cinnamomum zeylanicum): a systematic review. BMC Complementary and Alternative Medicine, 13(1), pp.1–10. https://pubmed.ncbi.nlm.nih.gov/24148965
[9] Singh, R., Singh, G. and Mahla, R.S., 2016. Moongbean (*Vigna radiata*): a promising pulse crop for nutritional security. Legume Research, 39(6), pp.855–865. https://www.researchgate.net/publication/336837277_Mungbean_Vigna_radiata_L_Wilczek_Retrospect_and_Prospects
[10] Chou, C.H. (1999) 'Roles of allelopathy in plant biodiversity and sustainable agriculture', *Critical Reviews in Plant Sciences*, 18(5), pp. 609–636. Available at: https://doi.org/10.1080/07352689991309414
[11] Imai, S., Tsuge, N., Tomotake, M., Nagatome, Y., Sawada, H., Nagata, T. and Kumagai, H. (2006) 'Plant biochemistry: Blue pigment formation in garlic and onion', Nature, 442(7102), p. 956. Available at: https://doi.org/10.1038/442956a
[12] Gornall, Allan G., et al. "DETERMINATION of SERUM PROTEINS by MEANS of the BIURET REACTION." Journal of Biological Chemistry, vol. 177, no. 2, 1949, pp. 751–766, https://doi.org/10.1016/s0021-9258(18)57021-6.
[13] Kozhuharova, L., et al. (2013) 'Eugenol and borneol as modulators of oxidative stress and NF-κB pathways', *Pharmaceuticals*, 6(7), pp. 845–862. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC3819475
[14] Kumar, P. and Singh, A. (2019) 'Effect of plant extracts on seed germination', Journal of Plant Physiology, 134, pp. 25-32. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0981942819303146
[15] Guardado-Fierros, Beatriz G, et al. "Comparative Study between Salkowski Reagent and Chromatographic Method for Auxins Quantification from Bacterial Production." Frontiers in Plant Science, vol. 15, 11 June 2024, https://doi.org/10.3389/fpls.2024.1378079.
[16] Waqas, Muhammad, et al. "Endophytic Fungi Produce Gibberellins and Indoleacetic Acid and Promotes Host-Plant Growth during Stress." Molecules, vol. 17, no. 9, 7 Sept. 2012, pp. 10754–10773, https://doi.org/10.3390/molecules170910754.
[17] Salvi, N.D., Singh, S. and Kumar, S. (2020) 'Auxin producing endophytic fungi and their beneficial role in plant growth: A review', Applied Microbiology and Biotechnology, 104(9), pp. 3803-3815.Available at: https://pubmed.ncbi.nlm.nih.gov/30335811/
[18] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) 'Antioxidant activity applying an improved ABTS radical cation decolorization assay', Free Radical Biology and Medicine, 26(9–10), pp. 1231–1237. Available at: https://doi.org/10.1016/S0891-5849(98)00315-3
[19] Lee, S.K. and Shibamoto, T. (2001) 'Antioxidant properties of aroma compounds isolated from clove buds (Syzygium aromaticum (L.) Merr. et Perry)', Food Chemistry, 74(4), pp. 443–448. Available at: https://doi.org/10.1016/S0308-8146(01)00161-3
[20] Prior, R.L., Wu, X. and Schaich, K. (2005) 'Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements', Journal of Agricultural and Food Chemistry, 53(10), pp. 4290–4302. Available at: https://doi.org/10.1021/jf0502698
[21] Cortés-Rojas, D.F., de Souza, C.R.F., Oliveira, W.P. (2014) 'Clove (*Syzygium aromaticum*): a precious spice', *Asian Pacific Journal of Tropical Biomedicine*, 4(2), pp. 90–96. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC3819475
[22] Zari, A.T., Zari, T.A. and Hakeem, K.R. (2021b) 'Anticancer properties of Eugenol: a review,' *Molecules*, 26(23), p. 7407. https://doi.org/10.3390/molecules26237407.
[23] Velho, Maiara Callegaro, et al. "Eugenol-Loaded Nanoemulsions: Antiproliferative Activity against Breast Cancer Cells and Hemocompatibility." Journal of Drug Delivery Science and Technology, vol. 101, 1 Oct. 2024, p. 106248, www.sciencedirect.com/science/article/abs/pii/S1773224724009171.
[24] Happy Kurnia, Permatasari, et al. "Eugenol Isolated from Syzygium Aromaticum Inhibits HeLa Cancer Cell Migration by Altering Epithelial-Mesenchymal Transition Protein Regulators." Journal of Applied Pharmaceutical Science, 5 May 2021, https://doi.org/10.7324/japs.2021.110507
[25] Debnath, Anirban, et al. "Eugenol's Anti-Cancer Properties, Its Modulation of Signalling Pathways, and Cascades across Various Cancers: A Review." Current Research in Biotechnology, 1 Aug. 2025, pp. 100330–100330, https://doi.org/10.1016/j.crbiot.2025.100330
[26] Hafsah Haleem, F. (2025). Anti-cancer activities of eugenol and potential immunomodulatory effects: a comprehensive review. Journal of Current Oncology and Medical Sciences, 5(1), 1067–1077. Retrieved from https://submission.journalofcoms.com/index.php/JCOMS/article/view/260
[27] Begum, Syeda Nurunnesa, et al. "A Comprehensive and Systematic Review on Potential Anticancer Activities of Eugenol: From Pre-Clinical Evidence to Molecular Mechanisms of Action." Phytomedicine, vol. 107, Dec. 2022, p. 154456, https://doi.org/10.1016/j.phymed.2022.154456.
[28] Chavda, Vivek P., et al. "Nano-Drug Delivery Systems Entrapping Natural Bioactive Compounds for Cancer: Recent Progress and Future Challenges." Frontiers in Oncology, vol. 12, 29 Mar. 2022, https://doi.org/10.3389/fonc.2022.867655.
[29] Khan, Zaitoon, et al. "Therapeutic Applications of Nanobots and Nanocarriers in Cancer Treatment." Analytical Sciences, 4 June 2025, https://doi.org/10.1007/s44211-025-00799-5.
[30] Abbasi, Naghmeh, et al. "Synthesis and Characterization of Q0-Eugenol Nanoemulsion for Drug Delivery to Breast and Hepatocellular Cancer Cell Lines." ACS Omega, vol. 10, no. 24, 9 June 2025, pp. 25299–25312, https://doi.org/10.1021/acsomega.4c11261
[31] Batiha, G.E.S. et al. (2020) 'Syzygium aromaticum L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities', Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7072209/

[32] Chandi Charan Kandar, and Dilipkumar Pal. Relation between Seed Life Cycle and Cell Proliferation. Metabolic Changes in Seed Germination. 1 Jan. 2024 www.researchgate.net/publication/383601368_Relation_Between_Seed_Life_Cycle_and_Cell_Proliferation_Metabolic_Changes_in_Seed_Germination?fbclid=IwZXh0bgNhZW0CMTEAAR0ZhPNPljrDOVJFSzzQCzvG3Uu2j246fQlnpDBY-8vAGIt_BddJjdqtvggaem_cN9mV8AquHKKsa4DgWq-gQ.

[33] Statistical Test: ANOVA and Standard Deviation were done using: Microsoft Excel, Tukey HSD was done using: https://astatsa.com/OneWay_Anova_with_TukeyHSD, The P- values were interpreted using: McLeod, S. (2025) *Understanding P-Values and Statistical Significance*. Simply Psychology. Available at: https://www.simplypsychology.org/p-value.html

[34] Spectrometer Imaging App: Dominant λ Light Spectrometer - Apps on Google Play Available at: https://play.google.com/store/apps/details?id=com.contechity.spectrometer