RAMAIAH
College of Arts, Science
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सत्यमेव जयते
DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
Government of India

ESSENTIALS OF BIOSCIENCES

LABORATORY MANUAL

BSc BIOTECHNOLOGY ELECTIVE
SEMESTER IV

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Department of Biotechnology

Certificate

Reg. No.

This is to certify that _____ _____

has satisfactorily completed the course of experiments in_____

_____***prescribed by the MSRCASC-Autonomous***

for B.Sc./M.Sc. _____

program in the Biotechnology laboratory during the year 20____- 20___.

Date: _____

Signature of the faculty in charge **Examiners** **Head of the department**

1. **1.**

2. **2.**

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DEPARTMENT OF BIOTECHNOLOGY AND GENETICS

COURSE: BSc BIOTECHNOLOGY

COURSE CODE: 24BITE402

ESSENTIALS OF BIOSCIENCES

LABORATORY MANUAL

ACADEMIC YEAR 2024-25 ONWARDS

Program: BSc

Semester: IV

Course: Biotechnology Elective Practical

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Course Code- 24BITE402: Essentials of Biosciences		
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Internal Evaluation Report

Sl. No.	Name of the Experiment	Date	Internal Evaluation			Sign
			Record/ Performance	Viva	Total	
1.	Effect of light on Plant's growth- Phototropism					
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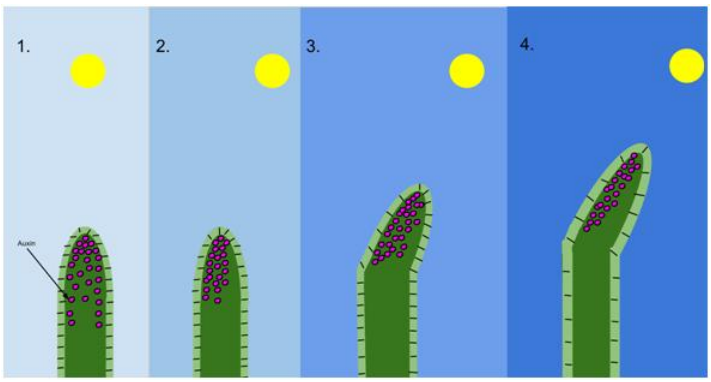
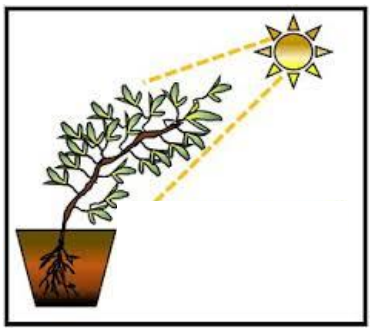
Exp.no. 1: Effect of light on Plant's growth- Phototropism

Aim: To determine the effect of light on plant's growth

Introduction:

"Photo" means light, and "tropism" means turning. Phototropism is the phenomenon by which the plant bends in the direction of light. The plant shoots grow toward a light source (positive phototropism) due to the hormone auxin, which causes cells on the shaded side to elongate. Light is required by the plants to stimulate energy production by the process of photosynthesis. The hormone called auxins that are present in the cells, respond to photosynthesis and react to produce more protein and generate energy for the plant. Almost all plants respond to photosynthesis in order to get more nutrition and energy. The stem and shoots usually react to positive phototropism by turning towards the sunlight, while negative phototropism takes place in the roots which turn away from the source of light. Here gravitropism or geotropism plays a major role in their growth.





Phototropic Response

Materials required:

Two pots, green gram, A cardboard boxes with a hole on one side

Procedure:

- Place Plant A (Control) in an open area receiving light from all sides.
- Place Plant B (Experimental) inside the cardboard box, ensuring the only light enters from the side
- hole.

- Water both plants equally.
- Observe over 5–7 days.

Observation: Plant A grows straight up, while Plant B bends towards the light hole.

Conclusion: Plants exhibit positive phototropism, responding to unilateral light by growing toward it.

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Exp. No. 2: Chlorophyll content through spectroscopy – extract, absorbance and plant physiology

Aim: To quantify chlorophyll pigments, a and b through spectrophotometer

Introduction:

Chlorophylls are green pigments present in a wide variety of plants, fungi, and cyanobacteria. Chlorophyll can be present in nearly any green component of a plant, such as the leaves and stems, as well as in the chloroplast, which is the only organelle that holds the majority of it. It is located in the centre of plant leaves in the mesophyll base. It transforms solar energy into chemical energy, which is then used to build vital carbohydrate molecules (glucose) that serve as the plant's primary food supply. Chlorophyll a and b are found abundant in all autotrophic plants. The molecular formula of chlorophyll is $C_{55}H_{72}O_5N_4Mg$ as shown in figure 1.

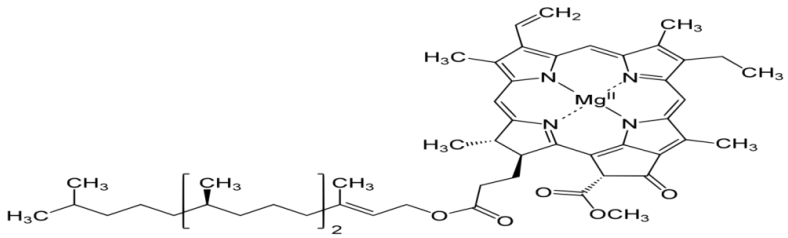

Figure 1: Structure of chlorophyll with hydrophobic hydrocarbon chain (tail) and hydrophilic porphyrin (head)

Chlorophyll a is essential for most photosynthetic organisms to release chemical energy and used in oxygenic photosynthesis. It absorbs most of the energy from wavelengths of violet-blue and orange-red light and chlorophyll b absorbs light same to chlorophyll a. *In vitro,* the characteristic absorption peak of chlorophyll b was observed at 453 nm and 625 nm, and *in vivo*, at 480 nm and 650 nm as shown in Figure 2.

Spectrophotometer

The simplest instrument for determining the concentration of chlorophyll in extracts of various plant specimens is a spectrophotometer. The instrument used is spectrophotometer and it measure how much a chemical substance absorbs light by measuring the light intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over certain range of wavelength.

The procedure to quantify spectrophotometric chlorophyll pigment a and b is based on absorption of light by chlorophyll extracts prepared in aqueous acetone 80%. The aqueous acetone breaks down the chlorophyll's lipid bond from the thylakoid structure which suspends the chlorophyll pigment highly soluble in organic solvent.

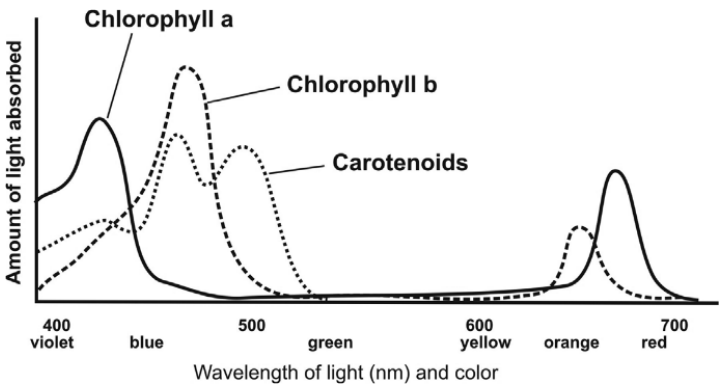

Figure 2: The absorbance curve of chlorophyll a and b at different regions of light

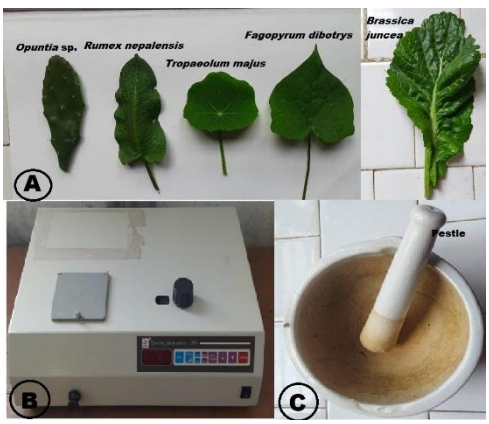

Figure 3: Material used in the experiment: A: Green leafy herbs. B: Spectrophotometer. C: Mortar and pestle

Materials required:

Green leafy herbs, mortar & pestle, 80% acetone, funnel, filter paper

Procedure

1. All the materials were collected
2. The spectrophotometer was switched on before 20 minutes

3. A 100ml of acetone of 80% concentration was prepared by adding 20ml distilled water in a conical flask and then added 80ml of acetone using a measuring cylinder
4. The midrib of plant leaves was removed using a blade
5. 1g of leaf of each specimen was measured using a weighing machine
6. 5ml of 80% acetone solution was added to the pestle along with the 1g of leaves of each specimen and it was grinded using a mortar. It was grinded thoroughly until the last bit of leaves were visible.
7. Then added the 5ml of acetone and mixed again thoroughly.
8. The mixture was filtered using a filter paper and funnel
9. The residue left on the filter paper was again grinded with addition of 5ml 80% acetone and filtered to obtain the enough filtered.
10. The cuvette was cleaned and rinsed using a tissue paper.
11. The filtrate was poured in the cuvette for the reading to be taken
12. The reading of absorbance was measured at two different wavelengths (663 and 645nm) using spectrophotometer
13. The amount of chlorophyll a, b and total (a + b) are determined using the formula given by Arnon (1949)

Observations and Calculations

The amount of chlorophyll a, b and total (a + b) are determined using the formula given by Arnon (1949) as follows:

$$Chorophyll\ 'a' = \frac{12.7 * D663 - 2.69 * D645 * V}{1000 * W}$$

$$Chorophyll\ 'b' = \frac{22.9 * D645 - 4.68 * D663 * V}{1000 * W}$$

$$Chorophyll\ 'a+b' = \frac{20.2 + (D645) + 8.02 + (D663) * V}{1000 * W}$$

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Observations & Calculations:

Table 1: Amount of chlorophyll content

Herbaceous plants	***Spinacia oleracea* (Palak)**	***Amaranthus cruentus* L. (Red Amaranthus)**	***Amaranthus viridis* L. Green Amaranthus**
Chlorophyll a			
Chlorophyll b			
Total Chlorophyll (a + b)			

Wavelength (nm)	***Spinacia oleracea* (Palak)**	***Amaranthus cruentus* L. (Red Amaranthus)**	***Amaranthus viridis* L. Green Amaranthus**
	Absorbance (nm)		

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Result: The amount of chlorophyll a, b and total amount (a + b) in different herbaceous plants are

a) b) c)

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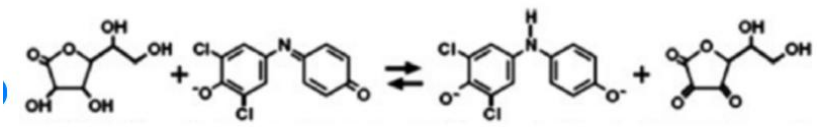
Exp. No. 3: Estimation of Vitamin C (Ascorbic acid) from Citrus fruits

Introduction

Vitamin C is a water-soluble vitamin, naturally present in some foods. Fruits and vegetables namely, citrus fruits, tomatoes, gooseberries, broccoli, strawberries are the best sources of vitamin C. In fact, humans are unable to synthesize vitamin C endogenously, so it is an essential dietary compound. Vitamin C is an essential nutrient in the diet, but is easily reduced or destroyed by exposure to heat and oxygen during processing, packaging, and storage of food. The instability of vitamin C makes it more difficult to ensure an accurate listing of vitamin C content on the nutrition label. The official method of analysis for vitamin C determination of juices is the 2,6-dichloroindophenol titrimetric method. While this method is not official for other types of food products, it is sometimes used as a rapid, quality control test for a variety of food products, rather than the more time-consuming microfluorometric method.

Principle

Vitamin C/ Ascorbic acid is a strong reducing agent that is oxidized to dehydroascorbic acid by 2,6 dichlorophenol indophenol dye and the dye itself is reduced to a colourless compound. Ascorbic acid reduces the indicator dye to a colorless solution. At the endpoint of titrating an ascorbic acid-containing sample with dye, excess unreduced dye is a rose-pink colour
in the acid solution. The end point of the reaction can be detected easily by observing the appearance of a pink colour which remains stable for approximately few minutes. The titer of the dye can be determined using a standard ascorbic acid solution. Food samples in solution then can be titrated with the dye and the volume for the titration used to calculate the ascorbic acid content.



Reagents required

- *Ascorbic acid standard solution:* (prepare only at time of use) Accurately weigh (on an analytical balance) approximately 50 mg ascorbic acid. Record this weight. Transfer to a 50-mL volumetric flask. Dilute to volume immediately before use with the metaphosphoric acid-acetic acid solution (see below for preparation of this solution).
- *Indophenol solution – dye:*

  To 50 mL deionized distilled (dd) water in a 150-mL beaker, add and stir to dissolve 42 mg sodium bicarbonate, and then add and stir to dissolve 50 mg 2,6-dichloroindophenol sodium salt. Dilute mixture to 200 mL with dd water. Filter through fluted filter paper into an amber bottle. Close the bottle with a stopper or lid and store refrigerated until used.

- *Metaphosphoric acid-acetic acid solution:*

To a 250-mL beaker, add 100 mL dd water then 20 mL acetic acid. Add and stir to dissolve 7.5 g metaphosphoric acid. Dilute mixture to 250 mL with distilled water. Filter through fluted filter paper into a bottle. Close the bottle with a stop-per or lid and store refrigerated until used.

- Orange juice samples**

  Use products processed and packaged in various ways (e.g., canned, reconstituted frozen concentrate, fresh squeezed, not-from-concentrate). Filter juices through cheesecloth to avoid problems with pulp when pipetting. Record from the nutrition label for each product the percent of the Daily Value for vitamin C.

  Standardization of Dye

1. Pipette 5 mL metaphosphoric acid-acetic acid solution into each of three 50-mL conical flasks.
2. Add 2.0 mL ascorbic acid standard solution to each flask.
3. Using a funnel, fill the burette with the indophenol solution (dye) and record the initial burette reading.
4. Place the Erlenmeyer flask under the tip of the burette. Slowly add indophenol solution

to standard ascorbic acid solution until a light but distinct rose-pink colour persists for < 5s (takes about 15–17 mL). Swirl the flask as you add the indophenol solution.

5. Note final burette reading and calculate the volume of dye used.

6. Repeat Steps 3–5 for the other two standard samples. Record the initial and final Burette reading and calculate the volume of dye used for each sample.

7. Prepare blanks: Pipette 7.0 mL metaphosphoric acid-acetic acid solution into each of three 50-mL Erlenmeyer flasks. Add to each flask a volume of distilled water approximately equal to the volume of dye used above (i.e., average volume of dye used to titrate three standard samples).

8. Titrate the blanks in the same way as Steps 3–5 above. Record initial and final burette readings for each titration of the blank, and then calculate the volume of dye used.

Analysis of Juice Samples

1. Pipet into each of three 50-mL Erlenmeyer flasks 5 mL metaphosphoric acid-acetic acid solution and 2 mL orange juice.
2. Titrate each sample with the indophenol dye solution (as did in Steps 3–5) until a light but distinct rose-pink colour persists for >5 sec.
3. Record the initial and final readings and calculate the difference to determine the amount of dye used for each titration.

Observations and Calculations

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Using the data obtained in standardization of the dye, calculate the titer using the following formula:

$$mg\ ascorbic\ acid(mL) = (X - B)\left(\frac{F}{E}\right)\left(\frac{V}{Y}\right) = (7.1\text{mL} - 0.1\text{mL})\ (0.130/2\text{mL})\ (7\text{mL}/7\text{mL}) = 0.455\text{mg/mL}$$

X- mL for sample titration

B- Avg mL for sample titration

F- titre of dye (=mg ascorbic acid equivalent to 1 mL indophenol std. solution)

E- mL assayed (2mL)

V- Vol. of initial assay solution (7mL)

Y- Vol. of sample aliquot titrated (7mL)

$$F = \frac{mg\ ascorbic\ acid\ in\ volume\ of\ std.solution\ titrated}{(avg.mL\ dye\ used\ to\ titrate\ std. - avg.mL\ dye\ used\ to\ titrate\ blank)}$$

$$F = \frac{(50.2mg/50ml)2ml}{(15.5mL - 0.10mL)} = 0.130\text{mg/mL}$$

Results:

The amount of Vitamin C present in

a) Sample 1 (Orange) =

b) Sample 2 (Lemon) =

c) Sample 3 (Tetra Pack orange juice) =

Exp. No. 4: Green Synthesis of Nanoparticles (Plant source)

Aim: To synthesize silver nanoparticles (AgNPs) using a green, eco-friendly method employing aqueous tea leaf extract as a reducing and stabilizing agent.

Principle: Green synthesis of nanoparticles utilizes biological materials such as plant extracts that contain natural reducing agents (polyphenols, flavonoids, tannins). These compounds reduce silver ions (Ag^+) from silver nitrate solution into metallic silver nanoparticles. The formation of nanoparticles is indicated by a visible colour change.

Requirements

Chemicals

Silver nitrate ($AgNO_3$) – 1 mM solution**,** Fresh tea leaves or commercial tea powder**,** Distilled water

Glassware & Equipment

Beakers (50 mL, 100 mL)**,** Measuring cylinder**,** Hot plate or water bath**,** Filter paper**,** Funnel**,** Dropper, Test tubes**,** Stirring rod

Procedure

A. Preparation of Tea Leaf Extract

1. Weigh 2 g of tea leaves.
2. Add to 50 mL of distilled water in a beaker.
3. Heat gently for 5–10 minutes (do not boil vigorously).
4. Cool and filter the extract using filter paper.
5. Collect the clear filtrate (tea extract).

B. Green Synthesis of Silver Nanoparticles

1. Prepare 1 mM silver nitrate solution (dissolve 0.017 g $AgNO_3$ in 100 mL distilled water).
2. Take 10 mL of $AgNO_3$ solution in a beaker
3. Add 2–3 mL of tea leaf extract dropwise with gentle stirring.

4. Add 2–3 mL of tea leaf extract dropwise with gentle stirring.
5. Keep the mixture at mild heating (40–50°C).
6. Observe the colour change within 10–30 minutes

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Result:

The reaction mixture changes from colourless/light yellow to brown, indicating the formation of silver nanoparticles due to surface plasmon resonance.

Discussion

Tea leaf extract contains polyphenols and antioxidants that act as natural reducing and capping agents. These biomolecules reduce Ag^{+} ions to Ag nanoparticles and stabilize them, preventing aggregation. The brown coloration confirms nanoparticle synthesis. This method is eco-friendly, cost-effective, and avoids the use of toxic chemicals, making it ideal for undergraduate laboratories.

Conclusion

Silver nanoparticles were successfully synthesized using tea leaf extract by a green synthesis method.

Precautions

- Handle silver nitrate carefully as it can stain skin.
- Avoid excessive heating.

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ABOUT THE DEPARTMENT

Department of Biotechnology and Genetics in the M S Ramaiah College of Arts, Science and Commerce, was established in the year 2000 offering both UG and PG programs. The main objective of the program is to provide conducive learning environment for the students and to mitigate the shortage of biotechnologists in the field of food, agriculture, medicine and environmental management. Highly qualified and experienced faculty members deliver the lectures and conduct the practical in various subjects as per the curriculum developed by the Bangalore City University. The department focuses mainly on teaching the basics, applications and hands-on training in a state-of-the-art classroom and laboratory environment. It also facilitates students to broaden their knowledge for multitasking.

ABOUT THE MANUAL

This laboratory manual for the course Essentials of Biosciences (24BITE402) is designed to provide hands-on understanding of fundamental biological concepts through a series of structured experiments. The manual covers plant physiology through studies on phototropism and chlorophyll estimation using spectroscopy, along with biochemical analysis such as estimation of Vitamin C from citrus fruits and assessment of antioxidant enzyme activity like catalase in plants. It introduces emerging techniques like green synthesis of nanoparticles using plant sources and explores enzyme by examining the effect of pH on salivary amylase activity. Practical applications extend to daily life with experiments on qualitative analysis of milk adulteration, soil texture analysis using the jar test, and health-based studies including surveys on lifestyle diseases among young adults. Additionally, it incorporates self-assessment techniques such as measuring BMI and pulse rate to correlate health indicators with lifestyle factors, thereby integrating theoretical knowledge with real-world biological and health-related applications. This manual is authored by Dr. Radha Dayanidhi & Dr. Muktha H, Assistant Professors, Department of Biotechnology and Genetics, MSRCASC, Bengaluru.

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Ranked #67 All India by National Institutional Ranking Framework (2nd in Karnataka)

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