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REVIEW ARTICLE

Characterization and evaluation of antimicrobial activity of composite blends of *aloevera* extract and eugenol loaded Polyhydroxyalkanoate (PHA)

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Abstract

Greece, Egypt, India, Mexico, Japan, and China are just a few of the civilizations that have employed *Aloe vera* for therapeutic reasons for millennia. Nefertiti and Cleopatra, two Egyptian queens, employed it as a regular component of their beauty regimens. It was used to cure soldiers' wounds by Alexander the Great and Christopher Columbus. Since Poly3-hydroxybutyrate (PHB) is biodegradable, effective in wet conditions, and includes an antibiotic element that is efficient against bacteria that cause skin infections. It is observed that by using these antimicrobial PHB blends in place of regular bandages have a better wound healing ability and have potential to reduce bio burden. In this paper, the methodology includes the application of PHB as a bio-based chemical, and the films have the potential to provide intriguing alternatives for used materials. Additionally, an attempt was made for different plant-based extracts to examine for a greater influence on skin-borne allergies, swelling tests, protein absorption investigations, etc. in order to establish the mechanical features of medicinal technique incorporated films and their role in enhancing wound healing capacity. It may also be possible to reduce the cost of such films by combining them with other biopolymers, such starch, to make them more practical and hence acceptable in everyday life.

Keywords: *Aloe vera,* Polymers, Hydroxybutyrate, Poly3-Hydroxybutyrate (PHB), Synthesis.

Introduction

The most notable phytochemical in the tulsi plant, eugenol, is thought to have some antibacterial properties. Numerous studies have postulated the mechanism of eugenol's, alpha terpinol's, and gamaterpine's bactericidal effect against test microorganisms, which are the three key components of tulsi essential oil (Alves et al., 2021; Regmi et al., 2021; Kurotani et al., 2021). The purpose of the research was to monitor changes in membrane fluidity by testing for protein and lipid leakage using the Bradshaw and van Boom methods, respectively. The oil's constituents might cause cell lysis by allowing the contents of its lipid and protein compartments to escape (Aloni et al., 2021; Chen et al., 2021; Callow et al., 2021). According to earlier research, eugenol at 2X MIC was very efficient in preventing protein leakage after 120 minutes

of exposure. Gamma terpinene and alpha terpinol had comparable effects at 2X MIC under the same circumstances. According to the results of the reference literature, the treated gram positive and gram-negative bacteria had extensively damaged cell walls and membranes.

Tulsi has long been acknowledged as one of India's most potent medicinal plants. Tulsi, which meaning "one that is incomparable," refers to a someone who does not accept or permit resemblance. The propagation of information about holy basil is necessary for the good of all humankind. The result is that various types of microorganisms respond differently to the antibacterial effects of tulsi extract. They exhibit various modes of action while dealing with various microorganisms. The study on the tulsi's antibacterial capabilities will be guided by the literature in the future (Bunsick et al., 2021; Mani et al., 2021; Minorsk et al., 2021).

Antimicrobial properties of *Aloe Vera* extract

A member of the Liliaceae family, *Aloe Vera* is a flower that thrives in hot, dry climates. This plant's existing mucilage tissue, which is also known as aloe gel, is utilized in a variety of cosmetic and medicinal products. This plant's peripheral leaf cells generate aloes, a bitter, yellow-colored latex. The most common plants that might be seen in this respect is aloe Vera. The herbaceous, perennial plant known as *Aloe Vera* or yellow aloe has long, thick, succulent leaves.

Its leaves have a little thistle curl on the border. Its blooms, which range in colour from green to yellow, are arranged in a lovely cluster at the end of the florescent stem axis. It is indigenous to parts of Africa and is also known as the desert lily (Hesperocallis). For the first time, the Egyptians employed the *Aloe vera* plant to heal infections, burns, and wounds. Following them, the *Aloe Vera* plant was used for a variety of uses by the Greek, Spanish, and African peoples. *Aloe Vera* has a hot and dry humor, and so its extract is employed for therapeutic purposes in Iranian traditional medicine (Ohtani et al., 2021; Sitzia et al., 2021; Hussain et al., 2021).

Chemical composition

The anthracene hydroxyl derivatives found in aloins A and B_2 and the 35%-55% of chromone hydrocarbons and derivatives found in aloe resins A, B2, and C make up the majority of the *Aloe Vera* plant's chemical makeup. Aside from the vitamins B_1, B_2, B_6, C, E, and folic acid, the *Aloe Vera* plant also contains multiple carbohydrates like glucose, mannose, and cellulose, as well as different chemicals like oxidase, enzymes, and catalase, as well as minerals like calcium, salt, aluminum, zinc, copper, and chrome.

Therapeutic and pharmacological actions

The *Aloe Vera* plant is thought to have pharmacological effects that include anti-inflammatory, anti-arthritis, antimicrobial, and hypoglycemic properties. *Aloe Vera's* antibacterial and antifungal capabilities shield the scalp against the development of dandruff. The *Aloe Vera* plant is beneficial for preventing fungal diseases like alopecia. One might assume that *Aloe Vera* fresh gel's healing properties in wounds and superficial skin injuries are among its other benefits. Similar to this, taking this medicine causes less pain at the site of the trauma. The Sudan black staining in fig. 1 reveals *B. megaterium* cells (pink) containing PHB granules (bluish black).

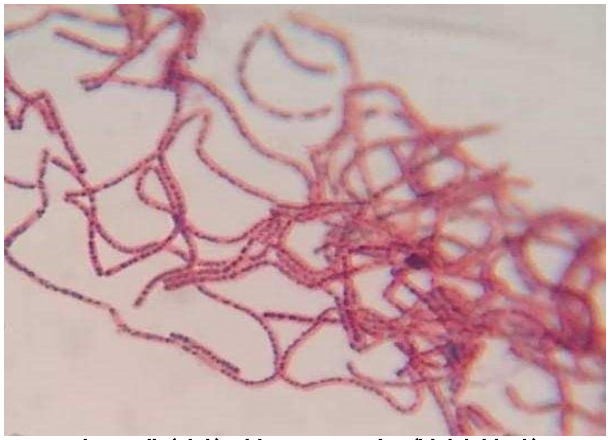

Figure 1. Sudan black staining showing *B. megaterium cells* (pink) with PHB granules (bluish black).

Aloe Vera's moisturizing properties have also been shown in its topical preparations. Aloe gel's skin-benefiting properties enhance medication absorption via the dermis as well. This effect of skin raising intake was detected in a research on the impact of Aloe Vera consumption on caffeine, colchicine, mefenamic acid, oxybutynin, and kinin medicines. This action of skin consumption may be related to a rise in water content (*stratum corneum*).

Aloe Vera, often known as yellow aloe, is a succulent, watery plant that resembles a cactus and has mucilage tissue on its leaves (gel). This mucus contains certain glycoproteins that guard against swelling and discomfort and quicken the process of healing. Additionally, it contains polysaccharides, which promote skin healing and development. This plant's mucilage may be applied to both internal and exterior wounds (Nardini et al., 2021; Smith et al., 2021; Xiang et al., 2021).

Production of biopolymer from dairy waste

Hectic polymers made their debut in the 1950s and have since become one of the materials we use the most in everyday life. According to reports, petroleum-based plastics are stable under adverse circumstances against microbiological and chemical deterioration, making them long-lasting, highly resistant, and persistent in the environment. Synthetic polymers have dominated the commodities market and advanced plastics production technologies because to their superior characteristics and broad variety of uses. Wastes of all kinds created by the dairy, oil, and agricultural industries have become a serious environmental problem. These wastes include components that may be used by microorganisms to produce Polyhydroxyalkanoates.

The biodegradable plastic raw material Polyhydroxybutyrate (PHB) may be created from the Polyhydroxyalkanoates (PHA). Polyesters called Polyhydroxyalkanoates (PHAs) are produced spontaneously by many bacteria during uncontrolled growth and have been shown to accumulate in their internal membranes as intracellular energy storage materials. Many bacteria spontaneously create PHA, a polyester made of hydroxy fatty acids, which serves as an internal carbon and energy store (Thomas et al., 2021; Hoffmann et al., 2021; Yang et al., 2021).

The most prevalent naturally occurring microbial PHA is poly 3-hydroxybutyric acid (PHB). PHA builds up in certain microbial species when there is an abundance of carbon and a deficiency in nitrogen sources. There are classes of bacterial polyesters collectively known as Polyhydroxyalkanoates (PHAs), which are collected intracellularly as reserve granules by certain bacteria under adverse environmental circumstances. Polyhydroxybutyrate (PHB) is a biodegradable and biocompatible thermoplastic. Lemoigne made the first discovery of Polyhydroxybutyrate (PHB) in *Bacillus megaterium* in 1926. The cost of producing PHB depends on a number of variables, including substrate, strain selection, growing technique, and post-processing.

The primary by-product of the dairy industry is whey, which is produced during the cheese-making process by precipitating and removing milk casein. Only 50% of the 120 million tons of whey generated yearly worldwide is utilized to make goods for food for people and animals.

In this research the author discussed about the remaining whey needs to be disposed of, which raises environmental concerns due to its relatively high organic load, as whey is mainly composed of lactose (69 g/L-89 g/L), fats (2.4 g/L-8.58 g/L), proteins (87 g/L-90 g/L), and mineral salts (9.6 g/L-10 g/L). From these cheese whey components, lactose is responsible for most of the biochemical oxygen demand of whey; therefore, it is essential to find a biotechnological use for lactose. The value of using whey lactose for PHA production is clear as it would dramatically decrease the costs of PHA production, without competing with the production of food for humans and simultaneously solving an environmental problem.

Literature Review

Maruri-López et al. in their study embellish that without any of the disputes that accompanied Staudinger's identification of macromolecules, the aliphatic polyester poly (3-hydroxybutyrate), or PHB, was discovered and chemically identified in the 1920s as a granular component of bacterial cells. PHB was recognized as the leading sustainable elastomer that may help with the problem of waste disposal four decades after it was created. Imperial Chemical Industries Ltd. was in charge of the project's development, which fostered a body of research that included the examination of the enzymes involved in biosynthetic pathways and phytoremediation as well as gene therapy and biotechnology. The same fundamental structure has been found in over 100 different aliphatic polyesters, all of which are derivations of the simple PHB homo-polyester that Maurice Lemoigne developed inside this middle of the 20s. Depending on the kind of bacteria

and the substrate, these molecular weight groups typically polyesters rank with oligonucleotides, polyetherimide, polyisoprenoids, phenol, polyphosphates, and polysaccharides as unique classes of natural polymers. This historical overview covers the 75-year discovery era, and the major chemical, physiological, and microbiology discoveries are linked to the persons and locations that were involved (Maruri-López et al., 2021).

Hiernaux and Quentin in their study embellish that the majority of the impetus for research into the creation of biodegradable polymers has come from regulatory changes in Europe and America. These policies have their origins in the Brundtland Report of 1987, which laid the foundation for a more sustainable society. A special generation of plant - based materials called biodegradable polymers aims to improve humanity, the environmental, and the economy. In the presence of ample carbon, bacteria often generate Polyhydroxyalkanoates (PHAs), which are naturally occurring biodegradable polymers, in reaction to an inorganic nutrient deficit. The bulk of the early studies on PHA accumulation and technical advancement for industrial-scale synthesis were carried out using virgin starting materials. Nowadays, commercial production of Polyhydroxybutyrate and composites like Polyhydroxybutyrate co-valerate uses glucose produced from maize (Hiernaux et al., 2021).

Daloso et al. in their study embellish that the environment has a significant impact on how plants function as biological systems because they are structured and controlled by a complex web of interactions that range from the genetic to the morphological level. Even while reductionist techniques have been widely used in plant genetics, they are still unable to pinpoint the mechanisms that allow plants to flourish in difficult conditions. The development of technology that enables thorough study of plant cells has resulted in the creation and accessibility of large amounts of data for in silico analysis and simulation techniques. Thus, it is becoming more clear that a variety of methods, including graph analysis and cluster based architecture, are required to understand this richness of information (Daloso et al., 2021).

In this research the author elaborates the non-reductionist techniques, such as those from systems biology, will be required to comprehend the complexity of plant metabolic reactions. Despite the methodological complexity and interpretability of such techniques, they have been effectively used in metabolic and gene expression networks in a variety of microorganisms, including plants more recently.

Methodology

Design

For assessing antibiotic activity, the bacterial strains Escherichia coli, Pseudomonas sp., Staphylococcus, and proteus were utilized. The nutrient agar medium was used to cultivate and support the bacterial cultures for 4 c. To maintain viability, the bacterial cultures were sub cultured once every 3 weeks. The author utilized sodium hypochlorite, acetone, and chloroform. We bought pancha tulsi extract from the neighborhood market.

Sample and instruments

PHA synthesis by *Bacillus megaterium* Ti3 was identified utilizing the Carbolfuchsin and Sudan black B stains. To ascertain the isolate's intracellular synthesis of PHA, carbolfuchsin stain was used. Carbolfuchsin stain was applied to a tiny smear of each isolate and left on for 45 seconds. PHA granules of a black colour were seen within the cells of isolates that could produce it. Using the Sudan black b staining technique, PHA-producing bacteria were further validated. A 0.3% solution (w/v) of Sudan black b stain was made in 60% ethanol. On the glass slides, a smear of the cultures was applied and heat cemented. The samples were stained with Sudan black b solution for 10 minutes, washed with water, then counter stained with 0,9% safranin for 6 minutes before being examined under a 100X light microscope.

Data collection

Using Lowry's approach, an assessment of whey protein content was made. The supernatant from the whey centrifuged at 8000 g for 10 minutes was used to calculate the amount of protein. 0.2, 0.4, 0.6, and 1.0 of the protein solution were pipetted into clean glass tubes, and water was added to bring the volume to 2 ml. Alkaline copper sulphate solution (4 ml) was added to each tube, well mixed, and left to stand for 10 minutes at room temperature. 30 minutes were given for the tubes to stand after adding 0.5ml of the FCR reagent and mixing right away. Fig. 2 discloses the Standard graph of Protein estimation in whey by Lowry's method with unknown values extrapolated.

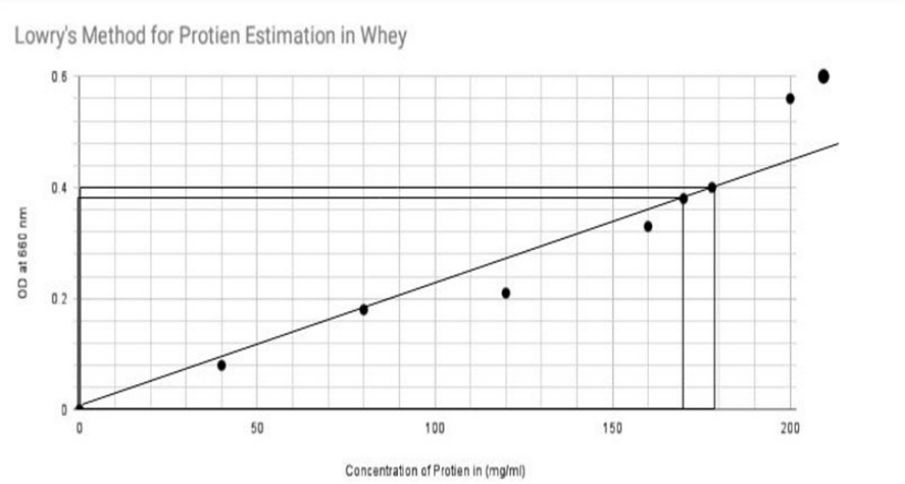

Figure 2. **Discloses the Standard graph of Protein estimation in whey by Lowry's method with unknown values extrapolated.**

Data analysis

Using the well diffusion technique, antibacterial activity was measured. Under aseptic circumstances, Mueller Hinton agar plates were created. A uniform bacterial inoculum was made, swabbed on the agar plates, and wells were bored in the agar medium using a sterile cork borer. The Mac Farland standard's concentrations were produced using bacteria cultures. *Aloe Vera* and tulsi extracts at 1mg/ml were tested against the bacterium. All of the bacterium plates underwent a 24-hour incubation period at 37°C to assess the zone of inhibition.

Results and Discussion

In the near future, it is anticipated that antimicrobial plastics, which now make up a minor portion of the plastic additives industry, will make up roughly 20% of the global plastics market. Antifungal polymers were formerly thought to be primarily used in the industrial sector, notably in the building and packaging sectors. But as people's awareness of pollution and diseases has grown, there has been a huge increase in the demand for antibacterial plastic items in other segments, including the healthcare and consumer goods sectors. Bandages traditionally had a problem with disposal and created a lot of bio load; however, PHB blends with faster biodegradability are viewed as a novel solution.

PHB has certain drawbacks due to its propensity for brittleness when used as plastic. But it is clear from earlier investigations that the plasticity of PHB may be increased by adding plasticizing substances like PEG. Incorporating PHV with PHB is the subject of several research because it might lessen PHB's brittleness. Our study's goal is to provide the bioplastic PHB with wound-healing characteristics a plasticity and antibacterial activity by adding various plant-based extracts as aloein, gingiverin, curcumin, eugenol, etc.

Sudan black discoloration

Sudan can distinguish cells by examine their cytoplasm and nucleus using black staining. Reserve fat found in the cells may be used to detect spore production by *Bacillus megaterium*. *Bacillus megaterium* cells have pink-colored bacterial cytoplasm after 48 hours of incubation in whey medium, while the spores are visible as being bluish-black in colour. Sudan Black is a somewhat basic dye that reacts with acidic groups in complex lipids to stain them as well. Being a lipid-derived molecule, PHB binds to Sudan Black, causing them to appear intracellularly as dense black deposits when Safranin is used as a counterstain. Nile red, sometimes referred to as Nile blue oxazone, is a lipophilic fluorescent dye that may be used as an alternative to Sudan black staining to see hydrophobic cell structures such membranes or lipid-like inclusions PHB, triacyl-glycerides. Fluorescence microscopy makes it simple to find Nile red because of its affinity for PHB granules.

The product produced by fermentation of two flasks containing 100 ml of medium kept in a shaker incubator for 48 hours allowed for the calculation of the PHB yield by measuring the total PHB and Dry Cell Weight (DCW) of the product. In order to acquire consistent readings owing to moisture being released by evaporation, the PHB was collected and weighed separately for total PHB and DCW for 5 days. It was discovered that 7.8 g/L of PHB was produced from 1 litre

of whey. Because PHB is an intracellular metabolite, cell disruption is crucial for the correct quenching out of the molecule.

Protein co-purification and cell debris, which may cause contamination, are difficulties. Bleaching agent sodium hypochlorite, which contains 4% active chlorine, causes cell walls to be disrupted. To get a greater purity yield, regular washes with water and acetone were used. The yield was then calculated using the determined DCW and PHB accumulation. PHB is dissolved in chloroform to create a solution since it dissolves in organic chloride solutions. PHB may be made into smooth sheets by applying heat to it, which causes the PHB to melt. The PHB sheets that were made were transparent and fragile. Agar diffusion experiment was used to investigate the inhibitory impact of *Aloe Vera* extracts made from Methanol, Ethanol, and Acetone on bacteria such *E. coli*, *S. aureus, Pseudomonas,* and *Proteus*. With higher concentrations of the extracts, the ethanol and acetone extracts had discernible antibacterial activity against the *Pseudomonas* and *Proteus* tested. Different sensitivity patterns for the three extracts were seen in these bacteria.

Conclusions

The disc diffusion of treated PHB sheets, which displayed higher area of inhibition zones, shows that the pathogens are practically eliminated, according to the halo technique. Developments in medical and pharmacological investigations will have an impact on the decision of which plastic or flexible bandage to use. There will always be new medical innovations that reflect changes in medical technology, lifestyle choices, governmental decisions, research and development, and environmental concerns. A multidisciplinary strategy that tackles these problems in the near future will work best to handle these concerns.

In the current study, the bioplastic PHB is given plasticity and antibacterial activity. From the methods and findings discussed above, it can be inferred that plant extracts and widely-used preservatives, either separately or in combination, make excellent choices for antimicrobial agents to be incorporated with bioplastics. As a result, the application of PHB can be enhanced by adding plasticizers and antimicrobial agents to the PHB. We advise using these antimicrobial PHB blends instead of standard bandages for improved wound healing and less bio-burden since PHB is biodegradable, effective in damp environments, and contains an antimicrobial ingredient that is effective against microorganisms that cause skin infections. Such films could provide interesting substitutes for synthetic materials since PHB is a bio-based substance. In addition, various plant-based extracts should be evaluated for a bigger impact on skin-borne allergies, swelling tests, protein absorption studies, etc. in order to establish the mechanical qualities of medicinal method included films and their function in increasing wound healing capacity. Efforts may also be made to lower the price of such films by blending them with other biopolymers, such as starch, to make them more economical and hence acceptable in daily life.

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