

**PREPARATION OF MICROBIAL MEDIA FOR CULTIVATION
OF BACTERIA USING FRUIT PEEL AND VEGETABLE
WASTE**

A RESEARCH PROJECT

Submitted by

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UNDER THE GUIDANCE OF

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**DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)**

YEAR 2025-2026

“Dissemination of education for knowledge, science and culture”

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR
(AN EMPOWERED AUTONOMOUS INSTITUTE)

DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE
OF
RESEARCH PROJECT COMPLETION

This is to certify that Ms. **PALLAVI R. HANDE** studying in M. Sc. part II, Sem- IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled “**PREPARATION OF MICROBIAL MEDIA FOR CULTIVATION OF BACTERIA USING FRUIT PEEL AND VEGETABLE WASTE**” during academic year 2025-26.

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Research Project Guide

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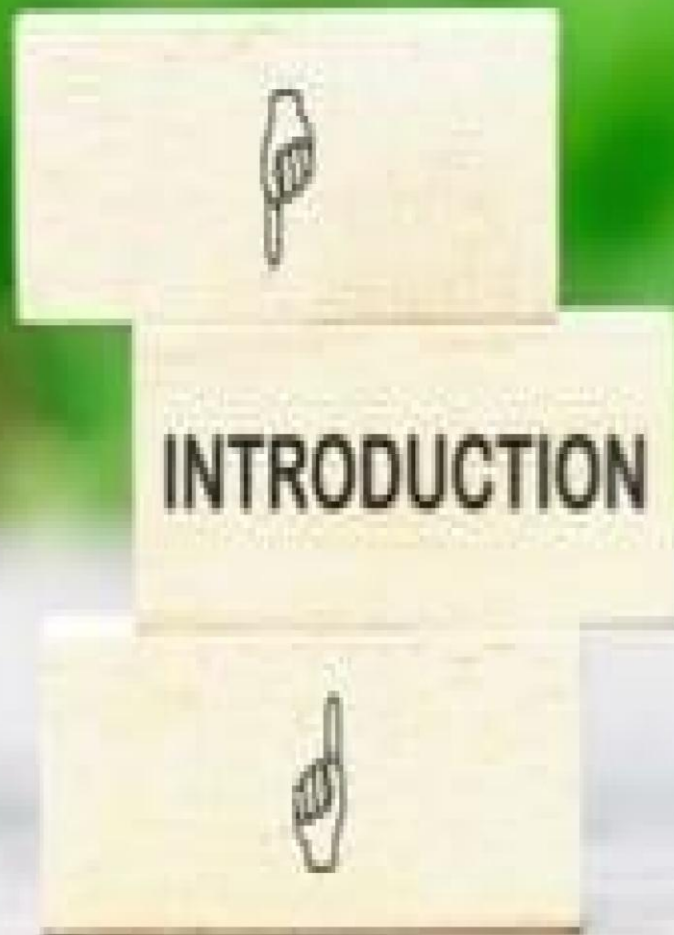
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Introduction

Fruit peels and vegetable waste, commonly considered agricultural waste, are actually rich sources of nutrients that can effectively support the growth of microorganisms. These materials contain significant amounts of carbohydrates, proteins, vitamins, minerals, fibers, and other organic compounds essential for microbial metabolism. Fruit peels are particularly rich in sugars such as glucose, fructose, and sucrose, making them excellent carbon sources, while vegetable waste provides additional nutrients including nitrogen, amino acids, and micronutrients. Because of this rich composition, these natural substrates can successfully support bacterial growth and often produce results comparable to commercial culture media.

Microorganisms are omnipresent and play a crucial role in various natural, environmental, and industrial processes. Their study, identification, and utilization require suitable culture media. Traditionally, commercial media such as nutrient agar and tryptic soy broth are widely used in laboratories; however, these are expensive and may not always be accessible, especially in developing regions and small educational institutions. Therefore, the development of low-cost, nutrient-rich alternatives using fruit and vegetable waste has gained increasing attention, as it significantly reduces laboratory expenses while maintaining efficient microbial growth.

Hence, there is a growing interest in formulating low-cost, nutrient-rich, and sustainable alternatives using natural sources such as fruit and vegetable wastes. Using fruit and vegetable waste for microbial growth but also contribute to waste reduction and environmental sustainability. This shift towards utilizing organic waste as a cultivation medium aligns with both sustainable practices and the need for cost-effective alternatives to traditional growth media. The use of agricultural byproducts, such as fruit peels and vegetable waste, presents an innovative way to address the dual challenges of waste disposal and microbial growth.

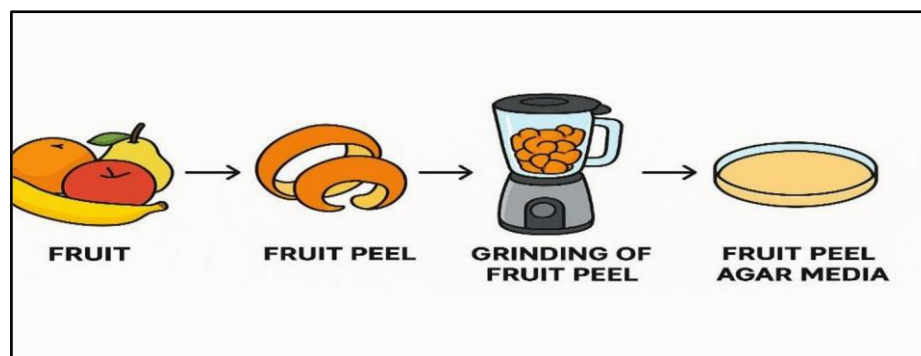


Fig1: preparation of bacterial culture media from fruit peel waste

Fruit Peel and Vegetable Waste as Potential Media for growth of bacteria. These organic wastes, often discarded in large quantities, could serve as a potential medium for the growth of a wide range of microbial species. The decomposition of plant-based waste results in the release of sugars, proteins, and amino acids, which can support bacterial development (Rahman et al., 2020). In particular, fruit peels are often considered nutrient-dense, with components like cellulose, pectin, and lignin that can be broken down by microorganisms, providing an ideal environment for bacterial growth (Pradeep et al., 2019). Studies have shown that various species of bacteria, including those from genera such as *Bacillus*, *Pseudomonas*, *Lactobacillus*, and *Escherichia*, exhibit significant growth when cultivated on fruit and vegetable waste (Ghosh et al., 2020). These bacteria can be utilized for multiple purposes, such as bioremediation, food preservation, and even biofertilizer production. For instance, certain strains of *Lactobacillus* are used in the fermentation of fruits and vegetables to produce probiotics.

The disposal of such waste materials, especially in urban areas, often leads to the accumulation of organic refuse in landfills, contributing to environmental pollution. According to a report by the Food and Agriculture Organization (FAO), approximately 1.3 billion tons of food are wasted globally each year, with fruit and vegetable waste representing a large portion of this total (FAO, 2019). Fruit and vegetable processing, packing, distribution and consumption in the organized sector in India, the Philippines, China and the USA generate approximately Improper disposal of these wastes contributes to environmental pollution and greenhouse gas emissions. By converting this biodegradable waste into bacterial culture media, the study promotes waste

valorisation turning waste into a useful, value-added product thereby reducing the environmental burden. Future research should focus on optimizing the use of these waste materials, exploring their biotechnological applications, and overcoming the challenges associated with their variability and microbial inhibition.

Using organic waste to cultivate bacteria reduces the environmental burden caused by food waste and helps decrease landfill waste. In addition, this approach contributes to circular economy principles by turning waste into valuable resources (Alvarez et al., 2016). Many fruit and vegetable waste materials. This makes them particularly suitable for growing both fastidious and non-fastidious bacteria (Siddique et al., 2020). Additionally, while there is considerable research on using agricultural waste for bacterial growth, more studies are needed to explore the scalability of these practices, particularly for large-scale biotechnological applications (Bhattacharya et al., 2020). This waste for the cultivation of bacteria represents an exciting and sustainable approach to microbial research.

From agricultural production to final household consumption, one third of the world's food produced for human consumption becomes waste every year. Underutilization of food leads to economic losses in terms of resources used in its production and, in addition, its management as waste entails environmental problems such as CO₂ and other greenhouse gas emissions. Therefore, the generation and accumulation of these organic wastes is not only a widespread and harmful environmental problem, but it is also important from an economic point of view in terms of collection, transport and final disposal costs. It has been estimated that the global production of municipal organic wastes will reach more than 1000 million tons per year by 2025. Specifically, in Europe approximately 50% of the food produced is being discarded (Laca et.al).

Research shows that microorganisms like bacteria, fungi, and yeast can efficiently grow on media prepared from such agro-wastes. For example, (Mondal et.al) used cucumber and orange peels to evaluate the production of single cell protein using *Saccharomyces cerevisiae* by submerged fermentation. The authors state that the bioconversion of fruit wastes into single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. Apple, turnip, papaya and banana peels were used for alcohol fermentation and biomass production by (Kondari et.al.,2012). The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported. The

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significance of this study lies in demonstrating how fruit peel and vegetable waste can be effectively utilized as alternative raw materials for bacterial culture media preparation. This approach offers both scientific and environmental benefits, making it an important contribution to sustainable microbiological practices.

This study is also important for promoting sustainable microbiology education and research. It empowers institutions to adopt cost-effective and eco-friendly laboratory practices and provides students with hands-on experience in sustainable science and innovation.

Additionally, this approach aligns with the global Sustainable Development Goals (SDGs) related to responsible consumption, production, and environmental protection.

The significance of this study includes:

- Providing a low-cost, nutrient-rich alternative to commercial bacterial media.
- Reducing laboratory expenses and promoting affordability in microbiological research.
- Utilizing agro-waste to minimize environmental pollution and waste disposal problems.
- Encouraging eco-friendly and sustainable laboratory practices.
- Supporting innovation and self-reliance in resource-limited settings.
- Contributing to the development of a circular bioeconomy through the conversion of waste into valuable scientific resources.

Therefore, the use of fruit peel and vegetable waste in bacterial culture media preparation represents a significant advancement toward sustainable, economical, and environmentally responsible microbiological research.

REVIEW OF LITERATURE

A JOURNEY THROUGH LITERATURE



Fruit peel, which are often considered agriculture waste, are actually rich sources of nutrients that can support the growth of various microorganisms. They contain significant amount of carbohydrates, proteins, vitamins, minerals, and other organic compounds that serves as essential nutrient for microbial metabolism and provide nitrogen and micronutrient required for microbial metabolism. Such natural substrate can successfully support the growth of many bacteria producing result comparable to those obtained from commercial media. Due to their high content of sugar such as glucose, fructose, and sucrose, fruit peels act as excellent carbon sources. Vegetable waste, which included peels, leaves stalk and other discarded part of vegetable, is rich source of nutrient that can support growth of microorganism. These waste contain high amount of carbohydrates, protein's, fibers, vitamins, making them suitable substrate for microbial growth. Microorganisms such as bacteria can utilize this organic material as carbon and nitrogen source for their metabolism and reproduction.

The bacteria cultivated on fruit peel and vegetable waste can be used in various biotechnological applications, such as bioremediation, the production of biofertilizers, or the fermentation of food products (Abid et al., 2019). This makes the use of agricultural waste not only beneficial from an environmental perspective but also from an industrial standpoint. Despite the promising potential of fruit peel and vegetable waste for bacterial cultivation, there are several challenges that need to be addressed. These include variations in the chemical composition of the waste, which can affect the consistency and quality of the growth medium, as well as the presence of antimicrobial compounds in certain fruits and vegetables that may inhibit microbial growth (Huang et al., 2019). Moreover, the optimization of conditions such as pH, temperature, and substrate concentration is crucial for maximizing bacterial growth and productivity on such waste materials (Wang et al., 2017).

Agro-waste materials exhibit significant biochemical variability due to differences in plant origin, maturity, and post-harvest handling (Adebayo-Tayo et al., 2019). The carbohydrate concentration in sweet lemon and watermelon peels is influenced by ripening stage, which directly affects fermentable sugar availability (Goyal & Kaushal, 2018). Similarly, the protein and mineral content of fenugreek and red amaranth varies depending on soil nutrients and cultivation practices (Singh & Gupta, 2018). This variability introduces inconsistency in nutrient composition when these wastes are used for culture media preparation (Kumar & Nirmala, 2021). In the

present project, this challenge necessitates reprocessing steps such as homogenization to ensure uniform nutrient distribution (Masurkar et al., 2023). Therefore, while agro-waste provides essential nutrients, its variability remains a critical limitation compared to standardized commercial media (Patel & Mehta, 2021). This analytical evaluation directly relates to the use of sweet lemon, watermelon, fenugreek, and red amaranth in the present study (Patel & Mehta, 2021).

History

The cultivation of microorganisms on artificial media has been an essential aspect of microbiology since the late 19th century. The foundation of microbial media preparation can be traced back to the pioneering work of Robert Koch (1881), who introduced solid culture media using agar to isolate pure bacterial colonies (Brock, 1988). Early culture media primarily consisted of nutrient sources derived from meat extracts, peptones, and yeast, which provided the essential nutrients for microbial growth. Over time, these conventional media became standardized and widely used in microbiological laboratories around the world (Madigan et al., 2015).

As microbiology advanced, the high cost and resource-intensive nature of traditional culture media became a limitation, especially in developing countries and large-scale industrial applications. In the mid-20th century, researchers began exploring natural and agricultural materials as potential substitutes for expensive synthetic components (Atlas, 1997). With the increasing global concern over waste management and environmental sustainability, attention turned to organic residues such as fruit peels and vegetable wastes.

The concept of using fruit and vegetable waste as a nutrient medium for microbial cultivation emerged in the early 2000s when scientists demonstrated that such wastes contain carbohydrates, proteins, and minerals sufficient to support bacterial growth (Okafor, 2007). Subsequent studies showed that banana peels, orange peels, and potato skins could effectively replace commercial media components for the cultivation of bacteria such as *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (Adebayo-Tayo et al., 2019; Kumar & Nirmala, 2021).

Need for Suitable Culture Media:-

A growth medium is a liquid or gel designed to support the growth of microorganisms. The commercially available media are very costly. Routine practical require large amount of media on regular basis for streak plate, pour plate, spread plate experiments. Availability of low cost media rich in nutrients, giving comparative results is the NEED OF THE DAY. (Berde & Berde, 2015). Microbial cultivation is a fundamental process in microbiology used to grow and study microorganisms under controlled laboratory conditions.

In addition to economic benefits, the development of culture media from fruit and vegetable wastes addresses critical environmental issues. The increasing generation of organic waste from households, markets, and food industries contributes to pollution and greenhouse gas emissions if not properly managed (Patil et al., 2023). Converting these wastes into valuable microbial media represents a sustainable waste management strategy aligned with the principles of a circular economy. Thus, the need for suitable culture media extends beyond scientific and industrial utility—it also contributes to environmental conservation and resource sustainability.

Nutritional Composition of Fruit and Vegetable Waste

Several studies report that fruit peels (e.g., orange, banana, mango, pineapple) and vegetable residues (e.g., potato, carrot, tomato, cabbage) are rich in sugars, cellulose, hemicellulose, pectin, and nitrogenous compounds. For example, Banerjee et al. (2015) found that orange peel extract contains 60–70% carbohydrates and traces of amino acids, making it suitable as a carbon source for bacterial growth. Similarly, (Singh and Gupta 2018) reported that vegetable wastes such as tomato and potato peels provide essential vitamins and nitrogen for microbial media formulation.

Adomi et.al., (2022) reported that the Fruit and vegetable wastes such as yam peels, plantain peels, pawpaw peels, sweet potato peels, and egg shells were analyzed for proximate and mineral composition.

Nutrient content (approximate):

- Carbohydrates: 48–55% (major source of carbon for microbial growth)
- Proteins: 10–21% (provides nitrogen for metabolism)
- Fats: 0.08–3.17%

- Crude fibre: 7–9% in peels, 1% in egg shells
- Ash: 3.98–5.94% (reflects mineral content)
- Moisture: 1.87–3.98%
- Minerals: Ca, Fe, Zn, Mg, Na, Mn present in significant amounts
- Vitamins: Vitamin C (2–4 ppm), particularly high in egg shells and plantain peels. These nutrients make the waste materials suitable for supporting microbial growth comparable to conventional nutrient media.

(Berde and Berde, 2015) reported that the Vegetable and fruit wastes such as onion peels, garlic peels, and corn peels were found to contain appreciable amounts of carbohydrates and proteins that support microbial growth.

- Carbohydrate content: ~24.2 mg/dl (by DNSA method)
- Protein content: ~28 mg/dl (by Folin-Lowry method)

The media prepared from these wastes had a pH of 6.8, suitable for microbial cultivation. The vegetable waste media were rich in sugars, organic compounds, and trace elements that act as sources of carbon and nitrogen, making them nutritionally comparable to standard commercial media.

Masurkar et.al., (2023) reported that the study revealed that fermented fruit peels (banana, chikoo) and vegetable wastes (cabbage, cauliflower) .Fermentation enriched the waste with soluble nutrients, making them suitable as a nutrient base for microbial media. These wastes provide carbon, nitrogen, and minerals necessary for microbial growth and plant tissue culture. The pH of prepared filtrates was adjusted to 7.4 for bacteria and 5.4 for fungi, ideal for cultivation. The wastes contained organic acids, carbohydrates, and proteins after fermentation, which enhance microbial utilization. Vegetable wastes are rich in starch, proteins, fibers, and essential minerals, which act as both carbon and nitrogen sources for microorganisms. They also contain amino acids, chlorophylls, and phenolic compounds that contribute to bacterial growth and enzyme synthesis. Leafy and root vegetables in particular provide micronutrients such as zinc, manganese, and phosphorus, which play roles in cellular metabolism.

Jadhav, (2018) reported that the Fruit and vegetable wastes such as cauliflower stalks, potato peels, fenugreek stems, orange peels, and drumstick seeds/peels were found to contain significant nutritional components that support microbial growth.

- Carbohydrate content: ranged from 7.2 to 10 mg/100 ml (by DNSA method).
- Protein content: ranged from 6.3 to 9.9 mg/100 ml (by Folin-Lowry method).

Plant-derived wastes consist of complex structural components such as cellulose, hemicellulose, and lignin that are not directly utilizable by all microorganisms (Rahman et al., 2020). Microbial utilization of these substrates requires enzymatic breakdown into simpler compounds (Atlas, 1997). In this study, simple sugars from watermelon and sweetlemon are readily metabolized, whereas fibrous components require longer degradation time (Banerjee et al., 2015). Fenugreek and red amaranth residues also contain structural polysaccharides that influence nutrient accessibility (Patil et al., 2023). This indicates that nutrient availability in agro-waste media depends not only on composition but also on structural complexity (Kumar et al., 2020). Thus, effective processing methods are essential to improve nutrient bioavailability (Masurkar et al., 2023). This analytical evaluation directly relates to the use of sweet lemon, watermelon, fenugreek, and red amaranth in the present study (Patel & Mehta, 2021).

Utilization of Fruit Peel Waste for Bacterial Media

Fruit peels, often discarded as waste, are rich in bioactive compounds and nutrients. Their chemical composition varies across species, offering potential applications in food, pharmaceuticals, and agriculture. This review synthesizes recent research on the chemical constituents of fruit peels, highlighting their nutritional and functional properties.

Fruit peels are a valuable source of bioactive compounds with diverse applications. Their rich chemical composition not only supports human health but also offers sustainable alternatives in various industries. Further research is needed to explore the full potential of fruit peels and to develop efficient methods for their utilization. They have been successfully used to prepare growth media for various microorganisms. The banana peel extract supported the growth of *Escherichia coli* and *Bacillus subtilis* comparable to commercial nutrient broth. Likewise, Kavitha and Gopinath (2020) showed that mango peel hydrolysate could replace up to 80% of conventional peptone in bacterial culture media, reducing costs significantly.

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peels especially yam, pawpaw, and plantain peels were used to prepare alternative media by mixing powdered peels with agar and distilled water. The formulated peel media supported growth of bacteria such as *E. coli*, *Staphylococcus aureus*, *Bacillus sp.*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The yam peel medium showed the highest nutrient support for microbial proliferation. Peels supplemented with *Cambarus sp.* (crayfish extract) enhanced bacterial growth better than when supplemented with glucose or NaCl. Thus, fruit peel-based media can serve as low-cost alternatives to commercial media for bacterial cultivation (Adomi et.al.,2022). peels like orange, apple, papaya, banana, and turnip can successfully be used for bacterial culture and fermentation: These wastes provide carbon and nitrogen sources essential for bacterial metabolism. They support the growth of bacteria such as *Bacillus sp.*, *Sarcina sp.*, and *Pseudomonas aeruginosa*. The bioconversion of fruit peels into microbial media contributes to protein production and sustainable bioprocessing. (Berde and Berde, 2015).

Morphological observations:

- *E. coli* – large, thick, grayish-white colonies on fruit agar.
- *Lactobacillus* – long, slender, yellow colonies.
- *Aspergillus* – cottony, black-yellow colonies.
- *Saccharomyces cerevisiae* – flat, moist, cream-colored colonies.

Carbon sources play a fundamental role in microbial energy generation and biomass synthesis (carbon utilization depends on both composition and microbial adaptability (Patel & Mehta, 2021). This analytical evaluation directly relates to the use of sweet lemon, watermelon, fenugreek, and red amaranth in the present study (Patel & Mehta, 2021). Thus, fruit peel powder agar effectively replaces commercial nutrient agar for bacterial and fungal cultivation.

Utilization of Vegetable Waste for Bacterial Cultivation

Vegetables are essential components of a balanced diet, offering a wide array of nutrients that support overall health. Nutritional Profile of Vegetables. Vegetables are naturally low in calories and rich in water content, often exceeding 70%. They typically contain about 3.5% protein and less than 1% fat. Key nutrients found in vegetables include:

- Vitamins: Notably vitamins A and C, which are crucial for immune function, skin health, and vision.
 - Minerals: Including calcium and iron, vital for bone health and oxygen transport, respectively.
 - Dietary Fiber: Essential for digestive health and maintaining healthy blood sugar levels.
 - Antioxidants: Such as flavonoids and carotenoids, which help combat oxidative stress and inflammation.
 - Broccoli: Rich in vitamins C and K, folate, and fiber. It also contains sulforaphane, a compound known for its anti-cancer properties.
 - Spinach: High in iron, calcium, vitamins A and C, and folate. It is also low in calories and rich in antioxidants.
 - Sweet Potatoes: Excellent source of beta-carotene (vitamin A), vitamin C, and potassium. They have a lower glycemic index compared to regular potatoes, making them beneficial for blood sugar control.
 - Carrots: High in beta-carotene, which the body converts into vitamin A, supporting vision and immune function.
 - Tomatoes: Contain lycopene, an antioxidant linked to reduced risk of certain cancers. They are also a good source of vitamins A and C.
 - Green Peas: Provide plant-based protein, fiber, and essential vitamins and minerals, supporting muscle health and digestion.
 - Cauliflower: Offers fiber and antioxidants like sulforaphane, contributing to digestive health and potential cancer protection.
- High-Fiber Vegetables for Digestive Health**
- Fiber is crucial for maintaining healthy digestion and preventing constipation. Vegetables high in fiber include:
 - Artichokes, Green Peas, Brussels Sprouts, Sweet Potatoes, Parsnips, Winter Squash, Cauliflower, Carrots, Kale, Spinach, Corn, Mushrooms,

These vegetables provide both soluble and insoluble fiber, which aid in digestion and support gut health. Aim to include a colorful array of vegetables in your meals to ensure a broad spectrum of nutrients. For locally grown, seasonal vegetables to enjoy peak freshness and nutritional value. By incorporating a diverse range of vegetables into your daily diet, you can enhance your nutrient intake and support overall health. Vegetables are integral to a balanced diet, offering a rich array of

nutrients that contribute to overall health. Their nutritional profiles vary across different types, but common benefits include:

- **Vitamins and Minerals:** Vegetables are abundant in essential vitamins such as A, C, E, and K, and minerals like potassium, magnesium, and folate. These nutrients play vital roles in immune function, bone health, and cellular processes. (Bhardwaj et.al.,2025)
- **Dietary Fiber:** High fiber content in vegetables supports digestive health, aids in maintaining healthy blood sugar levels, and contributes to heart health by lowering cholesterol (Javed et.al.2019).
- **Antioxidants and Phytochemicals:** Compounds such as flavonoids, carotenoids, and glucosinolates found in vegetables have antioxidant properties that help combat oxidative stress and inflammation, potentially reducing the risk of chronic diseases.
- **Low Energy Density:** Most vegetables are low in calories but high in volume, making them excellent choices for weight management and promoting satiety. (Thakur et.al.2022).

Nitrogen availability is essential for microbial protein synthesis and cellular growth (Atlas,1997). Fenugreek provides plant-based proteins that serve as nitrogen sources for microorganisms (Singh & Gupta, 2018). Red amaranth contributes additional amino acids and minerals that support enzymatic activity (Reddy et al., 2020). However, plant proteins are less readily available compared to hydrolysed nitrogen sources like peptone (Kumar et al., 2020). In this study, microbial growth depends on the ability to degrade these proteins efficiently (Masurkar et al., 2023). Thus, nitrogen source quality significantly influences growth performance in agro-waste media (Patel & Mehta, 2021). This analytical evaluation directly relates to the use of sweet lemon, watermelon, fenugreek, and red amaranth in the present study (Patel & Mehta, 2021).

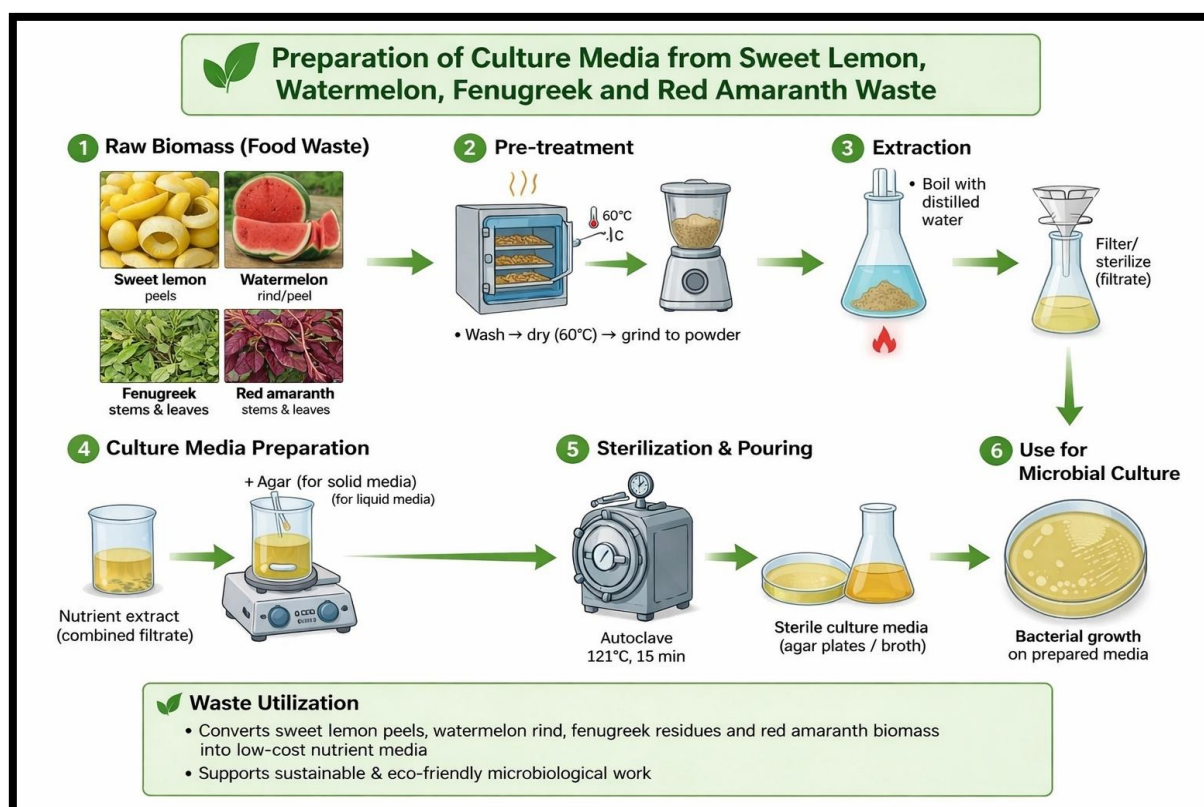


Fig.1-Preparation of culture media using fruit peel vegetable waste.

Combined Fruit and Vegetable Waste Media

Fruit peel vegetable waste are effectively used for preparation of media (Fig.1). Some studies have explored the synergistic use of mixed fruit and vegetable waste extracts. (Patel and Mehta 2021) reported that combined fruit–vegetable waste broth enhanced the growth of *Lactobacillus* species due to balanced carbon and nitrogen ratios. The combination also improved pH stability and supported longer microbial viability.

Combined use of fruit and vegetable waste (e.g., yam + sweet potato + plantain) or supplemented mixtures yielded better microbial growth. Formulations containing both fruit and vegetable sources provided a balanced nutrient profile high carbohydrates from fruits and higher proteins/minerals from vegetables. Media containing *Cambarus* sp. with fruit–vegetable blends supported faster and denser growth. This indicates that synergistic blending of fruit and vegetable waste extracts creates optimal conditions for bacterial cultivation (Adomiet.al.,2022).

The study highlights that combining different vegetable wastes (onion, corn, garlic) yields a balanced nutrient medium rich in both carbohydrates and proteins—

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supporting diverse microbial species. Such mixed waste media enhance microbial growth rate and sporulation, particularly for fungi and yeasts, compared to single-source waste media. Combined waste extracts also accelerate sporulation time for example, *Trichoderma* sp. sporulated in 48 hours on GCO media versus 72 hours on conventional Sabouraud's agar (Berde and Berde, 2015).

➤ Growth Observation

Bacterial species such as *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus* were able to grow successfully on media prepared from fruit peel and vegetable waste extracts. The colonies formed were similar in shape, colour, and size to those grown on standard media like Nutrient Agar or Nutrient Broth. Growth appeared within 24 hours at 35–37°C, showing that the waste-based medium provides sufficient nutrients for bacterial metabolism.

Nutritional Composition from fruit peels (banana, orange, mango, papaya) are rich in sugars, organic acids, and vitamins are good carbon source. Vegetable wastes (potato, spinach, carrot, tomato) contain proteins, amino acids, and minerals → good nitrogen and micronutrient sources. The combination of both provides a balanced nutrient medium similar to commercial peptone–beef extract media.

➤ pH and Optical Density

The pH of the waste-based media ranged between 6.5–7.2, suitable for most bacterial growth. Optical density (OD₆₀₀) and colony-forming units (CFU/mL) showed comparable growth rates to standard Nutrient Broth.

➤ **Cost and Environmental Impact** Preparation cost was 70–90% lower than synthetic media. Organic waste utilization reduces landfill load and greenhouse gas emission.

Demonstrates a sustainable, circular bioresource approach in microbiology.

Table no.-1 Comparison with Standard Media

Sr.no.	Parameter	Fruit Peel–Vegetable Waste Medium	Standard Nutrient Medium
1	Carbon Source	Natural sugars & starch (from fruit peels)	Glucose/peptone
2	Nitrogen Source	Proteins & amino acids (from vegetables)	Peptone, beef extract
3	Growth Rate	Moderate to high with (Varies waste ratio)	High Comparable in 24–36 hrs
4	Colony Morphology	Similar size, texture, and colour	Standard appearance, no major difference
5	pH Range	6.5–7.2	6.8–7.0
6	Cost Efficiency	Very low-cost (0), made from waste	High-cost chemicals ~80%
7	Sustainability Eco-friendly, renewable	Synthetic ingredients	More sustainable

The study found that media formulated from fruit peel and vegetable waste extracts effectively supported bacterial growth comparable to standard laboratory media. The mixed waste medium provided balanced nutrients, maintained favourable pH, and produced typical colony morphology for test organisms. In addition, it offered a highly economical and environmentally sustainable alternative. The comparative analysis confirmed that the growth performance in waste-based media is slightly lower but scientifically acceptable for routine microbiological work, educational laboratories, and resource-limited settings (Table 1).

The experimental findings revealed that bacterial isolates exhibited significant growth on the fruit peel and vegetable waste-based media. The colonies developed were comparable in appearance and density to those on Nutrient Agar, indicating adequate nutrient availability. The mixed extract medium provided a

balanced supply of carbohydrates and proteins essential for bacterial metabolism. When compared to standard media, no considerable differences were observed in pH stability, colony morphology, or incubation time. The growth rate was slightly lower, but within acceptable limits for laboratory use.

Applications of Fruit Peel and Vegetable Waste–Based Media

Environmental and Economic Significance

Using fruit peel and vegetable waste for media preparation reduces environmental pollution and disposal costs. It promotes waste valorisation, aligning with the principles of sustainable development and circular bioeconomy. As (Kumar et al.,2022) emphasized, such biowaste-based media not only lower production costs but also minimize dependence on animal-derived ingredients, offering a sustainable option for microbial industries.

- **Economic advantage:** Significantly reduces the cost of microbial media for laboratories, especially in developing countries.
- **Sustainability:** Promotes waste valorisation and supports circular bioeconomy by transforming organic waste into laboratory-grade nutrient media (Adomi et.al.,2022).
- **Environmental Impact:** Utilization of fruit and vegetable waste minimizes environmental pollution by reducing waste accumulation from markets and households. it encourages biowaste recycling, helping control odor, pest breeding, and open dumping issues. **Economic Benefit:** GCO liquid media costs only ₹80 per 100 L, compared to ₹3,180 per 100 L for commercial media. GCO solid media costs ₹9,700 per 100 L, compared to ₹12,800 for commercial solid media. This drastic cost reduction makes it an excellent low-cost substitute for educational, research, and industrial laboratories. (Berde and Berde, 2015)
- **Environmental Value:** Reuses agricultural and market waste that would otherwise contribute to pollution and landfill load. Reduces environmental hazards from organic waste decomposition. Encourages waste-to-resource recycling, promoting sustainable biotechnology and waste management.

- **Economic Value:** Considerably reduces cost of laboratory media preparation compared to commercial synthetic media. Enables resource-limited laboratories and educational institutions to conduct microbial cultivation effectively. Provides a pathway for industrial application in biofertilizer and fermentation sectors due to its low cost and nutrient richness (Masurkar, Pathade, Jadhav et.al.,2023)
- **Environmental Benefits:** Converts agricultural and household waste into productive resources, reducing landfill accumulation and pollution. Promotes biowaste recycling and decreases dependency on animal-based or synthetic media components. Supports sustainable laboratory practices and contributes to eco-friendly biotechnology.

The cost of alternative media was drastically lower than commercial nutrient media:

- Commercial Nutrient Broth (NB): ₹3,626 per 100 L
- Formulation B: ₹1,417 per 100 L
- Formulation D: ₹1,336 per 100 L
- Drumstick Peel (DP): ₹1,460 per 100 L

This represents up to 60% cost reduction, making it ideal for educational, industrial, and research laboratories. (Jadhav, 2018)

The aim of this review is to critically analyse and synthesize existing research on the development of alternative culture media using agro-waste materials, with specific emphasis on fruit peels such as sweet lemon and watermelon, and vegetable wastes including fenugreek and red amaranth, as utilized in the present study. The review focuses on understanding how these selected materials can act as economical and sustainable substitutes for conventional synthetic media used in microbiology.

The review further aims to examine the nutritional composition of sweet lemon and watermelon peels, particularly their richness in carbohydrates, pectin, and essential minerals, and to relate these components to their role as carbon and energy sources for microbial growth. Fruit peel wastes have been reported to contain sufficient nutrients to support microbial growth and can serve as effective substrates in media preparation (Anbu et al., 2017; Sadh et al., 2018). Citrus peel wastes, such as sweet lemon, are especially rich in fermentable sugars and bioactive compounds that enhance microbial growth efficiency (Sharma et al., 2020).

In addition, the review evaluates the biochemical composition of vegetable wastes like fenugreek and red amaranth, which are rich in proteins, vitamins, and micronutrients that function as nitrogen sources and growth-promoting factors. Agro-waste materials have been shown to replace conventional nutrient sources such as peptone and yeast extract in microbial media (Saranraj & Stella, 2012; Pundir et al., 2013). The presence of essential amino acids and minerals in leafy vegetable residues further supports microbial metabolism and growth (Kumar et al., 2019).

Finally, the review highlights the concept of waste valorisation by emphasizing how the utilization of these specific fruit and vegetable wastes contributes to eco-friendly and sustainable microbiological practices. The conversion of agro-waste into valuable laboratory resources reduces environmental pollution and supports circular bioeconomy approaches (Sadh et al., 2018; Sharma et al., 2020). The review also aims to identify research gaps, particularly in the combined use of fruit and vegetable wastes in a single culture medium formulation, which remains relatively underexplored and forms the basis of the present study.



OBJECTIVES

Objective

1. To collect and process agro-waste materials such as sweet lemon peel, watermelon peel, fenugreek leaves, and red amaranth.
2. To formulate and standardise nutrient media derived from this organic waste.
3. To formulate wet, dry waste-based media for respective waste.
4. To prepared combined media using all waste combinations
5. To compare the growth efficiency of selected bacterial species such as *E.coli*, *Bacillus*, *Staphylococcus aureus* on waste-based media.



MATERIAL METHOD

MATERIALS AND METHODS

Methodology:

The methodology of utilizing Sweet lemon peels (mosambi) waste depends on the specific application such as microbial media preparation, bio-product development, or other environmental uses. In this project, Sweet lemon peels waste is used for the preparation of culture media and study of microbial growth. Below is a general outline of the process followed in this study.

Culture media preparation from Sweet lemon peels.

1. Collection of Sweet lemon peels

- We collected sweet lemon peels from local juice shops, households, and market fruit vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Sweet lemon peels.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form sweet lemon peels. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry Sweet lemon peels and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism. Standard procedure performed as per Table 1.

Table 1:

Wet (Fresh) Sweet lemon peels Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table:2

Dry (Fresh) Sweet lemon peels Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The Sweet lemon peels (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of samples.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1 part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining

Procedure

1. A heat-fixed smear of the bacterial sample was prepared on a clean microscope slide.
2. The smear was flooded with crystal violet stain and left for about 1 minute.
3. The slide was gently rinsed with water to remove excess stain.
4. Gram's iodine solution was added and allowed to react for 1 minute.
5. The slide was again rinsed with water.
6. The smear was decolorized using ethanol or acetone for a few seconds until the purple color washed away.
7. The slide was immediately rinsed with water to stop the decolorization.
8. Safranin (counter stain) was then applied for 1 minute.
9. Finally, the slide was washed with water and gently dried using blotting paper.
10. The slide was then observed under a microscope to identify Gram-positive or Gram-negative bacteria.

Culture media preparation from waste Watermelon peels.

1. Collection of Watermelon peels.

- We collected Watermelon peels from local juice shops, households, and market fruit vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Watermelon peels.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form Watermelon peels paste. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry Watermelon peel and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism. Standard procedure performed as per Table3.

Table no:3

Wet (Fresh) Watermelon peels Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table no:4

Dry (Fresh) Watermelon peels Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The prepared Watermelon peels (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of sample.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1 part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining:

To perform gram staining the, above mentioned procedure (8) was followed.

Culture media preparation from Fenugreek waste.

1. Collection of Fenugreek Waste.

- We collected Fenugreek waste from local shops, households, and market vegetables vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Fenugreek waste.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form Fenugreek waste paste. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry Fenugreek waste and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism. Standard procedure performed as per (Table 5).

Table no:5

Wet (Fresh) Fenugreek Waste Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table no:6

Dry (Fresh) Fenugreek Waste Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The prepared Fenugreek waste (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of sample.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1 part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining

To perform gram staining the, above mentioned procedure (8) was followed.

Culture media preparation from Purslane waste.

1. Collection of Purslane waste.

- We collected Purslane waste from local shops, households, and market vegetables vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Purslane waste.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form Purslane waste paste. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry Purslane waste and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism. Standard procedure performed as per (Table 7).

Table no:7

Wet (Fresh) Purslane waste Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table no:8

Dry (Fresh) Purslane waste Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The prepared Purslane waste (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of sample.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1 part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining

To perform gram staining the, above mentioned procedure (8) was followed.

Combinations for Media formulations

We have prepared combinations of media as follows,

1. Sweet lemon and Watermelon peel waste

(Wet and Dry)

2. Fenugreek and Purslane waste.

(Wet and Dry)

Sweet lemon and Watermelon peel waste

1. Collection of Sweet lemon and Watermelon peel combine.

- We collected Sweet lemon and Watermelon peel combine from local shops, households, and market vegetables vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Sweet lemon and Watermelon peel combine.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form Sweet lemon and Watermelon peel combine paste. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry sweet lemon and Watermelon peel combine and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism. Standard procedure performed as per (Table 9).

Table no:9

Wet (Fresh) Sweet lemon and Watermelon peel combine Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh combine Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table no:10

Dry (Fresh) Sweet lemon and Watermelon peel combine Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh combine Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The prepared sweet lemon and Watermelon peel combine (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of samples.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

Preparation of Microbial Media for Cultivation of Bacteria Using Fruit Peel and Vegetable Waste

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining

To perform gram staining the, above mentioned procedure (8) was followed.

Fenugreek and Purslane waste.

1. Collection of Fenugreek and Purslane waste.

- We collected Fenugreek and Purslane waste from local shops, households, and market vegetables vendors.
- The wastes were separated from other waste materials to avoid contamination and ensure better quality of the sample.

2. Treatment on Fenugreek and Purslane waste.

Cleaning: The collected wastes were washed properly with clean water to remove dust, dirt, and pesticide residues.

Grinding: After washing, the wastes (Either wet/dried) were cut into small pieces and then grind using a grinder to form Fenugreek and Purslane wastepaste. This paste was used for further processing.

3. Media Preparation

Culture media preparation of different concentration using waste wet and dry Fenugreek and Purslane waste and distilled water (100ml) refers to creating nutrient rich growth medium for cultivation of micro-organism Standard procedure performed as per (Table 11).

Table no:11

Wet (Fresh) Fenugreek and Purslane waste Preparation for filter and unfiltered media. This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh combine Waste Paste (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

Table no:12

Dry (Fresh) Fenugreek and Purslane waste Preparation for filter and unfiltered media.

This chart follows your specific preparation details for fresh paste.

Concentration (%)	Weight of Fresh combine Waste Powder (g)	Total Consistency Description
10%	10g	Standard dilution for baseline testing.
20%	20g	Thoroughly blended for uniform consistency.
40%	40g	Highly integrated extract within the solvent.
60%	60g	Concentrated mixture; required careful mixing.

4. Boiling

Boiling: The prepared Fenugreek and Purslane waste (Paste/Powder) of different concentration was boiled in water for 10–15 minutes. To prepare different concentration of media.

Filter: After boiling, the mixture was filtered using filter paper and a glass funnel to separate the solid residue from the liquid extract for different concentration (10/20/40/60) filtrate media preparation.

Unfiltrate: Unlike typical filtered methods, the solid residue was kept in the liquid to observe how the total organic matter affects microbial growth for different concentration (10/20/40/60) unfiltrate preparation.

5. Sterilization.

Sterilization is an important step to remove unwanted contaminants and ensure purity of the culture media. In this study, sterilization was carried out using the autoclaving method. After sterilization agar poured in sterile petri plates.

6. Collection of samples.

Soil was collected from a waste disposal area. We chose well-drained soil with a neutral pH (6.0–7.5), as this is ideal for most bacterial growth. Large clumps, stones, and debris were removed for even mixing

Soil Dilution: After collection of soil serial dilution done in sterile tubes.

The organic material to soil ratio generally ranged from 10^{-4} to 10^{-8} (1 part organic material with 4–10 parts soil).

7. Spreading and Incubation:

Spreading: The diluted soil suspension was spread evenly on the agar plates using a sterile spreader to allow proper growth of microbial colonies.

Incubation: The Petri plates were incubated upside down (agar side up) at 37°C for 24–48 hours to allow microbial growth.

Colony Counting: We estimated the microbial population in the original soil sample based on Colony Forming Units (CFUs).

8. Staining:

Following the incubation period, well-isolated colonies were identified. A single representative colony was selected and used to create a suspension for Gram staining and motility testing to observe cellular characteristics.

Gram Staining

To perform gram staining the, above mentioned procedure (8) was followed.

RESULTS

The word "RESULTS" is rendered in a bold, 3D, red font. A magnifying glass with a silver frame and a black handle is positioned over the letters "SU", making them appear larger and more prominent. The entire graphic is centered horizontally on the page.

Collection of fruit peel and vegetable waste:

1.sweet lemon:

sweet lemon peel collected from were collected from household, market, juice centre, near Tawde hotel, Shahu market yard, Kolhapur and the collected peel grinded to make a paste. preparing sweet lemon peel media.

Wet sweat lemon peel

Filtered sweet lemon peel media:

Filtered sweet lemon peel extract was used to prepare different concentration media. The media was inoculated with soil sample and incubated at 37°C for 24-48 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered sweet lemon peel media supports the cultivation of bacteria (Fig no.1).

Media plate:

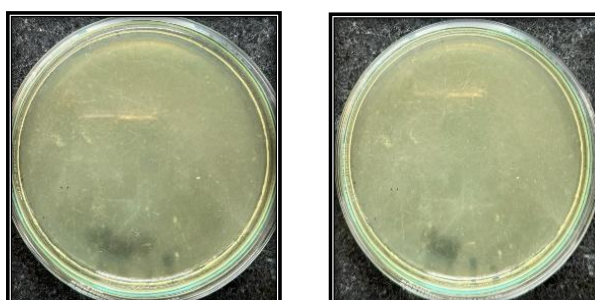


Fig no.1: sweet lemon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon wet peel agar media plate after incubation at 37°C for 24 hrs (Fig no.2).

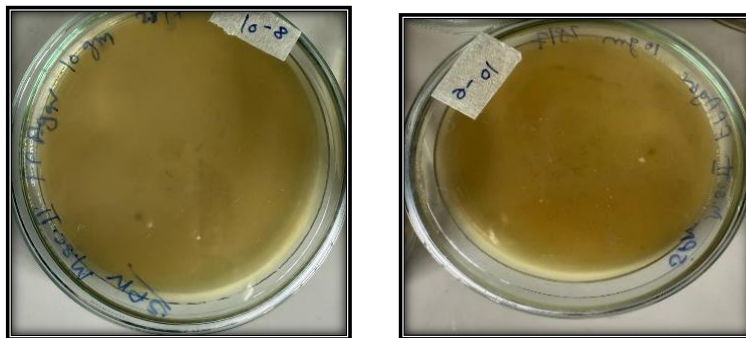


Fig no.2: Growth of organism on sweet lemon peel agar

For 10 % conc.:

Colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
4mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

Table 1: Gram nature of organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10	Gram negative	Short rod singly arranged
20	Gram positive	Cocci
40	Gram positive	Cocci
60	Gram positive	Short rod

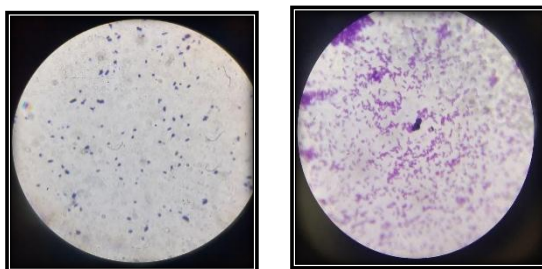
Gram staining:

Fig no.3: Gram staining of microorganism

Unfiltered sweet lemon peel media:

sweet lemon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that sweet lemon peel media supports the cultivation of bacteria (Fig no.4).

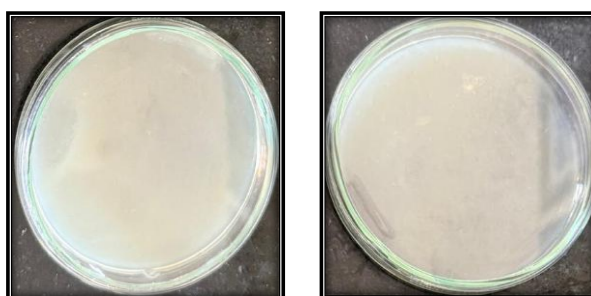
Media plate:

Fig no.4: sweet lemon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon wet peel agar media plate after incubation at 37°C for 24 hrs (Fig no.5).

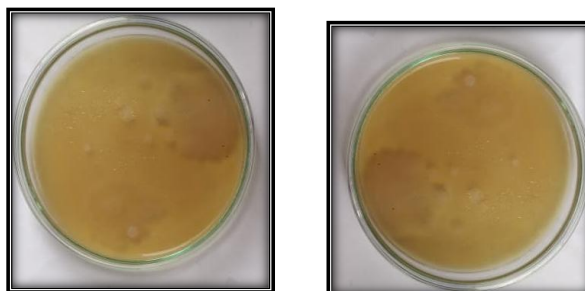


Fig no.5: Growth of organism on sweet lemon peel

For 10 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
5mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

Dry sweat lemon peel

sweet lemon peel collected from were collected from household, market, juice centre, near Tawde hotel, Shahu market yard, Kolhapur and the collected peel grinded to make a paste. preparing sweet lemon peel media.

Filtered sweet lemon peel media:

Filtered dry sweet lemon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered dry sweet lemon peel media supports the cultivation of bacteria (Fig no.6).

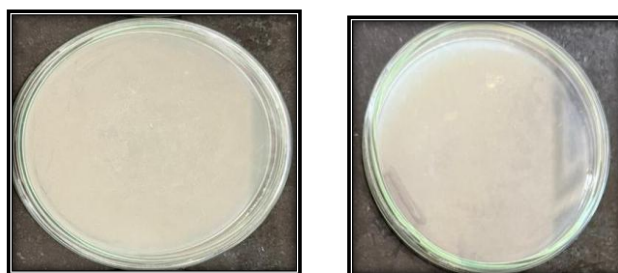
Media plate:

Fig no.6: sweet lemon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon dry peel agar media plate after incubation at 37°C for 24 hrs (Fig no.7).

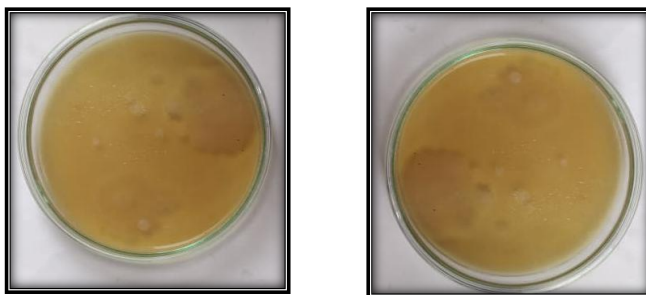


Fig no.7: Growth of organism on sweet lemon peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Low Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Low Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Low Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Low Convex	Moist	Opaque

Table 2: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10	Gram positive	Short rod
20	Gram positive	Short rod
40	Gram positive	Short rod
60	Gram positive	Short rod

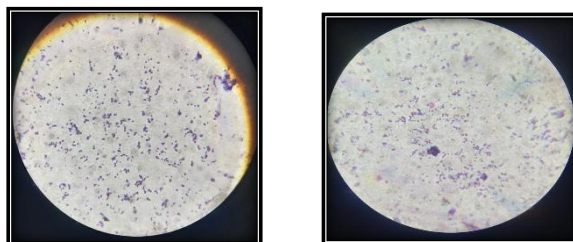
Gram staining:

Fig no.8: Gram staining of microorganism

Unfiltered sweet lemon peel media:

dry sweet lemon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that dry sweet lemon peel media supports the cultivation of bacteria (Fig no.9).

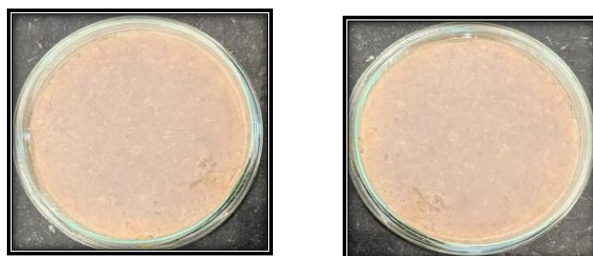
Media plate:

Fig no.9: sweet lemon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon dry peel agar media plate after incubation at 37°C for 24 hrs (Fig no.10).



Fig no.10: Growth of organism on sweet lemon peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

2.watermelon

watermelon peel collected from were collected from household, market, juice centre, near Tawde hotel, Shahu market yard, Kolhapur and the collected peel grinded to make a paste. preparing watermelon peel media.

Wet watermelon peel**Filtered watermelon peel media:**

Filtered watermelon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 48 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered watermelon peel media supports the cultivation of bacteria (Fig no.11).

Media plate:

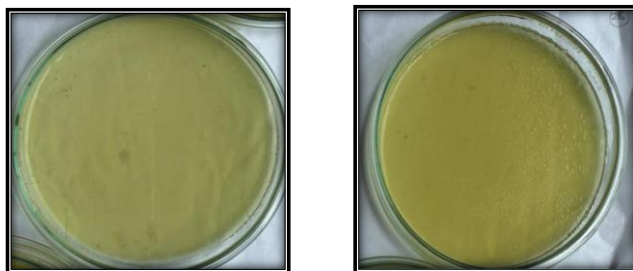


Fig no.11: watermelon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on watermelon peel agar media plate after incubation at 37°C for 48 hrs (Fig no.12).

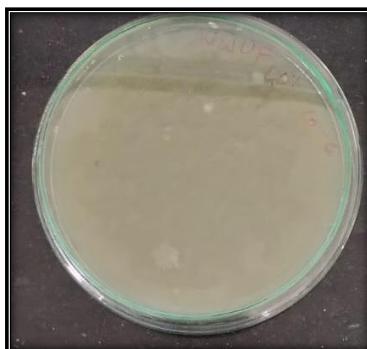


Fig no.12: Growth of organism on watermelon peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
1mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile watermelon on peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 3: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod
20%	Gram positive	Short rod
40%	Gram positive	Short rod
60%	Gram positive	Short rod

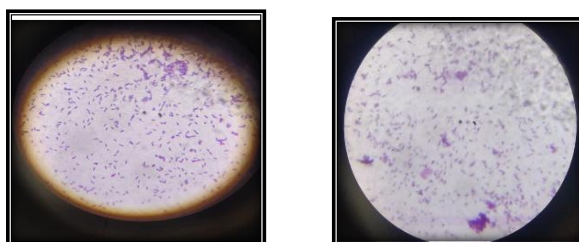
Gram staining:

Fig no.13: Gram staining of microorganism

Unfiltered watermelon peel media:

watermelon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 48 hours. The colonies showed distinct morphological characteristics. The growth indicates that watermelon peel media supports the cultivation of bacteria (Fig no.14).

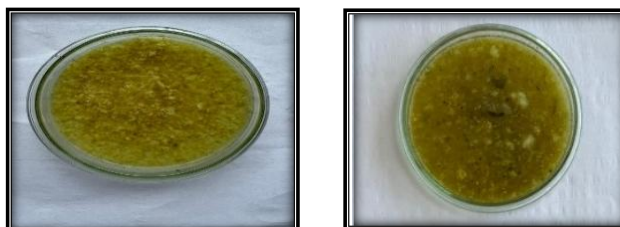
Media plate:

Fig no.14: watermelon peel media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on watermelon wet peel agar media plate after incubation at 37°C for 48 hrs (Fig no.15).

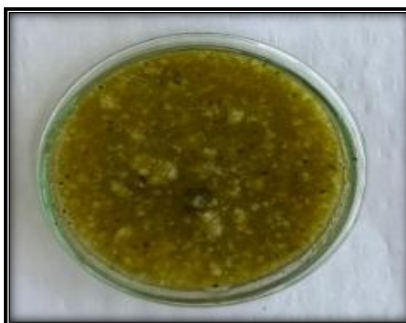


Fig no.15: Growth of organism on watermelon peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40% conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60% conc.:

colony characters of well isolated organisms grown on sterile watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

3.Fenugreek

Fenugreek peel collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing fenugreek peel media.

Wet Fenugreek stem

Filtered fenugreek stem media:

Filtered fenugreek stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that Filtered fenugreek stem media supports the cultivation of bacteria (Fig no.16).

Media plate:

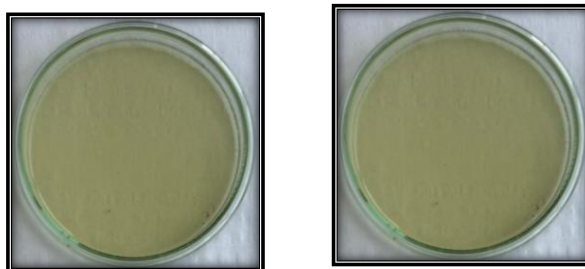


Fig no.16: Fenugreek stem media plate

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek stem wet agar media plate after incubation at 37°C for 24 hrs (Fig no.17).

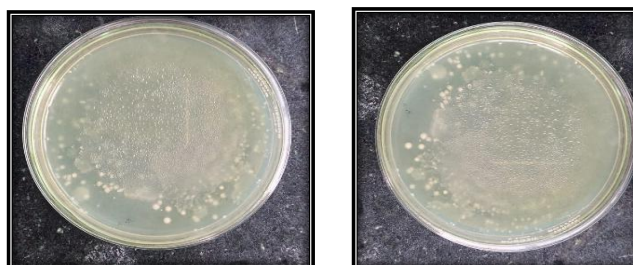


Fig no.17: Growth of organism on Fenugreek stem peel

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
1mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 4: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod
20%	Gram positive	Cocci
40%	Gram positive	Short rod
60%	Gram positive	Short rod

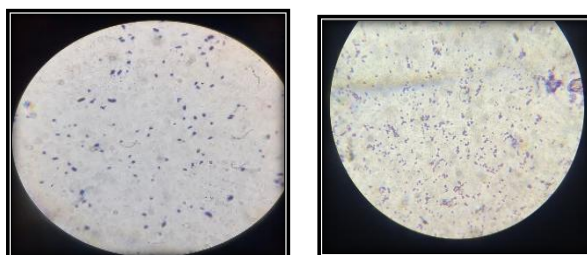
Gram staining:

Fig no.18: Gram staining of microorganism

Unfiltered Fenugreek stem media:

fenugreek stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that fenugreek stem media supports the cultivation of bacteria (Fig no.19).

Media plate:

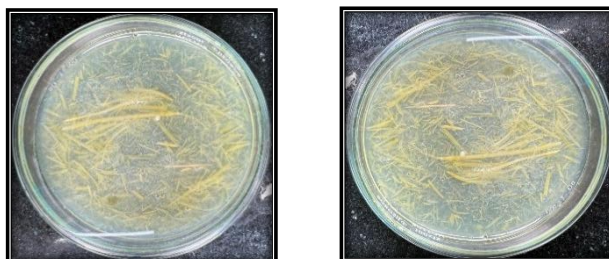


Fig no.19: fenugreek stem media plate

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek stem agar media plate after incubation at 37°C for 24 hrs (Fig no.19).



Fig no.19: Growth of organism on Fenugreek stem agar.

For 10 % conc.

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Dry Fenugreek stem

Fenugreek peel collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing fenugreek peel media.

Filtered fenugreek stem media:

Filtered dry fenugreek stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered dry fenugreek stem media supports the cultivation of bacteria (Fig no.20).

Media plate:

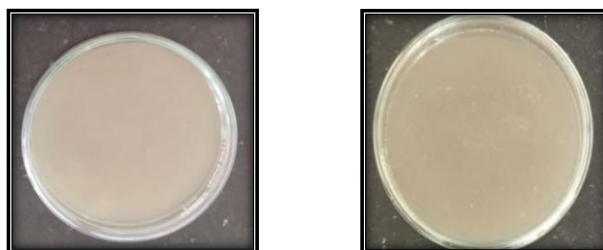


Fig no.20: fenugreek stem media plate

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek stem dry agar media plate after incubation at 37°C for 24 hrs (Fig no.21).



Fig no.21: Growth of organism on Fenugreek stem agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

Table 5: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod, arranged singly
20%	Gram positive	Cocci
40%	Gram positive	Short rod
60%	Gram positive	Short rod

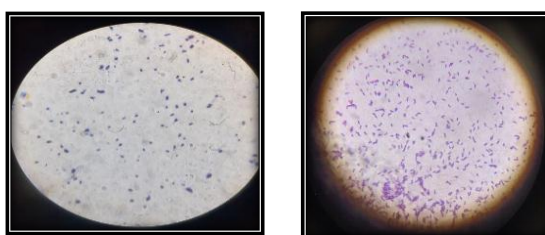
Gram staining:

Fig no.22: Gram staining of microorganism

Unfiltered Fenugreek stem media:

Dry fenugreek stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that dry fenugreek stem media supports the cultivation of bacteria (Fig no.23).

Media plate:

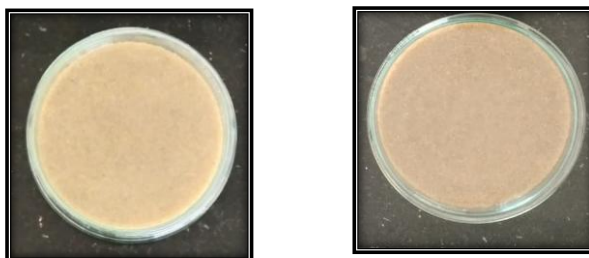


Fig no.23: fenugreek stem media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek stem agar media plate after incubation at 37°C for 24 hrs (Fig no.24).

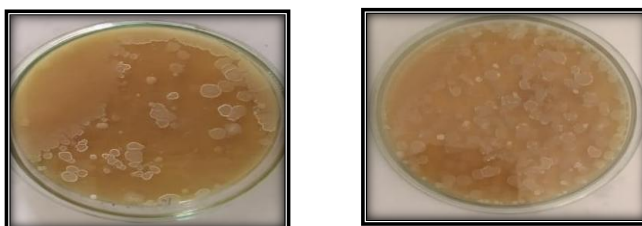


Fig no.24: Growth of organism on Fenugreek stem peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 20 % conc.:

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

4. Red amaranth

Red amaranth peel collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing red amaranth peel media.

Wet Red amaranth stem

Filtered Red amaranth stem media:

Filtered red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered red amaranth stem media supports the cultivation of bacteria (Fig no.25).

Media plate:

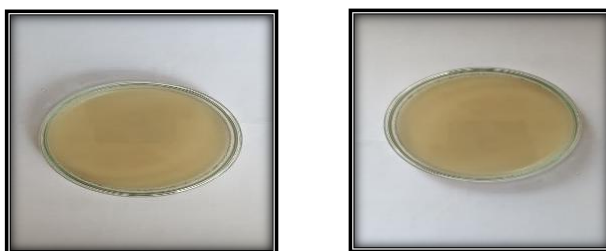


Fig no.25: red amaranth stem media plate .

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on red amaranth stem wet agar media plate after incubation at 37°C for 24 hrs (Fig no.26).

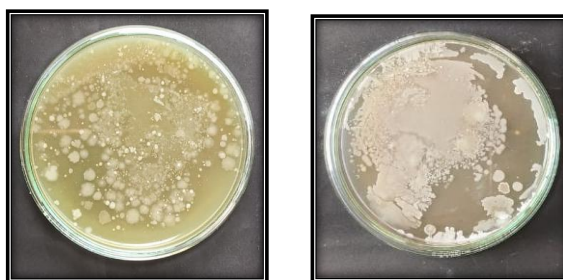


Fig no.26: Growth of organism on red amaranth stem peel agar.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 6: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	shape
10%	Gram negative	Short rod
20%	Gram positive	Short rod
40%	Gram positive	Cocci
60%	Gram positive	Short rod

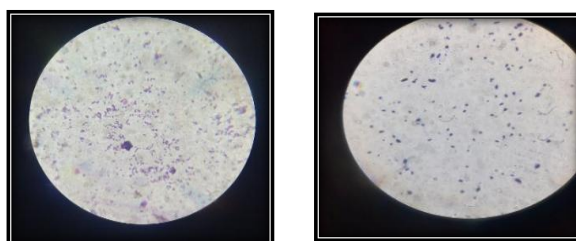
Gram staining:

Fig no.27: Gram staining of microorganism

Unfiltered Red amaranth stem media:

red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that red amaranth stem media supports the cultivation of bacteria (Fig no.28).

Media plate:

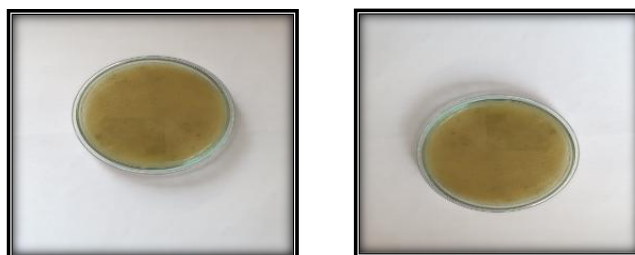


Fig no.28: red amaranth stem media plate.

Colony character

Colony characters of well isolated colony of organism isolated from soil sample observed on red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.29).



Fig no.29: Growth of organism on red amaranth stem peel

For 10 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Dry Red amaranth stem

Red amaranth peel collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing red amaranth peel media.

Filtered Red amaranth stem media:

Filtered dry red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered dry red amaranth stem media supports the cultivation of bacteria (Fig no.30).

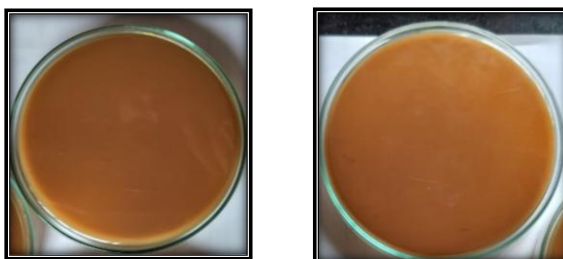
Media plate:

Fig no.30: red amaranth media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.31).

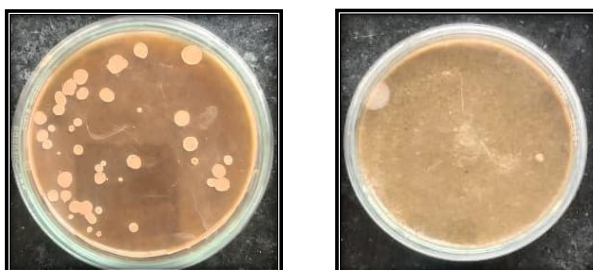


Fig no.31: Growth of organism on red amaranth stem peel

For 10 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Rough	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Unfiltered Red amaranth stem media:

Dry fenugreek stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that dry fenugreek stem media supports the cultivation of bacteria (Fig no.32)

Media plate:

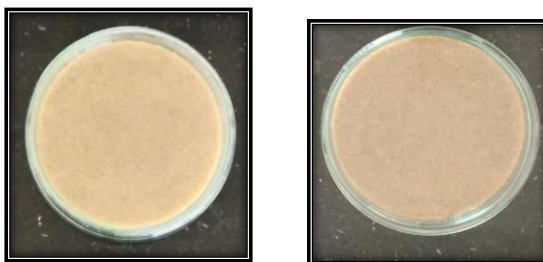


Fig no.32: red amaranth media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.33).



Fig no.33: Growth of organism on red amaranth stem peel

For 10 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

Combination of sweet lemon and watermelon:

Preparation of Microbial Media for Cultivation of Bacteria Using Fruit Peel and Vegetable Waste

Sweet lemon and watermelon peel collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing sweet lemon and watermelon peel media.

Wet Combination of sweet lemon and watermelon

Filtered sweet lemon and watermelon peel media:

Filtered Sweet lemon and watermelon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that Filtered Sweet lemon and watermelon peel media supports the cultivation of bacteria (Fig no.34).

Media plate:

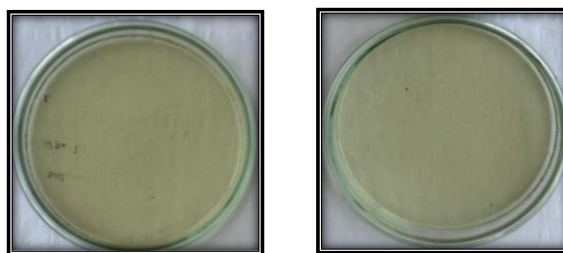


Fig no.34: combination of sweet lemon and watermelon peel media plate

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon and watermelon peel agar media plate after incubation at 37°C for 24 hrs (Fig no.35).



Fig no.35: Growth of organism on red amaranth stem peel.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 7: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod
20%	Gram positive	Short rod
40%	Gram positive	Short rod
60%	Gram positive	Short rod

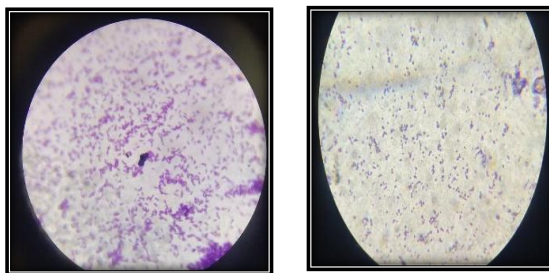
Gram staining:

Fig no.36: Gram staining of microorganism

Unfiltered sweet lemon and watermelon peel media:

Sweet lemon and watermelon peel extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that sweet lemon and watermelon peel media supports the cultivation of bacteria (Fig no.37).

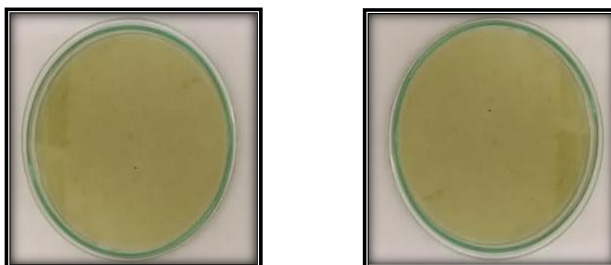
Media plate:

Fig no.37: combination of sweet lemon and watermelon media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on sweet lemon and watermelon peel agar media plate after incubation at 37°C for 24 hrs (Fig no.38).

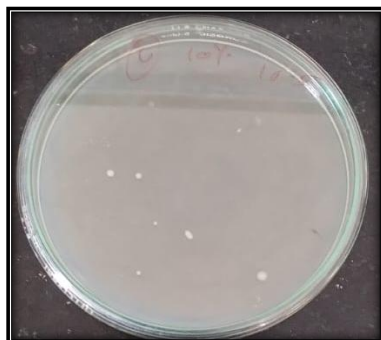


Fig no.38: Growth of organism on red amaranth stem peel media plate.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Sticky	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile sweet lemon and watermelon peel media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Combination Fenugreek and Red amaranth:

Fenugreek and Red amaranth stem collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing Fenugreek and Red amaranth stem media.

Wet Combination of Fenugreek and Red amaranth**Filtered Fenugreek and Red amaranth stem media:**

Filtered Fenugreek and Red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that

Filtered Fenugreek and Red amaranth stem media supports the cultivation of bacteria (Fig no.39).

Media plate:

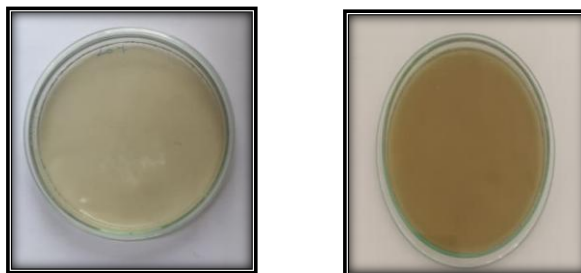


Fig no.39: Fenugreek and Red amaranth stem media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek and red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.40).

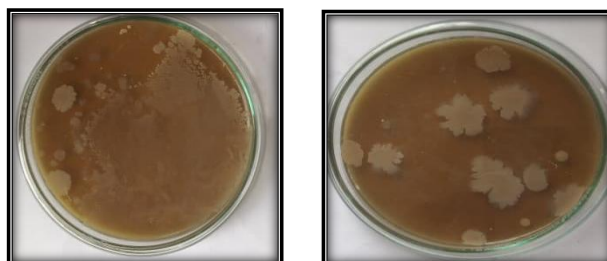


Fig no.40: Growth of organism on Fenugreek and Red amaranth stem media plate.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
3mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 8: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod
20%	Gram positive	cocci
	Gram positive	cocci
60%	Gram positive	Short rod

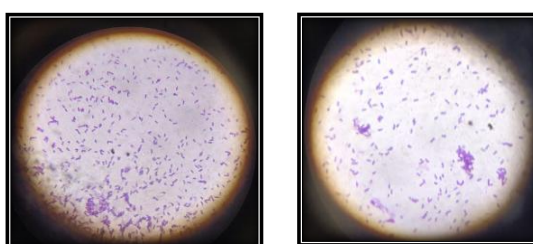
Gram staining:

Fig no.41: Gram staining of microorganism

Unfiltered Fenugreek and Red amaranth stem media:

Fenugreek and Red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that Fenugreek and Red amaranth stem media support the cultivation of bacteria (Fig no.42).

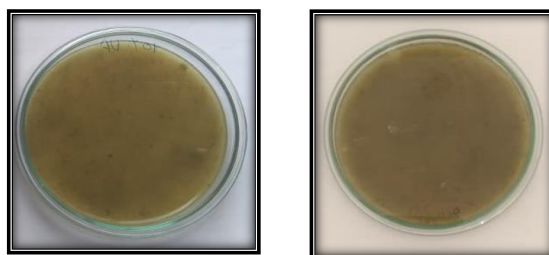
Media plate:

Fig no.42: combination of fenugreek and red amaranth media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek and red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.43).



Fig no.43: Growth of organism on Fenugreek and Red amaranth stem media plate.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Dry Combination of Fenugreek and Red amaranth

Fenugreek and Red amaranth stem collected from were collected from household, market, juice centre, Vegetable market, Nigave Khalsa, Ichalkarnji and the collected peel grinded to make a paste. preparing Fenugreek and Red amaranth stem media.

Filtered Fenugreek and Red amaranth stem media:

Filtered dry fenugreek and red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that filtered dry fenugreek and red amaranth stem media supports the cultivation of bacteria (Fig no.44).

Media plate:

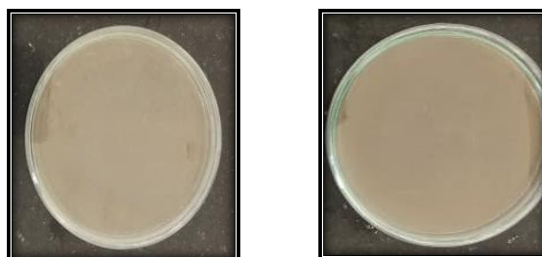


Fig no.44: combination of fenugreek and red amaranth media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek and red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.45).



Fig no.45: Growth of organism on Fenugreek and Red amaranth stem media plate.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20% conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Table 9: Gram nature organisms isolated on different concentration media plate.

Media concentration	Gram nature	Shape
10%	Gram positive	Short rod
20%	Gram positive	Cocci
40%	Gram positive	Short rod
60%	Gram positive	Short rod

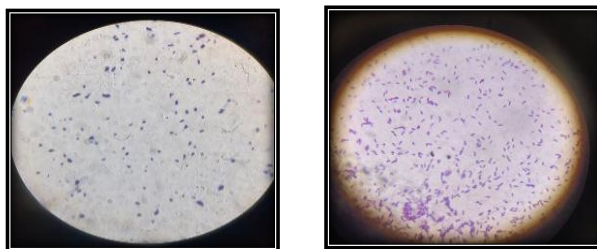
Gram staining:

Fig no.46: Gram staining of microorganism

Unfiltered Fenugreek and Red amaranth stem media:

Dry fenugreek and red amaranth stem extract was used to prepare agar media. The media was inoculated with soil sample and incubated at 37°C for 24 hours. The colonies showed distinct morphological characteristics. The growth indicates that dry fenugreek and red amaranth stem media supports the cultivation of bacteria (Fig no.48).

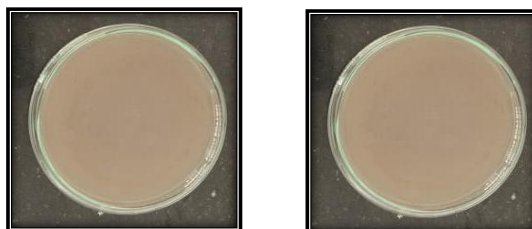
Media plate:

Fig no.47: fenugreek and red amaranth stem media plate.

Colony character:

Colony characters of well isolated colony of organism isolated from soil sample observed on fenugreek and red amaranth stem agar media plate after incubation at 37°C for 24 hrs (Fig no.48).



Fig no.48: Growth of organism on Fenugreek and Red amaranth stem media plate.

For 10 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 20 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 40 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

For 60 % conc.:

colony characters of well isolated organisms grown on sterile fenugreek and red amaranth stem media incubated at 37°C for 24 hrs.

Size	Shape	Colour	Margin
2mm	Circular	White	Entire
Surface	Elevation	Consistency	Opacity
Smooth	Convex	Moist	Opaque

Use of formulated media for growth of standard organisms.

We prepared media from fruit and vegetable waste. Microorganism isolated from soil sample and medium support their growth, weather the standard organism grow or not, so spot inoculation was performed and growth of organism observed (Fig no. 49).

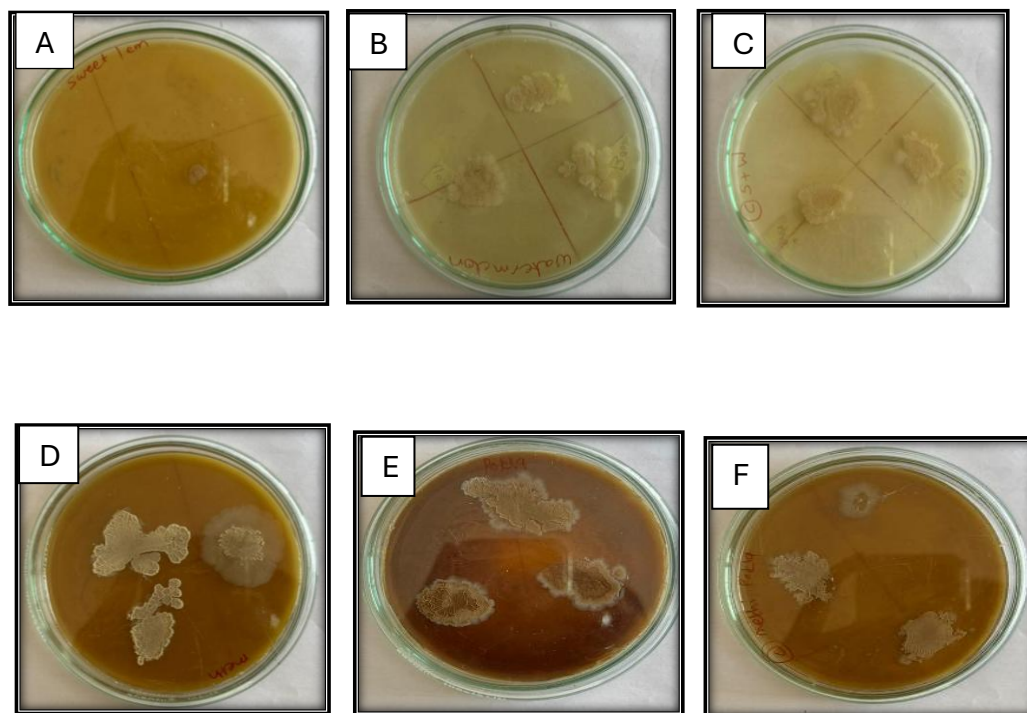


Fig no.49: Growth of organism on different media plate.

A = sweet lemon

B = watermelon

C = combination of sweet lemon and watermelon

D = fenugreek

E = red amaranth

F = combination of fenugreek and red amaranth

Table no.10: Comparison of growth of known organisms on different media formulated using waste

Media	<i>E.coli</i>	<i>Bacillus</i>	<i>Staphylococcus Aureus</i>
Sweet lemon	+	-	-
Watermelon	++	+	+
Combination of sweet lemon and watermelon	++	+	+
Fenugreek	++	++	+
Red amaranth	+	++	+
Combination of fenugreek and red amaranth	++	++	+

- = Indicates no growth.

+ = Indicates growth of organism.

++ = Indicates maximum growth of organism.



Discussion



The present study aimed to develop a low-cost culture medium using fruit peel and vegetable waste and to evaluate its ability to support microbial growth (Ravindran & Jaiswal, 2016; Sadh et al., 2018). In this study, fruit peels of sweet lemon and watermelon along with vegetable wastes of fenugreek and red amaranth were used for the preparation of culture media. These wastes were selected because they contain nutrients such as carbohydrates, vitamins, and minerals that can support microbial growth. Therefore, the prepared waste-based media were evaluated as a low-cost alternative to conventional culture media. Commercial microbiological media such as nutrient agar and potato dextrose agar are widely used for the cultivation of microorganisms in laboratories (Atlas, 2010; Madigan et al., 2018). However, these media are often expensive and may not be easily available in resource-limited settings (Ravindran et al., 2018). Therefore, alternative substrates derived from fruit peel and vegetable waste materials have been explored as potential culture media (Sadh et al., 2018; Pathak et al., 2019).

Fruit and vegetable wastes are rich in carbohydrates, proteins, minerals, and vitamins that can support microbial metabolism and growth (Ravindran & Jaiswal, 2016; Galanakis, 2012). In the present study, fruit peels of sweet lemon and watermelon and vegetable wastes of fenugreek and red amaranth were used for the preparation of culture media. These wastes contain nutrients that can support microbial metabolism and growth, making them suitable as alternative low-cost substrates for culture media preparation. The findings of the present study support the hypothesis that fruit peel and vegetable waste extracts can be utilized as alternative nutrient sources for microbial cultivation (Pathak et al., 2019; Sadh et al., 2018).

The successful microbial growth observed on the prepared waste-based culture medium indicates that fruit peels contain sufficient nutrients to support microbial metabolism and cell division (Ravindran et al., 2018; Galanakis, 2012). The observed microbial growth on this medium indicates that these wastes can effectively support microbial metabolism and cell division. Fruit residues contain simple sugars, cellulose, hemicellulose, organic acids, and trace minerals that act as carbon and energy sources for microorganisms (Sadh et al., 2018; Kumar et al., 2020). The observed microbial growth on this medium indicates that these wastes can effectively support microbial metabolism and cell division. These compounds are essential for microbial respiration, enzyme production, and biomass formation (Madigan et al., 2018). The observed microbial growth demonstrates that these natural wastes can effectively support

microbial development. Previous study reported that fruit peel waste contains high levels of carbohydrates and other nutrients that promote microbial growth (Ravindran & Jaiswal, 2016; Galanakis, 2012).

Experimental evidence suggests that fruit peel extracts can provide adequate nutrients to support the growth of bacteria, fungi, and cyanobacteria when used as culture media components (Pathak et al., 2019; Kumar et al., 2020). These results are consistent with the observations of the present study, where visible microbial colonies were obtained after incubation on the formulated waste-based culture medium (Ravindran et al., 2018). Similar results were observed in our study, where clear microbial colonies developed on our formulated medium. The results obtained in this study are also consistent with research comparing fruit-based media with standard laboratory media (Kumar et al., 2020). Certain microorganisms even showed enhanced growth in fruit-derived media because of the presence of natural sugars and bioactive compounds (Sadh et al., 2018). These findings suggest that fruit peel media can serve as viable alternatives to conventional microbiological media, especially for routine laboratory work and teaching purposes (Ravindran & Jaiswal, 2016).

Another important aspect of fruit peel-based culture media is their environmental and economic significance (Galanakis, 2012). The findings of our study are related to this observation, as the culture media prepared from sweet lemon and watermelon peels, along with vegetable wastes of fenugreek and red amaranth, also supported effective microbial growth. These natural materials contain sugars, vitamins, and other nutrients that help microorganisms grow well. Therefore, the results indicate that fruit and vegetable wastes can act as suitable nutrient sources for developing alternative culture media. Large quantities of fruit and vegetable wastes are generated globally from households, markets, and food processing industries (Sadh et al., 2018). Improper disposal of these wastes can lead to environmental pollution and microbial contamination of soil and water (Ravindran et al., 2018). Utilizing fruit peel waste as a substrate for microbial culture media represents a sustainable approach to waste management (Sadh et al., 2018; Galanakis, 2012).

Agricultural by-products such as banana peel, papaya peel, and citrus peel can be effectively converted into microbial growth media, reducing environmental waste while supporting sustainable biotechnology practices (Ravindran & Jaiswal, 2016). In our study, sweet lemon and watermelon peels, along with fenugreek and red amaranth waste, were successfully utilized as nutrient-rich substrates for culture media

preparation. This demonstrates a practical application of sustainable biotechnology by converting local organic waste into cost-effective and eco-friendly microbial growth media, aligning with the findings of Ravindran & Jaiswal (2016). In addition to laboratory applications, fruit peel substrates have significant potential in industrial microbiology and biotechnology (Kumar et al., 2020). Fruit peel hydrolysates have been used as alternative substrates for fermentation processes, enzyme production, and microbial biomass generation (Sadh et al., 2018). For instance, mango peel waste has been utilized as a low-cost medium for bacterial cellulose production (Ravindran et al., 2018). These findings highlight the broader biotechnological potential of fruit peel substrates beyond laboratory culture media preparation (Kumar et al., 2020).

The use of fruit peel media also offers several practical advantages (Ravindran & Jaiswal, 2016). The statement aligns with our project as it emphasizes the usefulness of fruit peel-based media in practical applications. In our work, sweet lemon and watermelon peels along with fenugreek and red amaranth waste were effectively used to prepare nutrient-rich culture media. This approach highlights affordability, easy availability, and sustainable utilization of organic waste, as supported by Ravindran & Jaiswal (2016). The raw materials required for preparation are readily available and inexpensive, making them suitable for educational laboratories and developing countries (Sadh et al., 2018).

Fruit peel-based media contribute to waste recycling and environmental sustainability by converting organic waste into useful laboratory materials (Galanakis, 2012). Such media can also promote innovative teaching approaches in microbiology by demonstrating the relationship between natural nutrient sources and microbial growth (Madigan et al., 2018). The given statement connects with our project by showing that using natural materials can improve understanding in Microbiology through practical demonstration.

Despite these advantages, several limitations are associated with fruit peel-based culture media (Kumar et al., 2020). One limitation is the variability in nutrient composition among different fruit wastes (Ravindran et al., 2018). Nutrient concentrations may vary depending on fruit type, ripeness stage, environmental conditions, and storage methods (Sadh et al., 2018). These variations may affect microbial growth rate and colony characteristics (Pathak et al., 2019).

In our project, the use of sweet lemon and watermelon peels along with fenugreek and red amaranth waste may lead to differences in nutrient composition, similar to the limitations mentioned in the citation. These variations can occur due to differences in the type and condition of the raw materials used. As a result, the prepared culture media may show changes in microbial growth and colony appearance.

Another limitation is the possibility of natural microbial contamination present on fruit surfaces (Madigan et al., 2018). Fruit peels naturally harbor microorganisms that may interfere with experimental results if not properly sterilized (Atlas, 2010). Therefore, careful washing, extraction, and sterilization procedures such as autoclaving are necessary to ensure sterile culture media preparation (Madigan et al., 2018). Further research is required to standardize preparation methods and nutrient concentrations to ensure consistent microbial growth results (Kumar et al., 2020).

Future research should focus on optimizing the formulation of fruit peel-based media tailored to specific microorganisms (Sadh et al., 2018). In the present project, culture media prepared from the peels of sweet lemon and watermelon, along with vegetable wastes such as fenugreek and red amaranth, highlights the potential of agro-waste as a nutrient source. Since different microorganisms have distinct nutritional requirements, the composition of these peel- and leaf-based media may need to be adjusted to achieve optimal microbial growth (Madigan et al., 2018). Furthermore, combining fruit peels (sweet lemon and watermelon) with leafy vegetable wastes (fenugreek and red amaranth) can improve nutrient balance and enhance microbial productivity (Ravindran et al., 2018). Biochemical analysis of these materials can help identify the specific nutrients responsible for supporting growth, thereby enabling further refinement of the media (Galanakis, 2012)

Overall, the results of the present study support previous research demonstrating that fruit peel and vegetable waste extracts can function as effective and economical alternatives to conventional microbiological culture media (Ravindran & Jaiswal, 2016; Sadh et al., 2018). The ability of these waste-derived media to support microbial growth highlights their potential applications in microbiology laboratories, biotechnology research, and waste management strategies (Kumar et al., 2020).

Conclusion

The present research project was successfully carried out with the objective of preparing an alternative culture medium for bacterial growth by utilizing agro-waste materials, specifically fruit peels of sweet lemon and watermelon along with vegetable waste of fenugreek and red amaranth. The study clearly demonstrates that these commonly discarded organic materials can be effectively transformed into a nutrient-rich culture medium capable of supporting bacterial growth. This approach not only provides a scientific solution for microbial cultivation but also addresses important environmental and economic concerns associated with waste management and laboratory resource utilization.

A key outcome of this research is the confirmation that sweet lemon and watermelon peels, along with fenugreek and red amaranth waste, contain sufficient nutrients required for microbial growth. Sweet lemon peels are rich in citric acid, sugars, and essential minerals, which contribute to the maintenance of suitable pH and provide energy sources for bacterial metabolism. Similarly, watermelon peels contain carbohydrates, moisture, and micronutrients that enhance microbial growth. On the other hand, fenugreek leaves and red amaranth residues are known to be good sources of proteins, vitamins (such as vitamin A and C), iron, calcium, and other essential nutrients. The combination of these fruit and vegetable wastes creates a balanced nutrient profile, which supports the growth and proliferation of bacteria in a manner comparable to standard nutrient media.

Another important outcome of this research is the cost-effectiveness of the prepared media. Commercial culture media such as nutrient agar are relatively expensive, which may limit their use in small laboratories, educational institutions, and developing regions. The waste-derived culture media developed in this study can significantly reduce the cost of microbial cultivation while maintaining satisfactory bacterial growth. Furthermore, the preparation method used in this research was simple and feasible, making it suitable for laboratory-scale as well as potential large-scale applications. The findings suggest that fruit peel and vegetable waste-based media

could be used for routine bacterial cultivation, preliminary microbiological studies, and teaching laboratories.

The experimental observations obtained during this study strongly support the effectiveness of the prepared waste-based culture medium. Bacterial growth was successfully observed on the media prepared from the selected agro-waste materials, indicating that these natural substrates can provide the necessary conditions for microbial survival and multiplication. The growth patterns, colony formation, and overall development of bacteria suggest that the prepared medium is capable of fulfilling basic nutritional and environmental requirements. This validates the concept that agro-waste materials, when properly processed, can act as efficient substitutes for commercially available culture media.

An Important aspect of this project is the direct correlation between the selected waste materials and their functional contribution to the culture medium. Sweet lemon peels contributed to acidity and carbohydrate content, which helps in maintaining an optimal growth environment. Watermelon peels enhanced the moisture content and provided additional sugars, supporting bacterial metabolism. Fenugreek waste contributed nitrogenous compounds and proteins, which are essential for cell growth and enzyme production. Red amaranth waste provided vitamins, minerals, and trace elements that play a crucial role in metabolic activities. Thus, each component used in this study played a specific and complementary role in improving the quality and effectiveness of the prepared culture medium. This synergy among the selected materials highlights the scientific relevance of choosing these particular agro-wastes for the project.

Several microorganisms have been successfully cultivated on fruit and vegetable based culture media. Studies have demonstrated that bacteria such as *Escherichia coli*, *Bacillus*, *Staphylococcus aureus*, can grow effectively on media formulated from fruit and vegetable waste extracts. In one investigation, sweet lemon peel agar, watermelon media, and fenugreek and red amaranth media supported the growth of multiple Gram-positive and Gram-negative bacterial species including *E. coli*, *Staphylococcus aureus*, and *Bacillus*. These results are consistent with the observations of the present study, where visible microbial colonies were obtained after incubation on the formulated waste-based culture medium.

The study also highlights the environmental benefits of utilizing agro-waste materials. Fruit and vegetable wastes are generated in large quantities from households, markets, and food industries, and their disposal often leads to environmental pollution. Converting these wastes into culture media not only reduces waste accumulation but also promotes waste recycling and sustainable laboratory practices.

In conclusion, the present research successfully demonstrates that culture media prepared from sweet lemon and watermelon peels along with fenugreek and red amaranth waste can effectively support bacterial growth. The study highlights the potential of agro-waste as a low-cost, eco-friendly, and sustainable alternative to conventional culture media. It establishes a strong correlation between the nutrient composition of the selected waste materials and their ability to support microbial growth. This approach not only reduces environmental pollution but also provides an economical solution for microbiological studies. Overall, the research supports the idea that waste materials can be transformed into valuable scientific resources, contributing to sustainable development, resource conservation, and innovation in microbiology.

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