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REVIEW ARTICLE

Characterization and Evaluation of *Aloe vera* extract and preparation of Eugenol loaded Polyhydroxyalkanoate (PHA) composite blends for antimicrobial activity

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Abstract

Aloevera extract and eugenol-loaded Polyhydroxyalkanoate (PHA) composite blends were prepared, characterized, and evaluated for the utility of antimicrobial activity. Such kind of films may provide intriguing alternatives to synthetic materials as PHA is biologically originated. In this research mechanical characterization of films were evaluated and explore the possibilities of biomedical applications for wound healing properties. It was also evaluated that the other plant-based extracts should be tested for greater impact on skin-borne allergies, swelling studies, protein absorption studies, etc. The cost of such films may also be decreased by blending them with other biopolymers, such as starch, to make them more economical and hence acceptable in daily life. The future potential of this research is the enhancement in the antimicrobial activity in the day to day life cycle.

Keywords: *Aloevera*, Biopolymers, Bacteria, Hydroxybutyrate, Polymers.

Introduction

Gram-positive and gram-negative bacteria from at least 79 different genera create Polyhydroxyalkanoates (PHAs), or Polyhydroxyalkanoates, which are hydroxyalkanoate polyesters. These polymers are stored intracellularly up to 90% of the dry weight of the cell in times of nutritional stress and act as a carbon and energy reserve. Escherichia coli's cytoplasm and cytoplasmic membrane have been shown to contain Poly3-hydroxybutyrate (P3HB), a non-storage PHA with a low molecular weight (Eljebbawi et al., 2021).

PHA's molecular weight varies according on the producer and ranges from 50,000 Da to 1,000,000 Da. With a molecular mass close to that of conventional polymers like Polypropylene, Polyhydroxybutyrate and other PHA produced by bacteria have similar polymer characteristics. Poly3-hydroxybutyrate is the PHA that has been investigated the most

(PHB). It is possible to produce copolymers of PHB that include either "3-hydroxyvalerate (3HV) or 4-hydroxybutyrate (4HB) monomers by co-feeding substrates. When 3HV is integrated into PHB, a poly 3-hydroxybutyrate co3-hydroxyvalerate [P (3HB-3HV)] is produced that is less stiff and more brittle than P (3HB)". Short side-chain PHA is a kind of PHA composed up of polymers that include these monomers (ssc-PHA). Polymers consisting of aliphatic carbon sources or C6-C16 3-hydroxy fatty acids are known as "medium-side-chain PHA" (mcl-PHA). The used growth substrate has an impact on the PHA's (Schnell et al., 2021; Burt et al., 2021; Brownlee et al., 2021).

PHA biodegradability

PHA differs from polymers generated from petroleum in that it is biodegradable. PHA begin to degrade when they come into contact with soil, compost, or marine trash. The quantity of larger surface area, hydration, climate, pH, molecular weight, and the microbial activity of the environment are only a few of the factors that influence how rapidly something degrades. PHA also heavily depends on polymer composition and crystallinity. It has been found that the kind of atoms also affects degradation (Zhan et al., 2021).

PHB has the beneficial virtue of being broken down in D-3-Hydroxybutyrate (HB), a component of human blood naturally. As a result, this homopolymer may be used in biological applications including drug transporters and scaffolds for tissue engineering. PHB has been utilized in packaging materials and tiny throwaway goods. By comparing PHB with PP, looked at the usage of PHB in food packaging. PHB had a deformation value that was almost 50% lower than PP's. PHB is less flexible and stiffer than PP. Under typical freezing temperatures, PHB often performs worse than PP does. PHB, however, outperformed PP at higher temperatures. PHB production costs are influenced by a number of important variables, including substrate, strain choice, growing technique, and post-processing. Fig. 1 discloses the Aloe vera plant and its extract.

Figure 1. Discloses the aloe Vera plant and its extract (Das et al., 2022).

PHB real estate

PHB is a partly crystalline substance with a high melting point and high crystallinity, as was already noted. It is 100% biodegradable, 100% optically active, piezoelectric, and has strong barrier qualities but is not water soluble. PHB has a Young's modulus and tensile strength that are equivalent to those for PP, however it has a substantially lower elongation at break (5%-10%). As a result, the non-externally imposed stress that causes the PHB spherulites to break is what is responsible for the material's stiffness. Brittleness increases with time when items are stored at room temperature. PHB doesn't include any catalyst residues since microorganisms are used as the manufacturing sources. It lacks chain branching and has an isotactic structure. For this reason, processing goes smoothly (Garagounis et al., 2021).

PHAs are well recognized to have a wide range of uses in packaging, medicine, and disposal. PHBV might be used for paper coating, film packaging, and blow-molded bottles. Additionally, the biocompatibility and gradual hydrolytic destruction of PHBV lead to its medicinal uses (potential in reconstructive surgery. MCL-PHAs may be used as temporary medical implants and coatings, such as scaffolding for the regeneration of arteries and nerve axons. Amphiphilic PHA copolymers have important uses in the medication delivery, tissue engineering, and cardiovascular fields, including pericardial patches, vascular grafts, implants, cardiologic stents, heart valves, and dressing tablets. PHB may be converted to the highly concentrated form of HB seen in human blood. Therefore, it is safe to implant in mammalian organs (Hiernaux et al., 2021; Kadirova et al., 2021; Maruri-López et al., 2021).

As the only structural polymer or a component of degradable composites, PHBV has shown successful applicability for disposable personal hygiene products. PHAs may be utilised in place of petrochemical polymers in composite materials since they exhibit similar qualities. PHA-based films have drawn attention for use in food packaging because of their recyclability, biodegradability, and water vapour barrier qualities. In the visible and ultraviolet light spectrum, PHB functions as a superior light barrier (Liu et al., 2021., Pise et al., 2021; Eckardt et al., 2021). Fig. 2. illustrates the Bioplastic films made of Eugenol in various concentrations are produced by combining PHB+PEG mixes.

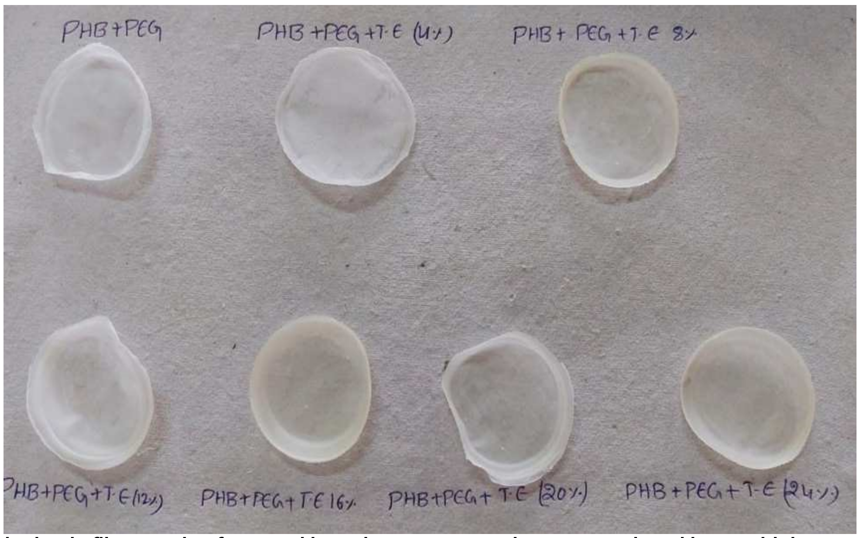

Figure 2. Illustrates the Bioplastic films made of Eugenol in various concentrations are produced by combining PHB+PEG mixes.

In contrast to dry wounds, moist wound circumstances result in an increase in endothelial and fibroblast cell populations. It is easier for epithelial cells to move across wound surfaces when the lesion is maintained wet and scab-free. This promotes the healing of wounds. Some people might develop noticeable scarring from superficial wounds. Maintaining moisture reduces the likelihood of scab development, which eases aesthetic problems. Patients' pain levels have been shown to decrease in moist conditions that promote wound healing. In order to prevent moisture from escaping from the wound, occlusive dressings are increasingly often employed.

As a result, the wound is protected against desiccation and further tissue damage caused by exposure to the surrounding environment. Desiccation and trauma prevent epithelial cells from migrating to the injured region, as was previously stated. When creating a dressing to cover the wound, this factor has to be taken into account since epithelial cells play a significant part in wound healing. The creation of a voltage difference between it humid conditions of the injured tissue and the dry region of the surrounding tissue prevents wound desiccation. This promotes epithelial cell migration to the location of the wound. After applying electrical stimulation, fibroblast cells express growth factors more often. Both of these elements promote wound healing. Although expensive, occlusive dressings have been demonstrated to cure wounds effectively and need less time for nursing care.

Artificial polymer sheets

Polymer films were first employed as coverings for surgical wounds. In research to determine if polymers may affect the speed at which wounds heal, polymers have been employed in dressings and medical equipment. They now have a position in the management of wound healing as occlusive dressings. Wound dressing has evolved recently and now mostly comprises of synthetic polymers.

In this essay, the author describes how Polyurethane (PU), a popular component in semi-permeable dressings, provides a superior barrier and oxygen permeability. One of these films' most important qualities is that they are impermeable to air and moisture vapour yet impermeable to germs and liquid. The fact that these materials are non-absorbent, which is a major drawback of such dressings, increases the risk of wound exudates accumulating underneath semi-occlusive films. Although it doesn't seem to encourage bacterial growth in the wound, the pressure of the seeping fluid may disturb the environment that the occlusive dressing is meant to maintain.

Literature Review

Koornneef and Maarten in their study embellishes that the advancement of genetics as a vital instrument for the comprehensive examination of plant biology. In this study, the findings demonstrate the discovery and physiological characterization of mutants in tomato and Arabidopsis with deficiencies in photonic function, hormone synthesis, or photoperiod also produced an Arabidopsis genomic map. The utilization of subsequent natural variation, including the molecular identification of underlying genetic differences. Over the last 40 years, the understanding of plant biology has become advanced significantly due to interdisciplinary approach and the use of Arabidopsis as the primary model species (Koornneef et al., 2021).

Autran et al. explore those biophysics and biomathematics has been built upon by the multidisciplinary discipline of quantitative plant biology. It now establishes a new benchmark in plant research owing to high spatiotemporal resolution instruments and computational modelling. In this research, the author applied a methodology in which they stated that whether molecular, geometrical, or mechanical, acquired data are measured, statistically evaluated, and integrated across scales and disciplines. The results show that the hypotheses for future experimental studies to follow. To take into consideration the internal dynamics of organisms and dynamic interactions with the outside environment, quantitative properties like variability, noise, resilience, delays, or continuous feedback are used. This research concluded that quantitative plant biology ultimately enables us to examine and comprehend our interactions with plants (Autran et al., 2021).

Zhu et al. studied that a Plant Natural Products (PNPs) are the hotspot area in synthetic biology. Plant Natural Products (PNPs) are the primary sources of medicines, food additives, and novel biofuels. Building microbial cell factories has allowed for the engineered biosynthesis of several PNPs during the last 20 years. In this paper, the author applied a methodology in which they reported that hosts have evolved from single-celled microorganisms to complex plant systems in tandem with the rapid development of plant physiology, genetics, and tools for genetic manipulation. The results show that a new area called "plant synthetic biology" blends engineering theory with plant biology. The author concluded an outline of current developments to understand the PNP biosynthetic pathway as well as the development of engineered PNP biosynthesis in plant cells (Zhu et al., 2021).

In this paper, the author elaborates the abnormalities in hormone synthesis, photoperiod, or photoreceptor function in tomato and Arabidopsis. The utilization of subsequent diversity, including the sequencing of underlying genetic differences and became a significant area of study.

Discussion

However, by straying from the "one size fits all approach," a dressing may get close to the ideal criteria provided it strictly complies with the demands of the wound and the patient. In order to facilitate wound healing with the least amount of time and money invested, a wound dressing should be functional. The many polymeric materials used as wound dressings in medicine are discussed in this article, along with the biological processes that arise from the dressing's interactions with the body's tissue. Thus, it has been shown how crucial it is to use synthetic polymer films, foam dressings, hydrocolloids, alginate dressings, and hydrogels.

Composites in wound healing

The activation of diverse cell types occurs throughout each successive phase of the intricate and strictly controlled physiological process that is wound healing. Any disruption in the normal flow of the healing processes might result in chronic wounds, which could have an impact on the quality of life of the patient and have repercussions for the management of wound care. The creation of totally biodegradable materials with improved bioactive potentialities and sustainability may take cues from nature itself. Biopolymers that are naturally derived are increasingly regarded as intelligent materials. They provide a flexible and solid foundation for designing the proper extracellular matrix capable of supporting tissue regeneration while preventing the beginning of negative events.

The development of biocompatibility based on living organisms' polymers, either originating from carbohydrates or starches, has gained a lot of popularity recently in order to address issues with wound healing. However, in the modern world, we definitely need to address the need for eco-friendly solutions to satisfy our demands moving ahead, includes applicability in the healthcare and gingival sectors. Next, a few protein-based biopolymers and several naturally occurring polysaccharides will be briefly addressed along with their key physical, chemical, and physiological functions. We will also discuss some of the most current methods for developing cutting-edge bio composites based on these bio-surfactants that are also environmentally friendly, with a focus on their applications in the fields of skin tissue regeneration. Finally, we'll discuss potential chances for creating a new class of sophisticated topical application that are produced sustainably and organically in the future. Fig. 3. discloses the Eugenol-incorporated PHB blends showing 12% MIC against Pseudomonas by contact method (P=Plain blend).

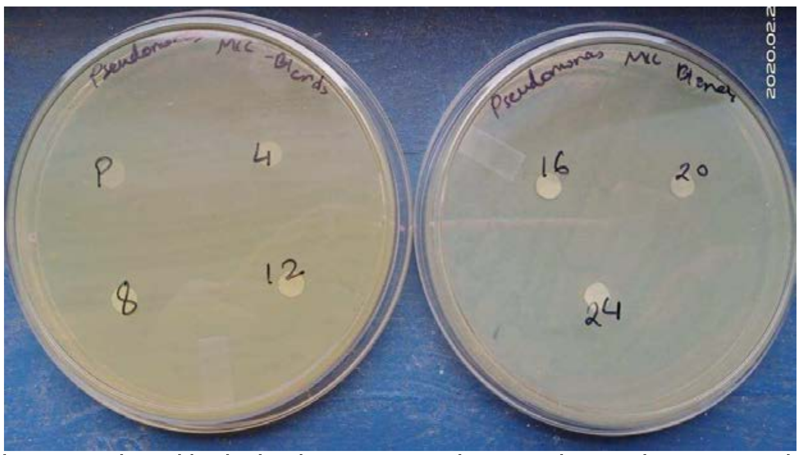

Figure 3. **Discloses the Eugenol-incorporated PHB blends showing 12% MIC against *Pseudomonas* by contact method (P=Plain blend).**

Physiologically, wound healing is a difficult process. Numerous irregularities might have an impact on this phenomena because of how intricate it is. In addition to cellular and molecular elements, several enzymatic channels also become active through repair and assist tissue recovery. The goals of this chapter are to introduce the newly emerging topic of self-healing materials to the area of biomaterials and to provide suggestions on how self-healing techniques may be utilized and modified to create various classes of nanomaterials. On the other hand, natural and manufactured gel like materials, productions, blends, and micro-/nanoparticulate systems have greatly facilitated the development of composites for cell therapy and other functions, such as wound healing. Given their high horizon ratios and great softness, nanofibrous membranes provide good carriers for medicinal medications or may expedite the healing of wounds.

As an alternative to conventional solutions for tissue repair and transplantation, biocompatible and biodegradable polymer scaffolds mixed with cells or biological signals are being studied. As created tissues that improve wound healing and skin regeneration, these methods are now in use in clinical settings. This chapter discusses current studies on the creation of biopolymers and biomaterials based on pharmaceutical dosage forms and wound dressings, as well as their biomedical uses.

Over the last 20 years, experimental research has helped to identify the fundamental stages of the healing. This complex network is structured by three sequential, overlapping methods. Primary sensory neurons recognize injury and send alert signals to the brain instantly, which stop bleeding and initiate inflammation. The inflammatory phase's early stage is represented by this.

The following objectives of the inflammatory phase, which is led by nk cells, are to get rid of the pathogens and clean the wound. The acute response will have passed at that point, and homeostasis will have been reestablished. The acute inflammation, the 1997 - 2002, aims to repair wound damage and begin tissue remodeling. Fibroplasia, reepithelialization, angiogenesis, and peripheral nerve healing are the main activities of this phase. The finishing phase's last objective is to complete tissue rebuilding and restore health of the skin.

Aloe vera extract is a crucial component in wound treatment and has been studied extensively. Natural products have a significant role as supplemental therapy in this respect. *Aloe vera* extract aids wound healing, particularly for cutaneous wounds, according to several research. The influence of *Aloe vera* on the speed of blood clotting and other processes have been studied in both clinical and experimental conditions, although further research is still required to verify the findings.

Compared to synthetic anti-microbial treatment, natural herbal therapies offer more promising anti-microbial characteristics and less negative side effects. Aloe vera has been used as a medical plant for treating a number of conditions because it contains anti-inflammatory, antibacterial, and immune-stimulating properties. Different quantities of *Aloe vera Gel* (AVG) were shown to have anti-microbial and inhibitive activities against oral pathogenic bacteria. Twenty patients had their subgingival calculi removed, their periapical and periodontal abscesses aspirated, and their samples were then transferred to thioglycolate broth and cultured in blood agar, MutansSanguis agar, and anaerobic gas chambers.

Eugenol India has been cultivating the subcontinental herb tulsi for both religious and therapeutic reasons. It belongs to the lameacea family and is an upright, numerous-branched shrub that is 30 cm-60 cm tall. About 54 chemicals have been found in tulsi leaves, flowers, and spikes, and they are thought to be the ones in charge of this sort of action. Essential oil of tulsi may be an effective standard antibacterial agent for treating skin and oral infections brought on by E. Coli and other organisms that cause infections of the skin, mouth, and soft tissues. Numerous studies and review papers have previously been written on this subject and have suggested that tulsi possesses antibacterial properties. The effectiveness of tulsi's antibacterial properties was investigated. *Salmonella enteritica*, *Vibrioparahaemolyticus*, *E. coli*, and *Listeria monocytogenes* were just a few of the microbial infections that tulsi's oil extract, chloroform extract, and alcohol extract all effectively combatted. All methods of extract production generally produced antibacterial activity against all examined pathogens. Finally, it was discovered that *ocimum* extract contains chemical components beneficial in food preservation and the creation of medications to combat pathogenic and food-borne microorganisms.

Conclusions

Agar diffusion experiment was used to determine the inhibitory impact of aloin and tulsi extract against bacterial and fungal food pollutants. The sensitivity of the bacteria to the two plant-based extracts differed. The films showed antimicrobial action in the concentrations of the two chosen phytoalexins included into the PHB films. Synergistic effects were sometimes seen, although inhibition was shown for all combinations single treatments as well as combinations. PEG was added to the PHB sheets, which produced more transparent and flexible sheets. Additionally, there was negligible antibacterial action in the PHB-PEG sheets. It is evident from the halo approach that the pathogens are virtually completely destroyed following disc dispersion of treated PHB sheets, which displayed larger areas of inhibition zones. The selection of a certain plastic or flexible bandage will depend on advances in pharmaceutical and medical research. There will always be new medical innovations that take into account changes in lifestyle, regulatory authorities' decision-making, medical technology, research and development, and environmental concerns. A multidisciplinary strategy that tackles these concerns in the near future will be the most effective way to handle these issues and challenges.

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