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Development of Biopolymer Sheet from *Bacillus sp* Using *Borassus Flabellifer* for Fruits and Vegetables Wrappers

Deepak R[1], Arunkumar P[2],Vishnupriyan A[3],Pritam dey[4]

[1,2,3]*UG Student, Department of Biotechnology, Muthayammal Engineering College, Rasipuram, Tamil Nadu, India.*

[4]*Assistant Prof.,Department of Biotechnology, Muthayammal Engineering College, Rasipuram, Tamil Nadu, India.*

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ABSTRACT: The study focused on the development of a biopolymer sheet using palmyra sprout (*Borassus flabellifer*) for application as fruits and vegetable wrappers. The biopolymer sheets are edible, biodegradable, easily compostable, and eco-friendly in nature. Ordinary plastic wrappers contribute to the creating issue of plastic contamination. By using polymer sheets, the entirety of plastic wastes in landfills and seas can be basically decreased, thereby making a difference to the Environment. Biopolymers used in these sheets are frequently derived from renewable sources like plant starch, cellulose, or sometimes from agricultural waste. This reduces dependence on fossil fuels and promotes a more sustainable production process and products. The methodology employed in the present study involved preliminary screening of *Bacillus* sp. as qualitative analysis using Sudan Black method, followed by the extraction of PHB using chloroform extraction method. The extracted PHB was characterised by the FTIR (Fourier Transform Infra-Red) spectroscopic analysis method. The palmyra sprout extract was collected by utilising the Soxhlet extraction method. A qualitative phytochemical screening of palmyra sprout extract was performed to identify the class of compounds present in extract.

KEYWORDS: Biopolymer, PHB, *Bacillus* Species, Palmyra sprout, FTIR, phytochemical analysis.

I.INTRODUCTION

1.1. BIOPOLYMER

Biopolymer sheets are typically biodegradable, they can break down normally within the environment without leaving behind harmful microplastic, this can be particularly critical for food and vegetable wrappers, as they are likely to conclude up within the waste stream. Conventional plastic wrappers contribute to the developing issue of plastic contamination. By utilising polymer sheets, the sum of plastic wastes in landfills and oceans can be essentially decreased, making a difference to relieve natural harm. Biopolymer-based edible films, formed from polysaccharides or blends of polysaccharides containing proteins, lipids, and food grade additives, are suitable for human consumption and can increase the shelf-life and quality of food products. Biopolymers utilised in these sheets are frequently derived from renewable sources like plant starch, cellulose, or even agricultural waste. This reduces dependence on fossil fills and promotes a more sustainable production process. Palmyra sprout cellulose is extracted from the fibres and transformed into a sheet in order to produce a biopolymer sheet. Processes including casting, chemical processing, and pulping can be used to accomplish this. A biopolymer sheet made from the extracted cellulose can subsequently be produced and used for a variety of purposes, including packaging and biodegradable products. Remember that depending on the desired sheet qualities and the tools at hand, the precise processes and methods may change. When biopolymers are applied to paper, it gains intriguing properties while retaining the material's environmentally favourable qualities, such as its capacity to be recycled and composted. When it comes to this project, starch has emerged as a possible candidate because it can be utilised to make edible food packaging from both conventional and unconventional sources. "Biopolymer" refers to polymers made by living things. Cellulose and other organic substances found in palmyra sprout, sometimes called the "Ice Apple" or "Tal palm," may be utilised to make biopolymers. Given that they are frequently biodegradable and more environmentally friendly than conventional

petroleum based polymers, these biopolymers may find use in a variety of industries, including packaging, textiles, and medical equipment. Research is still being done to see whether Palmyra sprouts can be used to make biopolymers.

1.2. BORASSUS FLABELLIFER (PALMYRA SPROUT)

Borassus flabellifer belongs to the family Arecaceae, commonly known as Palmyra palm is native to tropical Africa but cultivated and naturalised throughout India. The different parts of the plant are being used for medicinal properties like antihelminthic and diuretics. Toddy palm sprouts, sometimes referred to as palmyra sprouts, have a number of possible health advantages. They are a good source of vitamins (A, B, and C), minerals (potassium, calcium, and iron), and dietary fibres, among other important components. Palmyra sprouts may aid in better digestion, immune system support, and energy production. It's crucial to remember that there may not be much scientific research on these specific advantages. It has 98% dietary fibre and starch is the major carbohydrate present in palmyra shoot flour. Glucose, fructose, and sucrose are their major sugars. It has considerable fibre feeding low glycaemic index [1]. 3 Palmyra products are found to have anti-inflammatory, anti-arthritic, cytotoxic, antibacterial, analgesic, antipyretic, hypoglycemic, and antioxidant properties. The constituents of Borassus flabellifer are gums, albuminoids, steroidal glycosides, fats and carbohydrates like sucrose, spirostane-type steroids like brass sides and dioscin are found in the plant. Antimicrobial activity is noted in seed coat extract of the Borassus flabellifer [2]. The Palmyra palm, also known as the Borassus flabellifer, is treasured for its roots. The roots are important in crafts and construction due to their strength and durability. Because of their cultural significance, the Palmyra palm and its root have an impact on religious ceremonies and practices in various places. It is commonly employed in the cooking of food, especially in South Asian cuisine, where it can be consumed raw, added to salads and desserts, or used to flavour beverages.

FIGURE 1.1 : PALMYRA SPROUTS

1.3 PHB (POLYHYDROXYBUTYRATE)

Poly-(3-hydroxybutyrate) (PHB) was the first isolated and characterised amongst polyhydroxyalkanoates (PHAs). PHB is highly crystalline due to its linear chain structure, containing both amorphous and crystalline phases. It can be found as a virgin polymer or as part of copolymers and blends. It is generated as a carbon reserve in a wide variety of producing bacterial strains and is produced industrially through bacterial fermentation. PHB also has a number of advantages over synthetic polymers for the production of certain packaging applications including: PHB barrier permeability is superior to both polyethylene (PE) and polypropylene (PP), and they are also found to be more rigid and less flexible than PP . Besides that, PHB exhibits good barrier properties in comparison to polyethylene terephthalate (PET) and polyvinyl chloride (PVC) [3]. Another main characteristic of PHB material is its biodegradability, occurring within a reasonable timescale when the material is in contact with degrading microorganisms in biologically active environments such as soils, fresh water, and aerobic and anaerobic composting, designating them as sought-after eco-friendly alternative for synthetic polymers [4]. As a member of the PHA family, PHB is characterised by having a methyl functional group (−CH3) and an ester linkage group (−COOR), it is these functional groups that are responsible for the materials thermoplastic, hydrophobic, high crystallinity, and brittle characteristics. The thermal properties of semi crystalline materials, such as PHB and its derivatives, typically include two main temperatures: a glass transition temperature (Tg) for their amorphous phase and a melting temperature (Tm) for the crystalline phase [5]. There is also the degradation temperature (Td), the temperature at which the material can start to degenerate. The degree of crystallinity can also be calculated using

these analytical methods. In recent years it has also been used for the investigation of the crystallisation of PHA materials [6].

1.4. PHB AS A BIOPOLYMER

Polyhydroxyalkanoate (PHA) is a group of linear polyester biopolymers produced by bacteria during the fermentation of sugars and lipids that are accumulated in the cell as a carbon and energy storage body under circumstances of nutrient deficiency and in the presence of excess carbon sources [7] . Atfirst, PHA was discovered in *Bacillus megaterium*, which are synthesised and stored by both gram-positive and gram-negative bacteria as insoluble biopolymers [8]. Poly(3-hydroxybutyrate) (PHB) is a homopolymer of 3-hydroxybutyrate and the most significant member of the biodegradable thermoplastic polyhydroxyalkanoate family, with characteristics of high melting temperature, a high degree of crystallinity, and low permeability to O2, H2O, and CO2. The average molecular weight of PHB varies from 2 to 4 x 103 KDa. The molecular weight depends on the ability of microbes to accumulate the produced polymer, conditions of growth, and extraction method. Polyhydroxybutyrate can be classified into different monomers based on the carbon chain attached: short length (contains 5 carbon PHB units), medium length (contains 6–14 carbon PHB units), and long length (contains more than 14 carbon PHB) [9]. PHB is a good replacement for synthetic polymer, and its mechanical properties are comparable to that of polypropylene. PHB possesses the characteristics of thermoplasticity and biodegradability in compost which has attracted commercial attention. PHB has great potential in food packaging applications with better water vapour barrier properties than polypropylene and better oxygen barrier properties than both polyethylene terephthalate and polypropylene [10]. The typical properties of PHB are compared with polypropylene. PHB monomer is a chiral molecule which is insoluble in water and exhibits a high degree of polymerization. The PHB monomers can be used for the synthesis of complex chiral pharmaceutical compounds and also have the potential to be used as chiral precursors [11]. PHB is biocompatible and can be implanted in the human body without any inflammatory response. Degradation of PHB is a slow process inside the body, and therefore PHB can be useful in the slow drug release application as a carrier [12]. The use of PHB can be explored in the field of tissue engineering due to its biocompatible characteristics [13]. PHB in vitro biocompatibility has been established on different cell lines such as fibroblasts, osteoblasts, bone marrow cells, endothelial cells, smooth muscle cells, etc. The PHB homopolymer is rigid and brittle in nature [14]. PHB has limited chemical resistance as it is attacked by acids and alkalis and dissolves in chlorinated solvents. PHB's degradation rate at melt-processing temperature is high; thereby its copolymers and blends are ideal for general applications. Blending of PHB with other polymers or with plasticizers offers opportunities to reduce the brittleness and improve process-ability by lowering the processing temperature [15].

1.5 PHB AS A BIOPLASTIC

PHB can be a potential substitute for petrochemical-based plastic given their similarity in mechanical properties. PHB is both biocompatible and biodegradable and its degradation product 3-hydroxybutyrate could be a typical mammalian metabolite [16]. PHB all of which are ordinarily shaped as intracellular inclusions in bacteria under unbalanced development conditions. Recently, it became of industrial interest to evaluate the PHB polyesters as natural biodegradable plastics for a wide range of possible applications such as surgical sutures to packaging containers. Commercial production of PHB is based on sugars and fatty acids as carbon sources. PHB depolymerase produced by soil and water microbes degrades and reduces PHB back to CO2 and H2O from which it is synthesised. Hence, one can envision a plastic production that depends on completely renewable assets instead of the limited fossil fuel utilised in petrochemical-based plastics.

1.6 BIOSYNTHESIS OF PHB

PHB is produced in the cells of microorganisms [17], as a product of microbial secondary metabolism, usually in conditions when the cells are subjected to nutrient stress or in an unfavourable environment such as carbon-excessive with limited nutrients [18], which is possible in both gram-positive and gram-negative bacteria. The accumulation of the material is a natural technique used by microorganisms to store carbon and energy when essential nutrient supplies are imbalanced or depleted [19]. It is important to note that there are a number of different species of bacteria which have been known to accumulate materials, such as PHBs as intracellular 8 granules, with reports stating that this number may exceed 75 different genera [20]. There are several different approaches for the extraction and recovery of PHB materials and its derivatives from bacterial cells. PHB synthesis relies on a central carbon metabolite from acetyl-

CoA through a sequence of three enzymatic reactions: 1. The reversible condensation of two acetyl-CoA moieties forming acetoacetyl-CoA, catalysed by β-ketothiolase (PhaA); 2. Acetoacetyl-CoA reduction to (R)-3-hydroxybutyryl-CoA by an acetoacetylCoA reductase (PhaB); 3. The polymerization of (R)-3-hydroxybutyryl-CoA catalysed by the enzyme PHB synthase (phbC gene) to produce PHB. The biosynthetic pathway of PHB from acetyl-CoA [21].

1.7 CHEMICAL STRUCTURE OF PHB

PHB is characterised by having a methyl functional group (-CH3) and an ester linkage group (-COOR), it is these functional groups that are responsible for the material's thermoplastic, hydrophobic, high crystallinity and brittle characteristics.

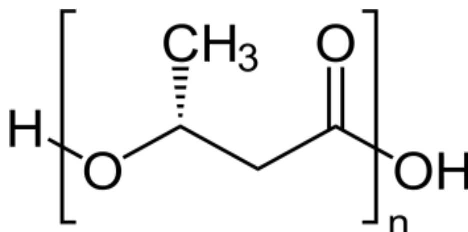

FIGURE 1.2: CHEMICAL STRUCTURE OF PHB

1.8 EXTRACTION OF PHB

According to Jahan Yousif (2018), PHB is extracted by several methods of extraction of poly beta-hydroxybutyrate from bacterial cells, including organic solvent extraction, enzyme digestion mechanic methods, and many other methods. The PHB extraction and purification step is a complementary step to production. In the last two decades, several methods of extracting the substance have been examined to find a viable economic method. The solvent extraction method is a commonly used method that can be applied to many microbiological organisms that produce polyhydroxybutyrate, PHB was extracted from R. eutropha bacteria after the digestion with hypochlorite preceded by an initial treatment substance such as sodium dodecyl sulphate (SDS). This method yielded a high molecular weight of the polymer due to the high cost of the solvent extraction method; it was replaced by enzymatic digestion and this replacement was developed by Imperial Chemical Industries (ICI). The enzymes used in digestion are lysosome, phosphorylase, phospholipase, Lecithin and protease. These enzymes work to digest most of the cellular components except the PHB which remains intact. The best method of extraction was obtained when using chloroform, methyl chloride and ethylene dichloride respectively.

1.9 APPLICATION OF PHB

PHB has many different applications in various sectors. Since PHB shares a lot of properties with some thermoplastics, it has been studied and developed as a candidate to substitute PP. For example, PHB has been tested for food packaging successfully and found to be more rigid than PP. Also, PHB is used for the fabrication of bottles, latex, and several products of agriculture and packaging. In industries, PHB alone or as a copolymer has been used as a diaper backs sheet and films. It has also been used as hot melt adhesives and as a toner in ion-conducting polymers[3]. PHB's are used as latex in paper coating applications, in dairy cream substitutes, food flavour agents and as raw materials for chemical and paint synthesis. In agriculture, PHB is commonly used for the release of pesticides and fertilisers. PHB in nitrogen fixing bacteria also plays an important role in nitrogen fixation efficiency, probably due to the participation of phaP gene in nodule formation .For all these medical applications, biocompatibility is a key aspect that PHB can fulfil. PHB's in general possess the biodegradability, biocompatibility and thermal properties that make them suitable for future pharmaceutical applications such as drug delivery and microencapsulation [22].

II. MATERIALS AND METHODS

2.1. ISOLATION OF PHB PRODUCING *Bacillus spp*. FROM SOIL

The sample is collected from the G.V. Residency near Sowripalayam, Mathampalayam. Coimbatore, Tamil Nadu, India, about 10-20 g of soil samples were collected, scraping to a 1-3 cm depth with a sterile spatula. The samples were placed in sterile plastic bags and stored at 4 °C and transported to the laboratory. Soil sample was taken from 10 cm depth with sterile spatula. Serial dilution technique was used considering different dilutions (10−1 to 10−7). The serially diluted sample was put in a petri plate with growth media and incubated at 37 oC[23].

2.2. PRELIMINARY SCREENING OF PHB USING SUDAN BLACK TECHNIQUE

0.1 g of sudan black powder was dissolved 75 ml of 95% ethanol & 25 ml of distilled water was added and it was made up to 100 ml. The mixture was filtered twice and it was stored. The plates were viewed under a microscope after 20 minutes[23].

2.3 QUANTITATIVE SCREENING OF PHB PRODUCING ISOLATES

The selected strains were taken and flooded with the sudan black for 20 mins. The stain was drained completely then it was viewed under the microscope[23].

2.4 EXTRACTION OF PHB BY CHLOROFORM EXTRACTION METHOD

Take 10 ml of broth in a centrifuge tube, and keep it in the centrifuge at 5000 rpm for 15 min. After centrifugation, add 2 ml of acetone & 2 ml of ethanol to the pellet and 2 ml of 4 % of sodium hypochlorite was added. The mixture was incubated in a water bath for 30 min under 40 oC. By adding 1 ml of ethanol & 1 ml of acetone & 2 ml of chloroform three layers were formed.The upper layer contains sodium hydrochloride and middle layer contains chloroform (undistributed dirt) and the bottom layer contains PHB. By using a micropipette the PHB was collected. The PHB were poured into the petri dishes and kept in a magnetic stirrer and heated for 10 min at 30 oC.The PHB was dried and collected in powder form.

2.5 CHARACTERIZATION OF PHB USING FTIR ANALYSIS

The extracted PHB was characterised by FTIR (Fourier Transform Infra-Red) Analysis. FTIR Spectroscopy is a form of vibrational spectroscopy, the sample was irradiated with infrared radiation from an infrared source, and absorption of the radiation stimulates Vibrational motions by depositing quanta of energy into vibrational modes. The functional groups of PHB were identified and compared with the standard references.

2.6 TO PREPARE & EXTRACT OF SLURRY BY SOXHLET METHOD

The Palmyra sprout is collected from the local shops of coimbatore.The Palm root is cut into small pieces and kept in the sunlight and dried for 72 hours. After 72 hours of drying, the dried Palm root grinds into powder form, and sieved twice and collected.

Extraction of palmyra sprouts by Soxhlet extraction using water

Take 50 g of sample and 150 ml of distilled water was added in Soxhlet tube. The sample solution is heated to reflux. And the solvent vapour travels up a distillation arm and floods into the chamber. The solid material in the chamber slowly fills with warm solvent. The solvent runs back down to the distillation flask. This cycle may be allowed to repeat many times, over a couple of hours or days. During each cycle, a portion of the compound dissolves in the solvent. After many cycles (24 hours) the desired compound is concentrated in the distillation flask. After extraction the insoluble portion was discarded and the solvent was collected.

2.7 PHYTOCHEMICAL ANALYSIS FOR PALMYRA SPROUTS

Phytochemical Analysis of Palmyra Sprout involves the identification of various bioactive compounds present in the sprouts.

Qualitative Screening Analysis for Palmyra Sprout

The qualitative analysis provides preliminary information about the presence of phytochemicals in palmyra sprouts.

Test For Tannins:

1 ml of plant extract and 3 ml of Bromine water are added in the test tube. The Disappearance of colour indicates the presence of tannins.

Test For Alkaloids:

1 ml of plant Extract & 1 ml of sodium hydroxide are added to the test tube. The formation of yellow colour indicates the presence of Alkaloids.

Test For Saponin:

1 ml of plant extract & 3 ml water added to the test tube. And shakes well the formation of foam after 10 min indicates the presence of saponins.

Test for Steroids:

1 ml of plant Extract and 2 ml of acetic acid & 1 ml of sulphuric acid added to the test tube. The presence of blue / green colour indicates the presence of steroids.

Test For Terpenoids:

1 ml of extract, 2 ml of chloroform & 1 ml of acetic acid then added 2 ml of sulphuric acid are added to the test tube. The presence of red, pink & violet indicates the presence of terpenoids.

Test For Glycoside:

1 ml of extract and 1 ml of ferric chloride & 1 ml of sulphuric acid are added to the test tube.The presence of blue/green colour indicates the presence of glycoside.

Test For Phenol:

1 ml of plant extract & 1 ml of ferric chloride are added to the test tube, observing the colour change.The black/green colour indicates the presence of phenol.

Test For Alkaloids:

1 ml of plant extract & 1 ml of iodine solution are added to the test tube, observe the colour change The blue colour indicates the presence of alkaloids.

Test For Quinine:

1 ml of plant extract & 1 ml of HCl are added to the test tube, The observation of green/ red, pink colour indicates presence of quinone.

Test for Protein:

1ml of plant extract & 1 ml of nitric acid are added to the test tube, The observation of the golden

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yellow colour indicates the presence of protein. **Test for Carbohydrate:**

1 ml plant extract & 2 ml of benedict's solution are added to the test tube, then observe if blue, green, orange indicate the presence of carbohydrate.

Test for Triterpenoids:

1 ml of plant extract & 1 ml of chloroform & 1 ml of acetic anhydride & conc. sulphuric acid is added to the test tube,The observation of violet colour indicates the presence of triterpenoids.

2.8 DEVELOPMENT OF SHEET WRAPPERS USING PHB AND PALMYRA SPROUT POWDER

Sample Collection of Sprout

Palmyra sprouts (borassus flabellifer) were collected from the streets of coimbatore.The root was cleaned and cut into small pieces and dried under the sun for 48 hours.Then the sprouts were powdered using the blenders.

Development of Bio Polymer Sheet (Palmyra Sprout)

Take 5 g of the sample (palm fine powder), and mix it with 50 ml of distilled water. Add 1.0 ml of glycerol, and 0.5 g of gelatin and 0.1 g of PHB to the solution. The mixture was stirred in a magnetic stirrer for 30 mins until the solid consistency formed. The mixture was poured into the aluminium foil and dried for 1-2 days. The sheet was peeled off.

2.9 ANTIMICROBIAL ACTIVITY

Agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly, to the procedure used in the disk diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. Generally, antimicrobial agents diffuse into the agar and inhibit germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured. It shows the growth media, temperature, period of incubation and inoculum size required by CLSI standards. The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be 20 uniformly circular as there will be a confluent lawn of growth. The diameter of the zone of inhibition can be measured in millimetres.

Nutrient Agar Medium

Composition of Nutrient Agar:

0.5% peptone and 0.3% of beef extract/yeast extract and 5% of agar is added into distilled water. The medium was prepared by dissolving 28 g of the commercially available Nutrient Agar Medium in 1000 ml of distilled water.Heat the mixture while stirring to fully dissolve all components. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25- 30ml/plate) while still molten. Culture of Test organisms. E.Coli (GRAM NEGATIVE) Staphylococcus aureus (GRAM POSITIVE) Petri plates containing 20 ml Nutrient Agar Medium were seeded with bacterial culture of E. coli and Staphylococcus aureus (growth of culture). Wells of approximately 10 mm were bored using a well-cutter and different concentrations of the sample such as 250 µg/mL, 500 µg/mL, and 1000 µg/mL were added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

Lawn culture method

A bacterial lawn culture is where an agar plate is inoculated with bacteria to produce a heavy uniform layer of growth over the whole surface of the agar without any individual colonies visible. This is often referred to as confluent growth. Lawn cultures are used to test the susceptibility of bacteria to antimicrobial substances such as antibiotics, antiseptics and disinfectants. Bacterial lawn cultures can be produced by 3 different methods: A pour plate technique where a bacterial suspension is flooded over the surface of the agar plate and any excess removed with a pipette. A spread plate method where the agar is inoculated with a sterile swab soaked with a bacterial suspension. A spread plate method where a bacterial suspension is spread over the surface of the agar using a sterile spreader. In this activity will produce a lawn culture using the spread plate method and a sterile swab soaked in a bacterial suspension.

III. RESULT AND DISCUSSION

3.1 ISOLATION OF PHB PRODUCING Bacillus sp. FROM SOIL

In the soil sample, the microorganisms were isolated and separate colonies were obtained by sequential dilution. In total, 5 bacterial colonies with various morphological characteristics were selected and the number was assigned to each colony. The colonies were stained on nutrient

adsorption plates and stored for further investigation.

TABLE 3.1: COLONY MORPHOLOGY

Strain No.	Colony morphology
B1	Large irregular colonies
B2	Small dried colonies
B3	Large dried edged colonies
B4	Large colonies
B5	Small irregular colonies

3.2 PRELIMINARY SCREENING OF PHB FROM (SUDAN BLACK TECHNIQUE)

Three colonies were displayed in Sudan black staining. They are B1, B2, and B5. These isolates tested positive for the presence of Sudan-black absorption patterns and lipophilic PHB granules.

TABLE 3.2 : PRODUCTION OF PHB

Strain No.	PHB production efficiency
B1	Moderate Production of PHB
B2	Minimum Production of PHB
B5	Maximum Production of PHB

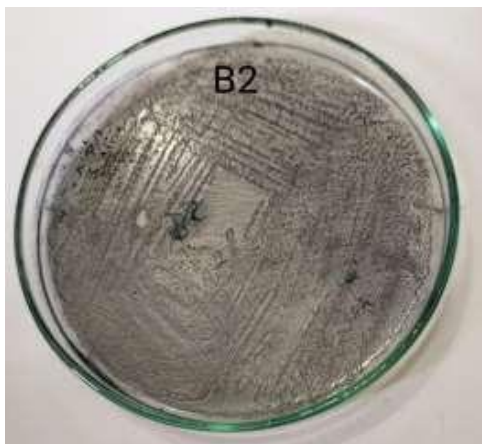

FIGURE 3.1: Strains of sudan black Stain B2

FIGURE 3.2 : Strains of sudan black Stain B5

3.3 EXTRACTION OF PHB HYDROCHLORIDE - CHLOROFORM EXTRACTION METHOD

Three phases are formed in the centrifuge tube. The upper phase contains sodium hypochlorite solution, the middle phase of chloroform containing undisturbed cells, and the bottom phase contains chloroform with the bottom phase containing (Chloroform with PHB) was precipitated into a Petri Plate and evaporated at a temperature of 70 °C in a Water bath for a period of 20 minutes. The PHB power was then collected in an Eppendorf tube.

FIGURE 3.3: EXTRACTED PHB

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3.4 FTIR ANALYSIS OF THE EXTRACTED PHB POWDER WITH A STANDARD PHB POWDER

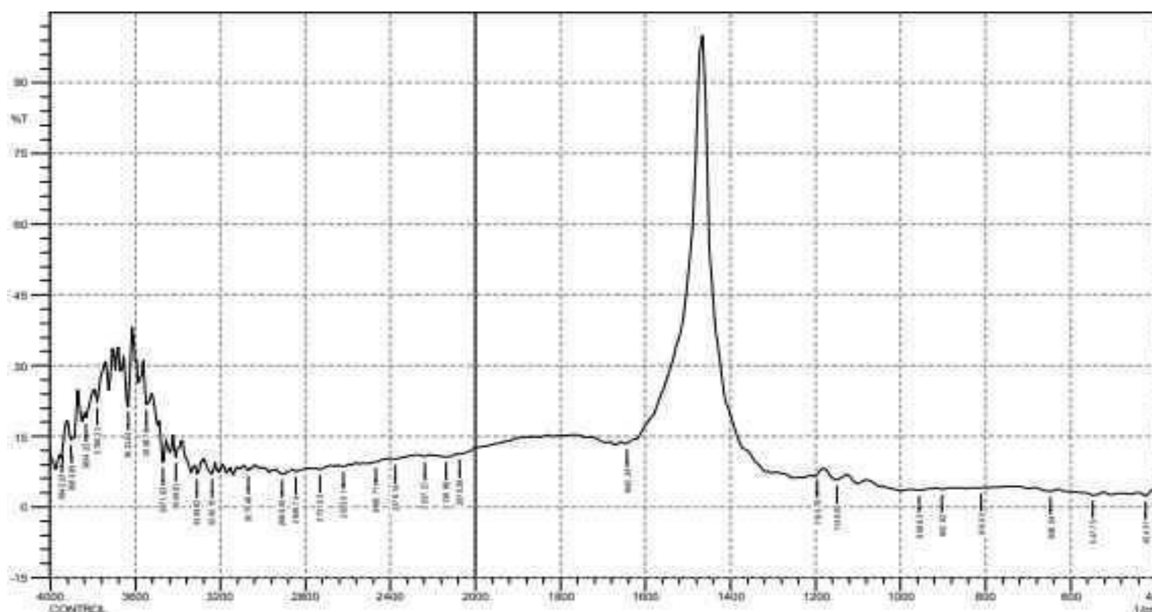

FIGURE 3.4: FTIR ANALYSIS STANDARD PHB

TABLE 3.3 COMPARISON OF STANDARD PHB AND EXTRACTED PHB

S. No.	PEAKS OF STANDARD PHB	PEAKS OF EXTRACTED PHB	FUNCTIONAL GROUP	DESCRIPTION
1	3873.06	3873.06	O-H stretching (alcohol)	Strong and bond
2	1689.64	1689.64	C-H bending (aromatic)	Overtone
3	1573.91	1573.91	C=O (ester)	Strong
4	1435.04	1435.04	C-H Methyl (alkene)	Medium
5	964.41	964.41	C=C Alkene	Strong (monosubstituted)

Peak I (3873.06) revealed the presence of OH Stretching in the extracted PHB, a similar peak observed with standard PHB. Peak 2 (1689.64) obtained shows the presence of C-H Bending reveals the presence of CH compound, the similar peak observed. Peak 3 (1573.91) shows the presence of C-O (thioester) in the extracted. It is also observed in the standard PHB. Peak 4 (1435.04) revealed the presence of the C-H (methyl-alkene) group, a similar peak observed in the PHB standard graph. Peak 5 (964.41) obtained shows the presence of C-C (alkene) reveals the presence of a similar peak observed.

3.5 TO PREPARE & EXTRACT OF SLURRY BY SOXHLET METHOD

Collection of Sample

FIGURE 3.5: THE PALMYRA SPROUT (*BORASSUS FLABELLIFER*)

FIGURE 3.6: PIECES OF PALMYRA SPROUT

FIGURE 3.7: POWDERED FORM OF PALMYRA SPROUT

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FIGURE 3.8: SOXHLET EXTRACTION METHOD

FIGURE 3.9: PALMYRA SPROUT EXTRACT

3.6 PHYTOCHEMICAL ANALYSIS FOR PALMYRA SPROUT

Phytochemical Analysis of Palmyra Sprout involves the identification of various bioactive compounds present in the sprouts.

Qualitative Screening Analysis for Palmyra Sprout

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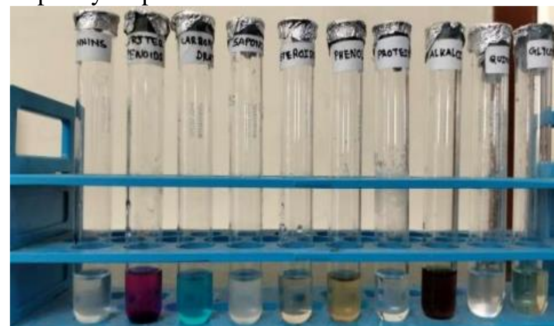

FIGURE 3.10: PHYTOCHEMICAL ANALYSIS OF *BORASSUS FLABELLIFER*

TABLE 3.4 RESULT OF THE PHYTOCHEMICAL ANALYSIS

Test no.	Test For	OBSERVATION	Result
1	**Tannins**	Disappearance of colour	Positive
2	**alkaloids**	Absence of Yellow colour	Negative
3	**Saponins**	Foam formation	Positive
4	**Steroids**	Absence of Blue/Green Color	Negative
5	**Terpenoids**	Absence of Red/Pink/Violet Color	Negative
6	**Glycoside**	Blue/Green Color	Positive
7	**Phenol**	Absence of Black/Green Color	Negative
8	**Alkaloids**	Blue Color	Positive

9	**Quinone**	Absence of Green/Red/Pink Color	Negative
10	**Protein**	Golden Color	Positive
11	**Carbohydrates**	Blue/Green/Orange	Present
12	**Triterpenoids**	Violet Color	Present

3.7 DEVELOPMENT OF BIO POLYMER SHEET (PALMYRA SPROUT)

FIGURE 3.11: DEVELOPMENT OF BIOPOLYMER SHEET

4.8 ANTIMICROBIAL ACTIVITY :

The antimicrobial activity was performed with two different strains. Antimicrobial activity for the developed biopolymer sheet. It shows the two different bacterial pathogens that were used. The anti-microbial activity was studied preliminarily against bacteria. The test organisms used are *Escherichia coli* and *Staphylococcus aureus*.

TABLE 4.5 SCREENING FOR ANTIMICROBIAL ACTIVITY

PATHOGEN	ZONE OF INHIBITION (mm)
Escherichia coli	NIL
Staphylococcus aureus	NIL

After 24 hours of incubation, no zone formation occurred in the plates. This shows that the developed biopolymer sheets are resistant to the pathogen (both) gram positive and gram negative). This provides good quality, longer shelf life for the Biopolymer laminates for further study.

FIGURE 4.12: (a)*Staphylococcus aureus* , and (b) *Escherichia coli.*

V. SUMMARY AND CONCLUSION

The development of a biopolymer sheet from *Bacillus* sp. Using Borassus flabellifer (palmyra sprout) for fruits and vegetables wrappers is a promising new technology with the potential to reduce plastic waste and improve food safety. The study found that the biopolymer sheet was strong, flexible, and biodegradable. It also had antimicrobial properties, which could help to extend the shelf life of fruits and vegetables. Biopolymer sheets are made from renewable resources and are biodegradable, making them a more sustainable alternative to traditional plastic wrappers. The biopolymer sheet was also found to be non-toxic and safe for food contact. This makes it a suitable

material for use in food packaging. It is important to scale up the production of the biopolymer sheet to make it commercially viable. The biopolymer sheet should be further tested to ensure its safety and effectiveness in a variety of food packaging applications. The study found that the biopolymer sheet developed using Bacillus sp. and Borassus flabellifer had good mechanical properties and water vapour barrier properties. The sheet was also found to be non-toxic and biodegradable. It is important to determine how the sheet performs in terms of protecting fruits and vegetables from moisture loss, microbial growth, and physical damage. If the biopolymer sheet can be produced at a competitive cost and on a large scale, it could have a significant impact on the fruit and vegetable packaging industry. By replacing traditional plastic wrappers with biopolymer wrappers, the industry could reduce its environmental impact and contribute to a more sustainable future. These results suggest that the biopolymer sheet has the potential to be used as a wrapper for fruits and vegetables.

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