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‘‘FORMATION AND STUDY OF PROPERTIES AND ANTIMICROBIAL ACTIVITY OF LIGNIN – CHITOSAN BIO-COMPOSITE FILM’’

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ABSTRACT: The increasing demand for sustainable and functional packaging materials has encouraged the development of biodegradable alternatives to conventional petroleum-based plastics. In the present study, lignin and chitosan were utilized to develop an eco-friendly bio-composite film with antimicrobial and antioxidant properties for food packaging applications. Chitosan derived from mussel shells was blended with lignin at varying concentrations, and composite films were fabricated using the solvent casting method. The prepared films were evaluated for water solubility and water content to assess their moisture resistance and structural stability. Optical and chemical characteristics of the films were examined using UV–visible spectroscopy and Fourier transform infrared (FTIR) analysis to investigate molecular interactions between lignin and chitosan. The antibacterial activity of the films was assessed against selected foodborne pathogens, including *Bacillus subtilis*, *Escherichia coli*, and *Salmonella typhi*, using agar diffusion assays, while antioxidant potential was determined through DPPH radical scavenging activity. Furthermore, the effectiveness of the bio-composite films in preserving food quality was evaluated using tomato as a model system by monitoring physicochemical parameters such as firmness and colour. The results demonstrate that lignin–chitosan bio-composite films exhibit enhanced functional properties and show significant potential as sustainable packaging materials for extending the shelf life of perishable foods.

Keywords- Lignin–chitosan films, Biopolymers, Antimicrobial packaging, Antioxidant activity, Shelf life extension, Sustainable food packaging

I.INTRODUCTION

The development of industrial expansion gave affluence to the food sector, which has since expanded and evolved. An increasing population necessitates more food. Food production and safety are therefore the most important tasks. Globalization and urbanization have increased the demand for food, making food safety critical. The preservation, storage, and transportation of food items without compromising their nutritional, aesthetic, or commercial value has long been a major challenge in the food industry. Food packaging has become critical for assuring food safety during transportation and protecting against contamination, dust, spillage, and weather conditions. It also decreases food waste while maintaining the nutritional value of meals. According to a report by the Centres for Disease Control and Prevention, more than 70 million people suffer from food-related diseases yearly. On a global scale, a variety of packaging materials are used to protect food from deterioration to save costs and avoid health hazards. This emphasises the critical function that food packaging plays in preserving food quality throughout time. Hence, the modern food system would be incapable of advancing without food [1].

Food packaging has traditionally relied on synthetic or semi-synthetic materials such as polyethene, also known as plastic or single-use plastic. Plastic use has been thoroughly investigated in terms of human health

and environmental impact. Over the past 70 years, it has been outlined that around 8 billion tons of plastic have been generated, and this number is projected to increase by 13% in the next two years[2]. Due to the immense demand for food packaging materials, manufacturers of packaging have emerged as one of the world's most important commercial sectors. The vast majority of food packaging materials are made from petroleum-based polymers. However, because petroleum-based plastics cause so many environmental problems, biodegradable polymers with a range of properties are now manufactured and used. Biodegradable polymers have received a lot of attention in recent years as materials for food packaging, but their high cost limits their use. The number of research studies on food packaging published throughout the previous 15 years[3].

In the modern era, attention has switched to the development of sustainable packaging materials, which are also garnering consumer interest. Polymeric materials are currently frequently used in food packaging due to their lightweight and superior thermal and mechanical properties. Polymers are complex branching structures made up of one or more monomer units. Each monomer has unique properties, such as ionic charge and polarity. These characteristics are critical for using these polymers in the food business. As a result, the development of biopolymers or natural polymers can meet the demand for sustainable and environmentally friendly alternatives to plastics.

Biodegradable packaging films have been created using a variety of biopolymers. The term biopolymer originates from the Greek words bio and polymer, representing nature and living organisms [4]. Biopolymer-based packaging materials are widely used as encompasses for a variety of food goods. Polysaccharide-based packaging materials have risen in popularity due to their low cost, biodegradability, and widespread availability. Furthermore, lipid- and protein-based compounds are employed to create edible and biodegradable films. Some naturally occurring bio-renewable and eco-friendly polymeric materials, such as cellulose, are classified as carbohydrates. Chitin/chitosan, hemicellulose, starch, glycogen, alginate, aromatic polymers such as lignin, and naturally occurring protein-based compounds such as gelatin, collagen, and keratin. One significant advantage of using these compounds is their abundance in the biosphere and the ease with which they can be retrieved from waste. Among several biopolymers outlined above, chitosan has shown remarkable potential and has been exploited extensively as a source of novel blends and composite materials in the last 15 years[5].

Chitosan is a natural, linear biopolymer of (14) -linked 2-amino-2-deoxy—D-glucopyranose that is generated by the deacetylation of chitin. Shells of crustaceans, marine invertebrates, insects, fungi, and yeasts can be made into film, fiber, bead, and powder. Chitosan is a naturally occurring biopolymer with excellent physiochemical characteristics that is biocompatible, biodegradable, and non-toxic[6]. Lignin is a rich source of phenolic antioxidants; nevertheless, these molecules cannot be active for long periods. Recently, it was shown that phenolic chemicals originating from agricultural waste can be used as chitosan film additives in food packaging applications. The advancement of research into lignin and chitosan is two essential elements of the polymer. It is never too late to use matrices to create environmentally friendly, low-cost polymer composites[5].

Chitosan is an effective biopolymer for active food packaging materials, despite its lack of antioxidant activity. Enhancing chitosan's antioxidant activity could be a significant barrier to expanding its use in active food packaging materials. The use of lignin in conjunction with synthetic/natural and traditional plastics may result in the production of biomaterials and sustainable green polymers. However, because of its fragility and uneven distribution in many composites, lignin is difficult to blend with other polymers. Thus, the current work is aimed at investigating the use of sugarcane bagasse-derived lignin (a phenolic polymer as a bulk) as a potential ingredient for chitosan films, as well as characterization and evaluation of the properties of the resulting films in terms of structure and antibacterial activity for food packaging applications.

II.MATERIALS AND METHODS

2.1 Extraction of chitosan

Mussel shells were procured from a local market and thoroughly cleaned by rinsing under warm running water to remove adhering organic matter, surface impurities, and soluble proteins. The cleaned shells were oven-dried at 35°C for 24–48 h, followed by crushing using a blender and sieving to obtain a fine powder. Deproteinization was performed by treating the shell powder with 10% (w/v) sodium hydroxide solution at ambient temperature (around 30°C) to eliminate residual proteins and organic components. The treated material was repeatedly washed (5–6 times) with distilled water until a neutral pH was attained, with the

appearance of a colourless wash indicating effective protein removal. The resulting solid was dried at 35–60°C for 24 h to achieve constant weight. Subsequently, demineralisation was carried out by stirring the deproteinized powder in diluted hydrochloric acid to remove mineral impurities such as calcium salts. A 10% HCl solution was applied at a solid-to-liquid ratio of 1:10 (w/v) at room temperature (around 30°C). After treatment, the solid fraction was thoroughly washed with distilled water until neutral and dried at 35–60°C for 24 h. The obtained chitin was then subjected to deacetylation using 50% NaOH at a solid-to-liquid ratio of 1:50 (w/v) at 80 °C for 24 h. Finally, the product was oven-dried at 65°C for 24 h to obtain chitosan powder.

2.2 Extraction of lignin

Sugarcane bagasse (SCB) was obtained from a local juice centre and initially dried in an oven at 42°C for three days. The dried biomass was then cut into small pieces and subjected to fractionation using a grinding machine to obtain particles with an average size of 2–3 mm through sieving. The powdered SCB was dried again at 42°C to remove residual moisture. Dewaxing of the biomass was performed using a Soxhlet extractor with 150 mL of toluene-ethanol mixture (2:1 v/v) for 6 hours, after which the solvent-extracted SCB was oven-dried at 42°C until complete evaporation of the solvents. For lignin isolation, the dewaxed SCB was delignified using 1% NaOH solution in an autoclave reactor at 121°C for 30 minutes, with a biomass-to-solution ratio of 1 g to 10 mL. Following delignification, the solid and liquid fractions were separated using a muslin cloth. The resulting black liquor was acidified with 1 M H_2SO_4 to precipitate lignin, which was subsequently collected by centrifugation and oven-dried at 42°C for 24 hours to obtain the final lignin product. The initial preparation of sugarcane bagasse was carried out with slight modifications based on previously reported methods[5].

2.3 Preparation of chitosan film at different fractions of lignin.

A 2% (w/v) chitosan solution was prepared by dissolving chitosan in 0.5% (v/v) aqueous glacial acetic acid and stirred overnight at 30 °C using a magnetic stirrer to ensure complete dissolution. Isolated lignin fractions were incorporated into the chitosan solution at concentrations ranging from 100 to 400 mg per 100 mL (w/v). The chitosan-lignin mixture was filtered through muslin cloth, stirred for 6 hours, and subsequently sonicated for 30 minutes at 57°C to achieve a homogeneous dispersion. Each prepared solution was then blended with 2% gelatin and pure glycerol. The resulting mixtures were cast onto 9 cm diameter plastic plates under dust-free conditions and allowed to dry in air at 30 °C for four days, yielding uniform chitosan-lignin composite films. The preparation of chitosan–lignin composite films was carried out with slight modifications based on previously reported methods [5].

2.4 To study the physical characterization of films.

The water solubility of the prepared films was evaluated by cutting them into 1 × 1 cm pieces and immersing each sample in 10 mL of distilled water at room temperature for 48 hours. After incubation, the remaining film pieces were collected, filtered, and oven-dried at 110 °C for several hours to obtain the final dry weight (Mf). The initial dry weight (Mi) of each film was measured prior to immersion. Water solubility (%) was calculated using the following equation:

$$\text{Solubility in water (\%)} = \frac{Mi-Mf}{Mi} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where Mi represents the initial dry weight (g), and Mf represents the final dry weight (g). The water content of the films was determined by weighing the samples before and after drying in a hot oven at 80 °C for 48 hours. The water content (%) was calculated using the equation:

$$\text{Water content (\%)} = \frac{Mi-Mf}{Mi} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where Mi is the initial weight of the film (g), and Mf is the weight after drying (g). The results were expressed as grams of water per gram of wet film (g H_2O/g wet basis).

The water solubility and water content of the films were determined with minor modifications according to previously reported methods [5].

2.5 To study the physicochemical parameters of the films.

The optical properties of the prepared films were analyzed using a Shimadzu UV-2600 spectrophotometer in the wavelength range of 200–500 nm, with a slit width set at 2 nm. Rectangular film samples (2 × 2 cm) were cut and dissolved in 0.1% (v/v) acetic acid to prepare the test solution. Quartz cuvettes with a path length of 1 cm were used for all measurements, and the absorbance spectra of the film solutions were recorded over the 200–500 nm wavelength range to assess their UV-visible light absorption characteristics. The optical properties of the films were analysed with slight modifications based on previously reported methods [5].

2.6 Film characterization

Fourier transform infrared (FTIR) spectroscopy was carried out using a PerkinElmer Spectrum 2 to investigate the chemical composition of the films and potential interactions with incorporated additives. The KBr pellet method was employed for sample preparation. Chitosan-lignin composite films with lignin concentrations of 100, 200, 300, and 400 mg/mL, as well as pure chitosan films, were analysed. Spectra were recorded over the range of 400–4000 cm^{-1}, and the peaks of interest were examined by calculating the area under the curve to assess the functional groups and interactions within the films. Fourier transform infrared analysis of the films was carried out with slight modifications based on previously reported methods [5].

2.7 To study the antibacterial and antioxidant activity of the film.

The in vitro antibacterial activity of the prepared polymer films was evaluated using the well diffusion method. Mueller-Hinton agar plates were prepared. Cultures of *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi* were spread onto sterile Mueller-Hinton petri plates and allowed to solidify at room temperature for 15 minutes. Wells of 10 mm diameter were created using a sterile cork borer, and 50 µL of polymer solution was added to each well. The plates were then incubated at 37 °C for 24 hours, after which the zones of inhibition were measured. The antibacterial activity was evaluated using the well diffusion method with slight modifications based on previously reported procedures [5].

The antioxidant activity of the films was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Film samples (20 × 20 mm) were immersed in 10 mL of methanol for 20 minutes at 25 °C to extract the soluble compounds. Subsequently, 500 µL of the film extract was mixed with 4 mL of 150 µM DPPH solution, stirred thoroughly, and kept in the dark for 30 minutes. The absorbance of the mixture was measured at 517 nm, with ascorbic acid used as the standard reference for comparison.

2.8 To study the food quality analysis using bio-composite films.

Food quality analysis was conducted using fresh tomatoes to evaluate the preservation effect of the prepared chitosan-lignin polymer films. Tomatoes were immersed in polymer solutions containing lignin at concentrations of 200, 300, and 400 mg/mL. The treated tomatoes were stored at room temperature and at 4°C as control conditions. The quality and preservation of the tomatoes, with and without the polymer coating, were monitored and recorded over the storage period.

III.RESULT AND DISCUSSION

3.1 Extraction of chitosan and lignin

Chitosan was successfully extracted from mussel shells through sequential deproteination, demineralization, and deacetylation processes. The final product appeared as an off-white powder shown in Figure 3.1, which is characteristic of purified chitosan and indicates effective removal of proteins and minerals. Similar extraction approaches and appearances have been reported for chitosan derived from other marine shell wastes such as prawns, oysters, and crustaceans[8].

Fig. 3.1 Extracted chitosan

Lignin extraction from sugarcane bagasse involved dewaxing, alkaline delignification, and acid precipitation, resulting in a brown-colored lignin powder shown in Figure 3.2. The dark colouration is typical of lignin and reflects the presence of aromatic phenolic structures. Comparable extraction strategies using alkaline and acid treatments have been widely reported for lignin isolation from agricultural residues [9]. The successful extraction of both biopolymers enabled their use in the preparation of lignin–chitosan bio-composite films.

Fig. 3.2 Extracted lignin

3.2 Bio-composite films

Bio-composite films were prepared using pure chitosan, pure lignin, and chitosan incorporated with varying lignin concentrations (100, 200, 300, and 400 mg/mL). The films obtained were uniform and flexible, as shown in Figure 3.3, confirming the compatibility of lignin with the chitosan matrix.

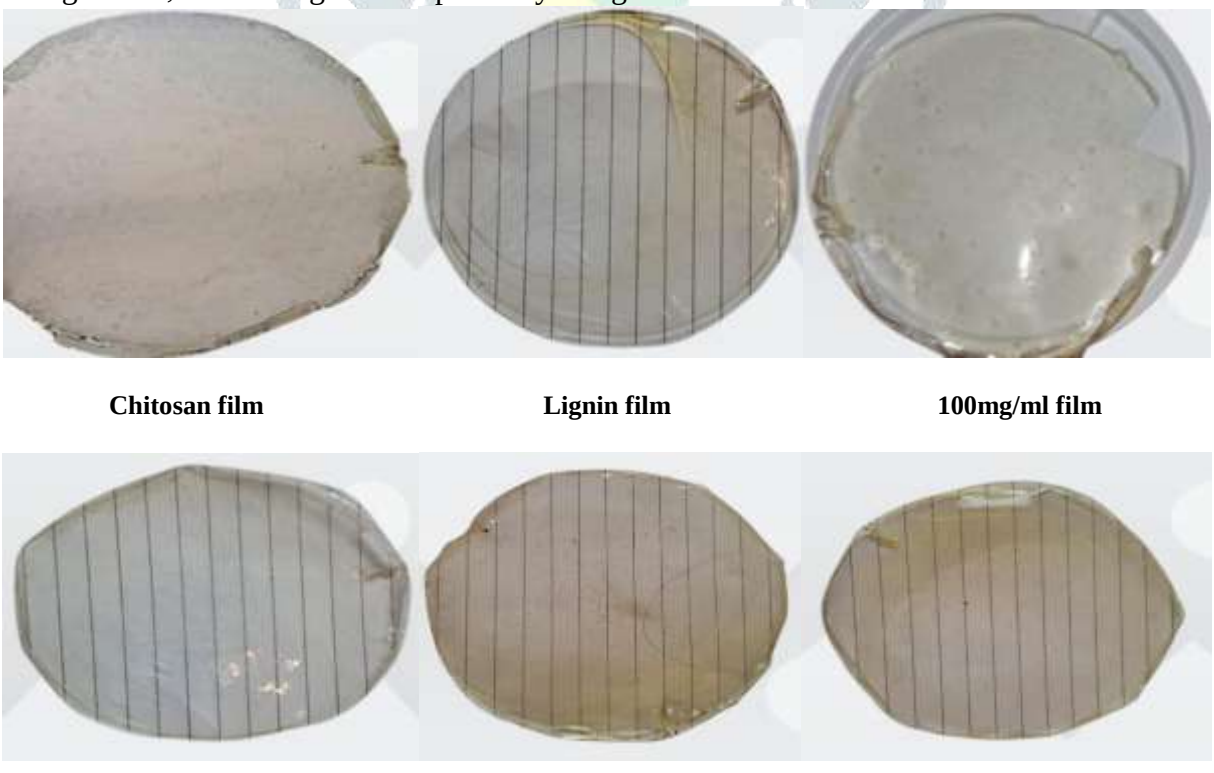

Fig:3.3 Bio-composite films

3.3 Water Solubility and Water Content

Water solubility and water content are critical parameters for evaluating the applicability of bio-composite films in food packaging. As presented in Table 3.1, pure chitosan film exhibited a water solubility of 90.17%, whereas pure lignin film showed lower solubility (82.81%). The incorporation of lignin into chitosan films resulted in a gradual decrease in water solubility as the lignin concentration increased, ranging from 96.21% (100 mg/mL) to 94.65% (400 mg/mL). This reduction in solubility can be attributed to a decrease in the number of hydrophilic functional groups and increased intermolecular interactions between chitosan and lignin. Similar observations have been reported by Campbell and Sederoff (1996) and [5].

Water content analysis further supported this trend. Pure chitosan film showed relatively higher water uptake due to its hydrophilic nature, whereas increasing lignin concentration significantly reduced water content. The water content decreased from 12.46% (100 mg/mL) to 7.55% (400 mg/mL). This reduction is attributed to the hydrophobic aromatic network of lignin, which limits moisture absorption by the film matrix. Intermolecular hydrogen bonding between hydroxyl groups of lignin and chitosan is also believed to restrict water penetration into the films [5].

Table 3.1: Water solubility and water content of chitosan, lignin, and lignin-containing chitosan films

Films	Water solubility (%)	Water content (%)
Chitosan	90.17	14.30
Lignin	82.81	10.58
100mg/ml lignin	96.21	12.46
200mg/ml lignin	95.86	10.98
300mg/ml lignin	95.51	9.57
400mg/ml lignin	94.65	7.55

3.4 UV–Visible Analysis

UV–visible spectroscopy (200–500 nm) was used to evaluate the optical and light barrier properties of the films shown in Figure 3.4. Lignin-containing films showed significantly higher UV absorbance compared to pure chitosan films, particularly around 280 nm. The UV barrier efficiency increased with increasing lignin concentration, with the 400 mg/mL lignin film exhibiting the highest absorbance. This enhanced UV-blocking ability is attributed to the chromophoric phenolic structures present in lignin, which effectively absorb UV radiation. Such UV shielding properties are desirable for food packaging applications, as UV light in the 280–350 nm range can induce lipid oxidation and degrade food quality. Similar findings have been reported for lignin-based composite films [8].

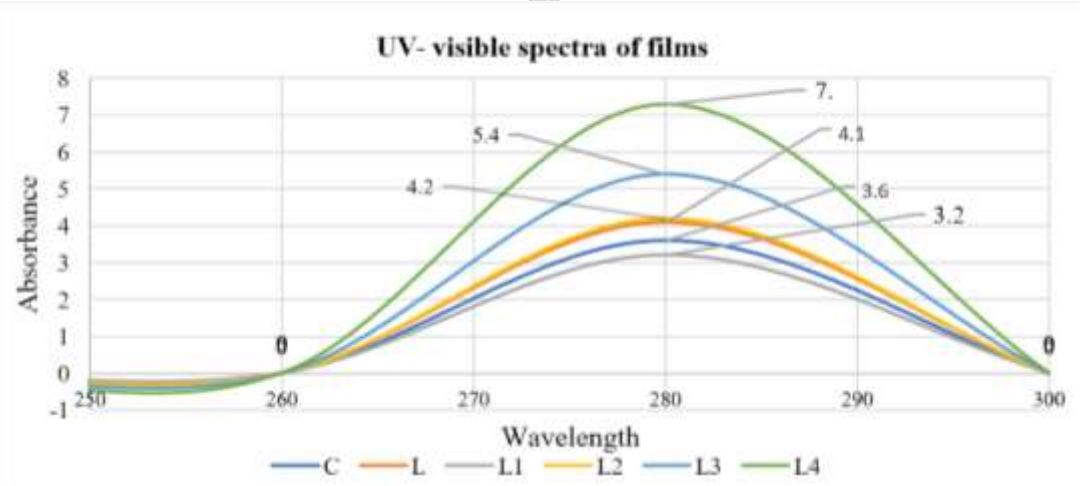

Fig. 3 .4 UV- visible spectra

3.5 FTIR Analysis

FTIR spectroscopy was employed to investigate chemical interactions between chitosan and lignin in the bio-composite films shown in Figures 3.5 and 3.6. The spectra revealed noticeable shifts and changes in peak intensities in lignin–chitosan films compared to pure components, indicating successful incorporation and interaction. The characteristic absorption band around 1543 cm^{-1} corresponding to the amino group stretching of chitosan showed modifications upon lignin addition. These spectral changes suggest intermolecular hydrogen bonding between hydroxyl (–OH), ether (R–O–R), and amino groups of chitosan and lignin, confirming the formation of a compatible bio-composite matrix.

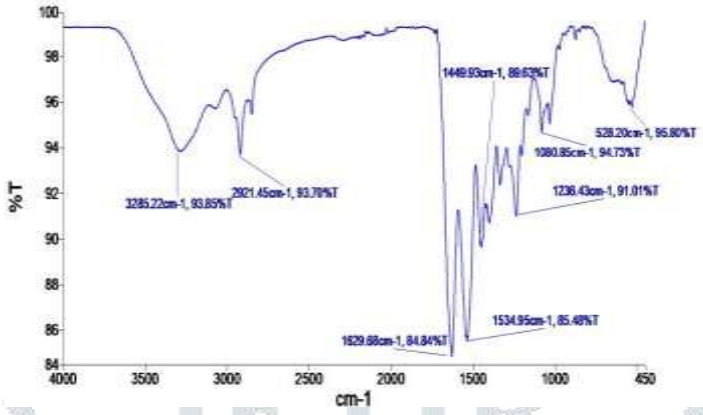

Fig. 3.5 FTIR spectra of chitosan

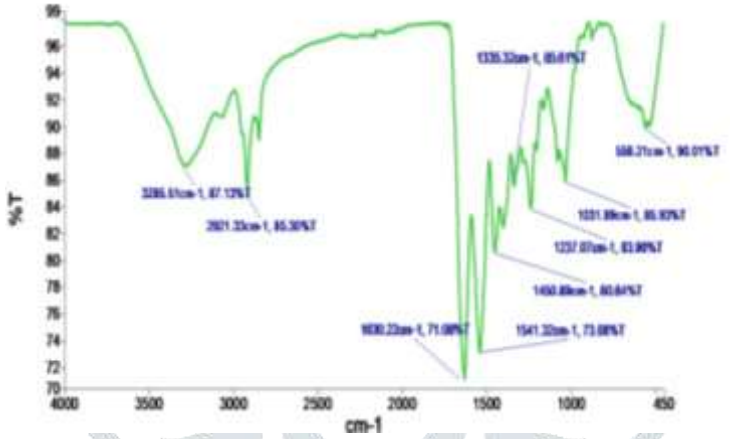

Fig.: 3.6 FTIR spectra of chitosan with 400mg/ml lignin

3.6 Antibacterial Activity

The antibacterial activity of polymer solutions was evaluated using the well diffusion method against *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhi* shown in Table 3.2. Pure lignin exhibited notable antibacterial activity against all tested organisms, as evidenced by clear zones of inhibition. In contrast, both extracted and commercial chitosan showed antibacterial activity under the tested conditions. Furthermore, the incorporation of lignin into chitosan films did not result in observable antibacterial zones. This suggests that the chitosan matrix may restrict the diffusion or activity of lignin's antimicrobial components. While [9] reported enhanced antibacterial activity in lignin–chitosan films against certain bacteria, the present findings indicate that antibacterial efficacy may depend on lignin availability, film structure, and interaction strength.

Films	Zone of inhibition in (mm)		
	E.coli	*B.subtilis*	*S.typhi*
Acetic acid (0.5%)	12	20	13.5
Chitosan	-	-	-
Commercial chitosan (1%)	-	-	-
Lignin	19	30	16.5
100mg/ml lignin	-	-	-
200mg/ml lignin	-	-	-
300mg/ml lignin	-	-	-
400mg/ml lignin	-	-	-

Table 3.2: Zone of inhibition (in mm) of polymer solutions

3.7 Antioxidant Activity

Antioxidant activity assessed using the DPPH assay revealed a clear concentration-dependent increase with lignin incorporation, shown in Figure 3.6. The highest antioxidant activity (55.29%) was observed for the film containing 400 mg/mL lignin, whereas pure chitosan showed minimal activity. This enhancement is attributed to the phenolic groups in lignin, which act as effective free radical scavengers. Although the antioxidant activity observed was lower than values reported by[14], the increasing trend with lignin concentration highlights the potential of lignin–chitosan films in reducing oxidative degradation in food systems.

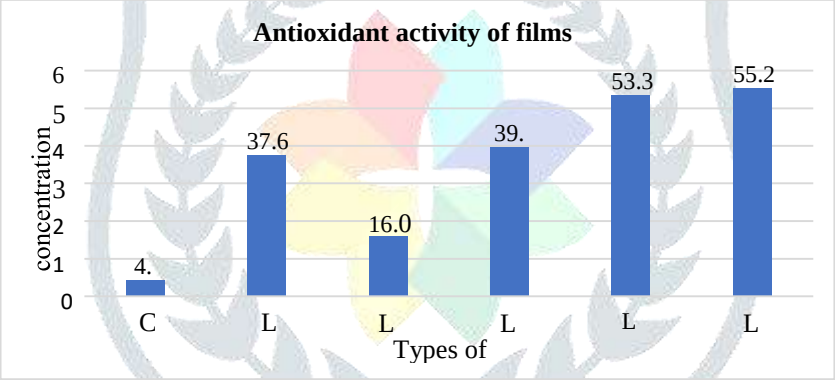

Fig.3.7 Antioxidant activity of films

3.8 Food Preservation Study

The practical applicability of the bio-composite films was evaluated through food quality analysis using tomatoes, shown in Figures 3.8 and 3.9. Uncoated tomatoes showed visible spoilage within 10 days and were completely spoiled by the third week at both room temperature and 4 °C. In contrast, tomatoes coated with lignin–chitosan films (200, 300, and 400 mg/mL) remained unspoiled for up to four weeks under both storage conditions. The 400 mg/mL lignin film exhibited the highest preservation efficiency. These findings align with previous studies demonstrating the effectiveness of chitosan-based edible coatings in extending the shelf life of fruits and vegetables[10],[11]. The combined antioxidant and moisture barrier properties of lignin–chitosan films contribute significantly to delaying spoilage and maintaining food quality.

Fig. 3.8 Food quality test on tomatoes at room temperature

After 1 week After 2 weeks After 3 weeks

Fig. 3.9 Food quality test on tomatoes at 4°C

IV. CONCLUSION

Extraction of chitosan from mussels was carried out using demineralization, deproteination, and deacetylation. The extraction of lignin was carried out by dewaxing using a Soxhlet apparatus with toluene-ethanol solvent and then delignified with NaOH, which resulted in black liquor. The black liquor was acidified by H_2SO_4, and lignin was obtained. The lignin was incorporated with chitosan at different fractions, and it was used for further study. Out of all the fractions of lignin, 400mg/ml lignin showed the least water solubility and water content. Among all the components used to prepare film, only pure lignin showed antibacterial activity against all three cultures. Among all the concentrations of lignin used, including 200mg/ml,300mg/ml, and 400mg/ml, all demonstrated a good amount of antioxidant activity. These properties could be favourable for the protection of certain types of food preparation in which the oxidation process may signify a limiting factor determining their shelf-life. Therefore, 200mg/ml,300mg/ml, and 400mg/ml lignin biofilms were used to determine the preservation of the tomato samples. 400mg/ml lignin-containing film shows the best and highest preservative activity as compared to 200mg/ml,300mg/ml, and the control. Thus highest concentration of lignin film is preferred to be used in packaging. Therefore, these bio-composite films can be used in the packaging of different food materials. The work highlights the utilisation of natural and waste-derived polymers, chitosan, and lignin as a whole in food packaging applications for storage, preservation, and enhanced shelf life of food.

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