Research Article

Product Optimization and Antimicrobial Efficiency of Starch-Based Active Packaging Film Prepared Using Amira (Plumbago Zeylanica) Root Extract

Melak Muche,1 Adamu Zegeye,2 Hirut Lemma,3 and Tilahun Gisila4

1Department of Food Engineering, Debre Berhan University, Debre Berhan, Ethiopia
2Department of Food Engineering, Addis Ababa University, Addis Ababa, Ethiopia
3Traditional and Modern Medicine Research Directorate, Ethiopian Public Health Institute, Addis Ababa, Ethiopia
4Department of Chemical Engineering, Debre Berhan University, Debre Berhan, Ethiopia

Correspondence should be addressed to Tilahun Gisila; tile2224@gmail.com

Received 18 May 2022; Revised 11 July 2022; Accepted 22 July 2022; Published 5 September 2022

Academic Editor: Guosong Wu

Copyright © 2022 Melak Muche et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

This study aimed to develop food packaging from cornstarch combined with methanol extract of Plumbago zeylanica root as antimicrobial agents. The antimicrobial activity of the extract was tested against three bacterial strains, namely, Staphylococcus aureus, Salmonella typhi, and Escherichia coli and one fungal strain called Aspergilla niger. Microorganism growth inhibition was examined by employing a zone of inhibition check on solid media. Two hundred microliters of assorted concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml) of plant extracts were added into an eight-millimeter hole diameter employing a micropipette. Hence, 200 μl of 200 mg/ml extract inhibited 13.5, 8.75, 10.5, and 27 mm diameter for S. typhi, E. coli, S. aureus, and A. niger, respectively. The minimum inhibitory concentrations 1.25 mg/ml, 2.5 mg/ml, 1.25 mg/ml, and 0.625 mg/ml of crude extract utterly suppressed the growth of S. aureus, E. coli, S. typhi, and A. niger, respectively. Later on, the antimicrobial agent films and the control media were prepared by the casting method. The prepared antimicrobial packaging films exhibited inhibitory zones (a wide clear zone on solid media) for S. typhi and A. niger growth inhibition; however, inhibitions for E. coli and S. aureus were not effective. Antimicrobial activity decreased while decreasing the solubility of the film and antimicrobial concentration with increasing cornstarch content in the solution. The tensile strength of the films increased with increasing antimicrobial content in the solution. Higher antimicrobial inhibition activity was found at a 1.5 glycerol/starch ratio, 1.5 gram content of P. zeylanica, and 0.5 mm film thickness. Generally, the plasticized cornstarch antimicrobial packaging film combined with P. zeylanica extract had a significant effect on the growth of each selected microorganism and the physical properties of the material (P < 0.05). A preprint of this research has previously been uploaded to the Addis Ababa University database (Yirdaw et al. 2018). In this section, only the summary and updated content of the research findings are presented.

1. Introduction

Food safety and spoilage triggered by food-borne microorganisms are stern problems [1, 2]. On the other hand, the shelf life of most food products depends on the biological, chemical, and physical interactions between the food package and the ambient environment [3, 4]. Synthetic packaging has good mechanical properties and effectiveness as a barrier to oxygen and water [4]. However, these materials have led to serious environmental problems due to their nonbiodegradability [4]. Consequently, the purpose of modern food quality preservation and assurance with packaging technologies is to enhance food safety and quality in as natural a way as possible [2–5]. Antimicrobial packaging (AMP) has been introduced to provide an interaction between food and packaging materials to control bacterial and fungal food spoilage [1, 4, 6]. AMP has more advantages compared to directly adding AM agents to foods because antimicrobial agents added to food surfaces by spray or drip are not effective enough to inhibit microorganisms [1, 7].
 Whereas, AMP offers slow and continuous migration of antimicrobial agents from packaging materials to food surfaces, which enables the antimicrobial agent to maintain a high concentration over a long period [6, 7]. The film technique is considered to be more effective, although more complex to apply [8]. The antimicrobial capacities of food packaging depend on antimicrobial agents, which can be classified as natural antibacterial agents and synthetic antibacterial agents based on their sources [1]. Antimicrobial agents have been developed from natural sources because natural antimicrobial agents are relatively safe and are easy to obtain [1, 9, 10]. *Plumbago zeylanica* is a natural antimicrobial extract and plays an important role in the traditional system of medicine against various diseases, such as anti-inflammatory, antimalarial, antioxidant, wound healing, memory enhanced, anticancer and antimicrobial, antifungal, and antidiabetic [9, 11–13].

The functionality of raw starch alone does not properly suit and fulfill product demands, mainly due to its high hydrophilicity and poor mechanical properties, which pose critical problems in starch commercialization [14]. Currently, the food processing industries require the development of economically viable modified packaging materials to meet the increasing demand for protective, biodegradable packaging materials that are highly specific to each application [4, 5, 15]. In this experimental trial, an antimicrobial packaging film was prepared from starch, glycerol, and *P. zeylanica* root crude extract using a casting process, and its effectiveness was tested on food infectious microorganisms.

### 2. Materials and Methods

#### 2.1. Raw Materials Collection and Preparation.

*P. zeylanica* (root) was collected from Addis Zemen, which is located 634 km northwest of Addis Ababa (latitude and longitude of 12°07′N 37°47′E). The species of *P. zeylanica* were approved by the Department of Botany, at the College of Natural Science, AAU and Ethiopian Public Health Institute (EPHI). *Escherichia coli* (ATCC29212), *Staphylococcus aureus* (ATCC25923), and *Salmonella typhimurium* (ATCC13311) bacterial and *Aspergillus niger* (ATCC10535) fungal species were used for the test. The organisms were obtained and identified in the department of microbiology at the Ethiopian Public Health Institute (EPHI). The root of *P. zeylanica* was washed with distilled water and blotted with filter paper and then dried in shade. After drying, it was grounded into powder by using a universal grinder. Finally, the sample was stored in a sealed plastic bag at room temperature before use. Moreover, the bacterial strains in Muller–Hinton agar (MHA) and the fungus strain in Sabouraud Dextrose Agar (SDA) were preserved and stored at 4°C until used.

#### 2.2. Extraction of Root of *P. Zeylanica* Root.

A hundred grams of dried and powder root of *P. zeylanica* was used for extraction. The extraction was carried out with 600 ml methanol for 8 hr using a soxhlet extractor. The extract was filtered through Whitman No. 1 filter paper and then concentrated at 50°C using a rotary evaporator. Finally, it was transferred to glass vials and kept at 4°C before use.

#### 2.2.1. Preliminary Phytochemical Screening *P. Zeylanica* Root Extract.

The alkaloids, flavonoids, saponins, phenolic components, plumbagin, and glycosides content were determined as described in reference [11].

#### 2.2.2. Preparation of Stock Solution for Antimicrobial Agent.

The stock solution for the preliminary antimicrobial test was prepared by dissolving a thousand milligram of the extract in 5 ml dimethyl sulfoxide (5% concentration). Then, different concentrations of methanol extracts of the root such as 200 mg/ml, 100 mg/ml, 50 mg/ml, and 25 mg/ml were prepared. MIC was determined by taking 2 ml of each concentration per plate. Then, the stock solution was calculated according to CLSI by using the following equation:

\[
C_2xV_2 = C_1xV_1
\]

where \( C_2 \) is the final concentration of stock solution per liquid medium, \( V_2 = 20 \text{ ml} \), and \( V_1 = 2 \text{ ml} \).

#### 2.2.3. Preparation and Standardization of Inoculum.

Microorganisms were refreshed to get the young strain using a slant test tube. Five milliliters MHA for bacteria and 5 ml SDA for fungal were prepared in a separate test tube for each organism and incubated at the standard condition for 24 h. After refreshment, cultures of the test organisms were prepared following the method adopted by Chikezie [16].

#### 2.2.4. Preliminary Screening Test of Antibacterial and Antifungal Activities.

A preliminary screening test of antibacterial and antifungal activities was carried out for the crude plant extracts by using the hole plate diffusion method [9]. The test organisms were *S. aureus* (ATCC 25923), *E. coli*...
2.3.5. Minimum Inhibition Concentration (MIC) of P. zeylanica Extract. The MIC of P. zeylanica root extract against the tested organisms was determined using the tube dilution method [17] by inoculating the standardized test organisms with different concentrations of the extract per plate. To determine the MIC, 2 ml antimicrobial agent per 20 ml plate was taken according to CLSI. Therefore, the crude plant extract was prepared to have the final concentration of 0.625, 1.25, 2.5, and 5 mg/ml in liquid media when 2 ml of different concentrations were added to 18 ml of presterilized molten agar at 45°C. All plates were incubated at 37°C for 24 h for bacterial strains and 28°C for 72 h for A. niger in an aerobic environment. Finally, the presence or absence of growth at each concentration of P. zeylanica was inspected. The MIC that completely inhibited the growth of bacteria for the test compound was recorded. All tests were carried out in three duplicates and the results were reported as the average of these replications.

2.4. Antimicrobial Packaging Film Production. The cornstarch-based films were prepared by the conventional solution-casting technique. A film-forming dispersion was prepared using the procedure adapted from Naz et al. [17] having a different ratio of glycerol/starch and antimicrobial agent. The films were prepared with 0.5 mm and 1 mm thicknesses. The level of glycerol/starch ratio and antimicrobial concentration were 0.67, 1, 1.5 (w/w) and 0.5, 1, 1.5 Grams, respectively.

2.5. Antimicrobial Activity of the Packaging Films. The cornstarch film without antimicrobial compounds was used as a control. The inhibition zone of bacteria species was measured after incubation at 37°C for 24 h. Besides, the inhibition zone of the fungus was measured after 72 h incubation period at 28°C [18]. For this test, three different concentrations (0.5 Gram, 1 Gram, and 1.5 Gram) of antimicrobial were prepared.

2.6. Characterization of Antimicrobial Packaging Films Properties

2.6.1. Moisture Content of Antimicrobial Packaging Films. The moisture content was determined by the moisture analyzer. The films were analyzed for 10 minutes at 100°C and recorded the value of the moisture content.

2.6.2. Swelling of Antimicrobial Packaging Films. The swelling of the film was determined by soaking a known weight of the film in distilled water at room temperature for 30 minutes. The starch film was blotted with a filter paper to remove the excess water and weighted immediately [19]. The average values of the duplicates were determined. The percentage of water absorption $w_{sw}$ was calculated using the following equation:

$$Swelling(w_{sw}) = \left( \frac{w_{i} - w_{0}}{w_{1}} \right) \times 100,$$

where $w_{i}$ is the weight of the starch film after 30 minutes of absorption and $w_{0}$ is the initial weight of the starch film.

2.6.3. Solubility of Antimicrobial Packaging Films. A piece of film with the dimension of 2 cm × 3 cm was immersed in distilled water for 24 h with slow mechanical stirring using a shaker at room temperature. Samples were removed from the solution by filtration and then dried in an oven dryer (60°C for 24 h). The film solubility was calculated via weight difference [20] using the following equation:

$$Solubility = \left( \frac{W_{ti} - W_{tf}}{W_{ti}} \right),$$

where $W_{ti}$ is the initial weight of the starch film and $W_{tf}$ is the weight of the film after immersion.

2.6.4. Tensile Strength of Antimicrobial Packaging Films. Tensile strength was performed using a texture analyzer. Uniform film specimens of 100 mm by 5 mm size were prepared from the starch-based antimicrobial film samples. The film strips were placed in the pneumatic grip of the texture analyzer, which was set at the initial separation of 50 mm. The crosshead speed was set at 50 mm min$^{-1}$ [21]. Values were reported as the means of duplicate determination.

2.7. The Process Variables of Antimicrobial Packaging Film. The glycerol/starch ratio, concentration of the antimicrobial agent, and film thickness were taken as factors to study the antimicrobial activity of the film as well as the physical and mechanical properties of the packaging film. The experimental design selected for this study was a general factorial method with three factors having mixed levels and the response was described by the inhibition zone. The Design Expert 6.0.8 software was used in the analysis of variance (ANOVA). The physical and mechanical properties of the packaging films are also described graphically using Microsoft Excel factors.
3. Results and Discussions

3.1. Phytochemical Constituents of P. Zeylanica Root. The results of Phytochemical screening showed that saponins, phenols, and tannins were found in the methanol extract of P. zeylanica root. Tannins and phenols content was determined by the Braymer’s test method and therefore dark green color was formed, which indicates the presence of phenols and tannins. This exhibited that crude extract P. zeylanica using methanol can have the potential to inhibit the growth of microorganisms.

3.2. Preliminary Test Results of Crude Extract of P. Zeylanica. The activity of P. zeylanica was effective on A. niger and the average inhibition diameter was measured at 27 mm. Moreover, P. zeylanica has also been more effective on S. typhi than S. aureus (ID = 13.5 mm) and E. coli (ID = 8.75 mm) next to A. niger. From the results obtained, P. zeylanica had a nearly similar effect on E. coli and S. aureus. The inhibition diameter for negative control was null for all microorganisms. Sulfamethoxazole (as positive control) was used for both diameter for negative control was null for all microorganisms.

3.3. Determination of MIC of P. Zeylanica Crude Extract. The MIC results are listed in Table 2. The results showed that 2.5 mg/ml of liquid medium completely inhibited the growth of E. coli and it was taken as a minimum inhibition concentration for E. coli. The growth of S. aureus and S. typhi was completely inhibited at 1.25 mg/ml of liquid medium and it could be considered as minimum inhibition concentration for both microorganisms. The growth of A. niger was inhibited at 0.625 mg/ml of liquid medium. E. coli was highly resistant to antibiotics than S. aureus, S. typhi, and A. niger.

The current results presented on S. typhi and S. aureus were relatively enhanced when compared to the data reported by Rao et al. [12], 5 mg/ml and 5 mg/ml, respectively.

3.4. The Main Effect of Process Variables on Inhibition Zone

3.4.1. Effect of Glycerol/Starch Ratio on Inhibition Zone. The glycerol/starch ratio is one of the important factors that affect the activity of the packaging film (Figure 1). The graph shows that the inhibition diameter of each organism increased as the ratio of glycerol/starch increased. An increase in the ratio of glycerol/starch decreases the formation of strong hydrogen bonds with hydroxyl groups on starch chains without decreasing the crystalline level, which in turn increases the release rate of antimicrobial agents. Films with greater glycerol content exhibited higher film solubility. As reported by Nemet et al. [22], hydrophilic plasticizers, such as glycerol, enhanced the water solubility of dry matter in the film.

![Figure 1: Effect of Glycerol/Starch Ratio on Inhibition Zone](image)

3.4.2. Effect of Antimicrobial Concentration on Inhibition Zone. The concentration of antimicrobial agents significantly affected the antimicrobial activity of the packaging film. As the concentration of extract in the packaging film raised from 0.5 gram to 1.5 gram, the mean inhibition diameter also increased (Figure 2). The mean inhibition diameters reached 9.93, 16.60, 11.23, and 18.54 at 0.5 gram of extract for E. coli, S. aureus, S. typhi, and A. niger, respectively. The mean inhibition diameters reached around a peak of 21.63 mm, 25.80 mm, 32.21 mm, and 36.50 mm at 1.5 gram of extract for E. coli, S. aureus, S. typhi, and A. niger, respectively. Consequently, the highest inhibition diameter was found at 1.5 grams keeping other process variables constant. Similarly, the effect of antimicrobial concentration was the most dominant factor on inhibition diameter among the other factors except in the case of S. aureus.

3.4.3. Effect of Film Thickness on Inhibition Zone. The inhibition zone of each organism gradually decreased as the film thickness increased. When the thickness was large enough, the physical and mechanical properties of the film packaging were altered. The thin layer exhibited high water solubility, sorption, and poor mechanical properties, whereas the thick films found low water solubility and sorption with good mechanical strength. Hence, the release rate of the antimicrobial agents from the film to the medium is considerably increased.

3.5. Physiochemical and Mechanical Properties of Antimicrobial Packaging Film

3.5.1. Color of Antimicrobial Packaging Film. The color of different combinations of glycerol, starch, and antimicrobial agents of the packaging films was detected visually. Therefore, color of the packaging film was changed as the concentration of antimicrobial agents increased. The film without an antimicrobial agent was highly transparent and

| Concentration (mg/ml) | S. typhi | E. coli | S. aureus | A. niger |
|-----------------------|---------|---------|-----------|---------|
| 200                   | 13.5    | 8.75    | 10.5      | 27      |
| 100                   | 9.75    | 6.75    | 6         | 14.5    |
| 50                    | 3.8     | 2.75    | 3         | 4.5     |
| 25                    | 1.5     | 0       | 1         | 3.5     |

**Table 1: Inhibition diameter (ID) of microorganisms at different concentrations.**

| Concentration (mg/ml) | Visible growth of microorganisms |
|-----------------------|---------------------------------|
|                       | E. coli | S. aureus | S. typhi | A. niger |
| 5                     | No      | No        | No       | No       |
| 2.5                   | No      | No        | No       | No       |
| 1.25                  | Yes     | No        | No       | No       |
| 0.625                 | Yes     | Yes       | Yes      | No       |
| Control (without AMA) | Yes     | Yes       | Yes      | Yes      |

**Table 2: Minimum inhibition concentration for each organism.**

Note: AMA, antimicrobial agent.
colorless than films with an antimicrobial agent, as shown in Figure 3. The films prepared from glycerol/starch ratio (1.5 w/w), antimicrobial concentration (0.5 mg), and film thickness (0.5 mm) were more transparent than other formulations. However, as the thickness and concentration of the antimicrobial agent were raised, the transparency of the packaging film was declined. The film without an antimicrobial agent was used as a control to compare the effect of antimicrobial agents on the color of packaging films.

3.5.2. The Moisture Content of Antimicrobial Packaging Films. The moisture content of the films affects the physical properties of starch-based antimicrobial films. The highest moisture content (14.5%) was recorded at 1.5

---

**Figure 1:** Effect of glycerol/starch ratio on inhibition diameter of (a) *E. coli*; (b) *S. typhi*; (c) *S. aureus*; (d) *A. niger*.

**Figure 2:** Effect of antimicrobial concentration on inhibition diameter of (a) *E. coli*; (b) *S. typhi*; (c) *S. aureus*; (d) *A. niger*. 
glycerol/starch ratio, 0.5 grams of antimicrobial concentration, and 1 mm film thickness (Figure 4). The lowest moisture content was recorded at 0.67 glycerol/starch ratio, 1.5 Gram antimicrobial, and 0.5 mm of film. These results indicate that an increasing glycerol/starch ratio had a significant effect on the equilibrium water content as its concentration enhanced. Schaefer et al. [5] reported that the moisture content is mainly influenced by the amount of plasticizer added, which favors the adsorption of water in the film matrix, forming hydrogen bonds. Glycerol contains three hydrophilic alcoholic hydroxyl groups, which are responsible for its solubility in water and its hygroscopic nature. When the glycerol/starch ratio increased, the moisture content of the packaging films increased. This may be associated with glycerol increasing the content of hydrophilic hydroxyl groups of polysaccharides, which increased the water absorbability of starch-based antimicrobial packaging films [22, 23]. Farrahkny et al. [20] reported that higher levels of plasticizer increased the film’s moisture affinity and these results could be attributed to the hydrophobicity of the plasticizers, with an accessible hydroxyl group. Moreover, the films prepared with glycerol were more soluble than films without plasticizers. Generally, the moisture content increased as the film thickness also increased and the effect of the glycerol/starch ratio had a significant effect on the moisture content of the films.

3.5.3. Solubility of Antimicrobial Packaging Film. The water solubility of the cornstarch via incorporating P. zeylanica crude extract film as a function of glycerol content is shown in Figure 5. It indicates that glycerol, in all concentrations, increased the water solubility of starch films. It is probably because increasing the plasticizer content in the film increased the water-soluble dry content. The solubility of the film was influenced by the number of antimicrobial agents incorporated in the film. The films containing a small amount of the crude extract had a higher solubility. On the other hand, the films containing a larger amount of

![Figure 3: Color of packaging films without AM and with AM agent. (a) Film without AM. (b) AM packaging film.](image_url)
antimicrobial agent had a lower solubility. It is probably because since *P. zeylanica* root extract has fat and oil, increasing the extract amount might increase the lipid content in the film. Based on the results, it can be recommended that films having low solubility are favorable for the packaging purpose.

3.5.4. Swelling of Antimicrobial Packaging Films. The effect of glycerol, antimicrobial concentration, and film thickness on the swelling of the film was presented in Figure 6. The highest swelling capacity of the film (35%) was recorded at 1.5 w/w glycerol/starch ratio, 0.5 g of antimicrobial extract, and 0.5 mm of film thickness and the lowest swelling percentage (13%) was measured at 0.67 w/w glycerol/starch ratio, 1 gram of antimicrobial agent, and 1 mm of film thickness. The swelling capacity of the films was increased as the glycerol content and film thickness increased. However, the swelling capacity decreased as the antimicrobial concentration increased because the higher concentration increased the crystallite of the films. Moreover, the swelling capacity of the packaging film was dominantly affected by glycerol/starch ratio than antimicrobial agent and film thickness.

3.5.5. Tensile Strength of the Antimicrobial Packaging Film. The tensile strength (TS) of the antimicrobial films prepared from cornstarch via incorporating *P. zeylanica* crude extract is shown in Figure 7, and the tensile strength (TS) of the films is affected by glycerol/starch ratio, antimicrobial concentration, and film thickness. Hence, as the glycerol content is increased, the tensile strength of the films was decreased.

The presence of plasticizer at a lower concentration of 0.67 (glycerol/starch ratio), the higher concentration of antimicrobial concentration (1.5 gram), and film thickness (1 mm) demonstrated a high tensile strength value of 19.52 MPa. The possible reason for the high tensile strength...
at low plasticizer concentration is the domination of strong hydrogen bonds produced by a starch-starch intermolecular interaction over starch-plasticizer attraction. Moreover, the presence of plasticizer at a higher concentration of 1.5 (glycerol/starch ratio), lower concentration of antimicrobial concentration (0.5 gram), and film thickness (0.5 mm) demonstrated a low tensile strength value of 5.2 MPa. Therefore, these results also describe that high antimicrobial concentration and film thickness had a positive effect on the tensile strength of the antimicrobial packaging films.

4. Conclusions

The antimicrobial starch-based film showed good film-forming properties due to the presence of high inter- and intramolecular hydrogen bonding. The release rate of antimicrobial agents from the films could be made by changing the composition of the initial casting solution. Antimicrobial activity decreased while decreasing the solubility of the film and antimicrobial concentration with increasing cornstarch content in the solution. P. zeylanica root extract containing antimicrobial film can be used as an active food packaging material. The shortcomings of the films, the long-term effectiveness of antimicrobial properties, and the physical and chemical properties could be minimized by further studies on the improvement of cornstarch-based packaging film products.

Data Availability

The data used to support the findings of this study are statistically valid and available from all authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Melak Muche and Adamu Zegeye did the experiments and wrote the main part of the paper. Hirut Lemma worked on the species identification microbial test of the research. Tilahun Gisila Abebe prepared and developed the structure and content of the manuscript.

Acknowledgments

A preprint of this research has previously been uploaded to the Addis Ababa University [25] database. In this section, only the concise and restructured contents of the research findings are presented.

References

[1] B. Malhotra, A. Keshwani, and H. Kharkwal, “Antimicrobial food packaging: potential and pitfalls,” Frontiers in Microbiology, vol. 6, pp. 611–619, 2015.
[2] S. Haghighi-Manesh and M. H. Azizi, “Active packaging systems with emphasis on its applications in dairy products,” Journal of Food Process Engineering, vol. 40, no. 5, Article ID e12542, 2017.
[3] L. Motelica, D. Ficai, A. Ficai, O. C. Oprea, D. A. Kaya, and E. Andronescu, “Biodegradable antimicrobial food packaging: Trends and perspectives,” Foods, vol. 9, no. 10, pp. 1438–1536, 2020.
[4] E. W. Schaefer, J. M. F. Pavoni, C. L. Luchese, D. J. L. Facchin, and I. C. Tessaro, “Influence of turmeric incorporation on physicochemical, antimicrobial and mechanical properties of the cornstarch and chitosan films,” International Journal of Biological Macromolecules, vol. 148, pp. 342–350, 2020.
[5] S. Y. Sung, L. T. Sin, T. T. Tee et al., “Antimicrobial agents for food packaging applications,” Trends in Food Science & Technology, vol. 33, no. 2, pp. 110–123, 2013.
[6] B. Kloeckner, “Optimal transport and dynamics of expanding circle maps acting on measures,” Ergodic Theory and Dynamical Systems, vol. 33, no. 2, pp. 529–548, 2013.
[7] D. S. Cha and M. S. Chinnan, “Biopolymer-based antimicrobial packaging: a review,” Critical Reviews in Food Science and Nutrition, vol. 44, no. 4, pp. 223–237, 2004.
[8] S. R. d. Paiva, M. R. Figueredo, T. V. Aragão, and M. A. C. Kaplan, “Antimicrobial activity in vitro of plumbago isolated from plumbago species,” Memorias Do Instituto Oswaldo Cruz, vol. 98, no. 7, pp. 959–961, 2003.
[9] K. I. Suhr and P. V. Nielsen, “Antifungal activity of essential oils evaluated by two different application techniques against rye bread spoilage fungi,” Journal of Applied Microbiology, vol. 94, no. 4, pp. 665–674, 2003.
[10] B. Eldhose, V. Notario, and M. S. Latha, “Evaluation of Phytochemical Constituents and in Vitro antioxidant Activities of Mentha Arvensis,” International Journal of Biology, Pharmacy and Allied Sciences, vol. 10, no. 9, pp. 157–161, 2021.
[11] D. H. Rao, T. Vijaya, B. R. Naidu, P. Subramanyam, and D. J. Rayalu, “Phytochemical screening and antimicrobial studies of compounds isolated from Plumbago zeylanica L,” International Journal of Analytical, Pharmaceutical and Biomedical Sciences, vol. 2, no. 3, pp. 4–6, 2014.
[12] R. Tyagi and E. Menghani, “A review on Plumbago zeylanica,” A Compelling Herb, vol. 5, no. 04, pp. 119–126, 2014.
[13] M. F. Ramadan and M. Z. Sitohy, “Phosphorylated Starches: preparation , properties , functionality , and Techno-applications,” Starch - Starke, vol. 72, no. 5-6, Article ID 1900302, 2020.
[14] M. Sitohy, M. Fawzy, and R. Hassanien, “Degradability of different Phosphorylated Starches and Thermoplastic films prepared from Corn starch Phosphomonoesters,” Starch - Starke, vol. 53, no. 7, pp. 317–322, 2001.
[15] I. O. Chikezie, “ Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method,” African Journal of Microbiology Research, vol. 11, no. 23, pp. 977–980, 2017.
[16] R. Naz, A. Bano, H. Yasmin, Samiullah, and U. Farooq, “Antimicrobial potential of the selected plant species against some infectious microbes used,” Journal of Medicinal Plants Research, vol. 5, no. 21, pp. 5247–5253, 2011.
[17] E. Salleh, I. Muhamad, and N. Kairuddin, “Structural characterization and physical properties of antimicrobial (AM) starch-based films,” World Academy of Science, vol. 3, no. 7, pp. 428–436, 2009, http://waset.org/journals/waset/v31/v31-76.pdf.
[18] G. Yehuala and S. Admassu, “Antimicrobial activity, physicochemical and mechanical properties of Aloe (Aloe debraana)
based packaging films,” *British Journal of Applied Science & Technology*, vol. 3, no. 4, pp. 1257–1275, 2013.

[19] A. Farahnaky, B. Saberi, and M. Majzoobi, “Effect of glycerol on physical and mechanical properties of wheat starch edible films,” *Journal of Texture Studies*, vol. 44, no. 3, pp. 176–186, 2013.

[20] D. F. Parra, C. C. Tadini, P. Ponce, and A. B. Lugao, “Mechanical properties and water vapor transmission in some blends of cassava starch edible films,” *Carbohydrate Polymers*, vol. 58, no. 4, pp. 475–481, 2004.

[21] N. T. Nemet, V. M. Sošo, and V. L. Lazić, “Effect of glycerol content and pH value of film-forming solution on the functional properties of protein-based edible films,” *Acta Periodica Technologica*, vol. 41, pp. 57–67, 2010.

[22] G. Kavoosi, S. M. Dadfar, and A. M. Purfard, “Mechanical, physical, antioxidant, and antimicrobial properties of Gelatin films incorporated with Thymol for potential Use as Nano wound Dressing,” *Journal of Food Science*, vol. 78, no. 2, pp. E244–E250, 2013.

[23] A. L. Brody, B. Bugusu, J. H. Han, C. K. Sand, and T. H. McHugh, “Scientific Status summary,” *Journal of Food Science*, vol. 73, no. 8, pp. R107–R116, 2008.

[24] T. Huang, Y. Qian, J. Wei, and C. Zhou, “Polymeric Antimicrobial food packaging and its applications,” *Polymers*, vol. 11, no. 3, p. 560, 2019.

[25] M. M. Yirdaw, “Addis Ababa Institute of Technology School of chemical and Bio Engineering process Stream,” *Materials Science*, vol. 2357, 2018.