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Antimicrobial Activity, Physicochemical and Mechanical Properties of Aloe (*Aloe debrana*) Based Packaging Films

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAE designed and supervised the research; author GA managed the literature search, performed the experimental analyses, conducted the statistical data analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To develop aloe based packagings films and evaluate the antimicrobial activity, physicochemical and mechanical properties at different proportions of *Aloe debrana* extract, papaya leaves extract, gelatin and glycerol.
Study Design: Completely randomized design (CRD)
Place and Duration of Study: Addis Ababa University, between May 2010 and November 2012.
Methodology: The Aloe and papaya leaves extracts were analyzed for their antimicrobial activity, transparencies and colours. The packaging films were developed by adding various concentrations of gelatine and glycerol into the solution of *Aloe debrana* and papaya extracts. Films were evaluated for their antimicrobial activity, physicochemical and mechanical properties. The antimicrobial activity of Aloe based films were tested on *E. coli*, *S. typhi*, *S. aureus*, *C. albicans*, and *F. xylarioides*
Results: All films exhibited inhibitory zones on the test microorganisms used in this study. A wide inhibition zone (6.52cm^2) was observed on *S. typhi* growth whereas the least (4.20 cm^2) was found on *C. albicans*. The developed films were soluble in water with maximum solubility (90.49%) for $P_{1,1}$ and lowest (44.57%) for $P_{0,2}$. The film solubility

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significantly increased as the concentration of glycerol augmented and decreased as the concentration of gelatine increased. Film with $P_{0.5,2}$ has revealed highest tensile strength where as the lowest was obtained by film made-up from $P_{0.5,1}$. Increasing gelatine concentrations significantly increases the tensile strength but glycerol has an opposite effect on the tensile strength. Film $P_{1,2}$ showed the maximum elongation (180%) and the minimum elongation (89%) was obtained for $P_{0.5,1}$.

Conclusion: The outcomes indicated that *Aloe debrana* extract and papaya leaves extract with gelatin and glycerol could be good raw materials for the development of effective antimicrobial packaging films. The developed packaging films exhibited their potential for antimicrobial activity which can be used as one of the most promising packaging systems.

Keywords: Aloe extract; antimicrobial activity; mechanical properties; papaya extract; packaging films; pathogenic microorganisms; physicochemical properties.

1. INTRODUCTION

Food packaging has developed strongly during recent years, mainly due to increased demands on product safety, shelf-life extension, cost-efficiency, environmental friendliness, and consumer convenience. Novel packaging technologies have great commercial potential to ensure the quality and safety of food with fewer or no additives and preservatives, thus reducing food wastage, food poisoning and allergic reactions [1].

The prevention of microbial and chemical deterioration of foods is always the major research subjects in food engineering and science fields. The microbial spoilage of foods occurs mostly on the food surfaces. According to [2], edible films and coatings are especially valuable in controlling the surface microbial contamination because of the capability to serve as additive carriers and then release the active compounds on food surfaces where they are actually needed for impeding microbial growth.

Active packaging systems based on the application of packaging materials with incorporated antimicrobial agents provides one of promising trends in food processing. Antimicrobial food packaging reduces, inhibits or retards the growth of pathogenic and/or spoilage microorganisms that may be present on food surfaces due to releasing of antimicrobial components. These systems can contribute to the shelf-life extension, the quality maintenance and storage stability improvement of packaged food stuffs [3]. Even though most of the packaging films used today to preserve foods are of synthetic origin, it is worth to say that in recent years; bio-based materials have gradually if not extensively, been tested and experimented to develop biodegradable films which had been proven to have more versatile properties [4]. This has undoubtedly set off and given rise to the diverse utilization of packaging films made of bio-based materials. Research and development of antimicrobial materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymer materials and antimicrobials.

To formulate edible films or coatings with functional properties, film forming base materials as well as the bioactive ingredients must be carefully chosen. With several decades of studies, proteins, polysaccharides and lipids are the major types of base materials used in fabricating edible films and coatings [5]. As for the selection of bioactive compounds such as antimicrobial agents, it should be noted that the bioactive compound for edible coating or

films must also be edible materials. It is preferable to select materials with antimicrobial activity, because synthetic food preservatives always raise safety concerns by consumers. It is highly interested in the use of bio-preservatives in the preparation of antimicrobial packaging films [6].

Therefore, a big effort to extend the shelf life and enhance food quality for international as well as local market, while reducing packaging waste has encouraged the exploration of new bio-based packaging materials, such as aloe based packaging films, which is the aim of this study. Moreover, an increase in the cost of petroleum, awareness of environmental issues, and international regulatory pressure banning biodegradable plastics in packaging applications has promoted the development of non petroleum based materials and biodegradable packaging. The purpose of this work was to develop antimicrobial aloe based packaging films at various concentrations of gelatin and glycerol and evaluate the antimicrobial activity, physicochemical and mechanical properties of the developed packaging films.

2. MATERIAL AND METHODS

Aloe debrana is an endemic aloe species to Ethiopia. The fresh leaves of *Aloe debrana* were collected from Chancho, 40 km to North of Addis Ababa, Ethiopia. Fresh papaya leaves (*Carica papaya*) were obtained from Melkassa Agricultural Research Center. All the leaves samples were packed and transported to Addis Ababa University, Food Process Engineering laboratory. Gelatin powder (Bluluxr, blulux laboratories Pv Ltd, Product code no: 121001) and glycerol (product code: 38454 L05, Batch noE10A/0110/1801/08) were purchased from Neway PLC, Addis Ababa. Test cultures for the antimicrobial activity assay were selected from both bacterial and fungal strains, which are common food spoilage and pathogenic microorganisms. The bacterial strains used in this investigation were *Escherichia coli* (American type culture collection (ATCC) 25922), *Staphylococcus aureous* (ATCC25923) and *Salmonella typhi* (ATCC6539). These test organisms were obtained from microbiology department of Ethiopian Health and Nutrition Research Institute (EHNRI).The fungal pure cultures (yeast and mold) taken for the antimicrobial assay were *Candida albicans* (ATCC62376) and *Fusarium xylarioides*. These test cultures were obtained from Biology Department of Addis Ababa University, Science Faculty.

2.1 Aloe and Papaya Leaves Extraction Methods

2.1.1 Aloe gel extraction

Aloe gel extraction was performed by using the method described by [7]. Freshly harvested leaves of *Aloe debrana* were washed with distilled water. The lower 2.54 cm of the leaf base (the white part attached to the large rosette stem of the plant), the tapering point (5.08 -10.16 cm) of the leaves top and the short, sharp spines located along the leaves margins were removed by a sharp knife. Then the knife is introduced into the mucilage layer below the green rind avoiding the vascular bundles and the top rind was removed to get colorless hydroparenchyma. The colorless hydroparenchyma obtained was homogenized using food blender (Philips HR2021, China) and the solution was filtered using cheesecloth. According to the method described by Sai et al., (2011), the clear aloe extract was pasteurized at 70^0C for 45 minutes in order to avoid enzymatic degradation. Finally it was stored at 4^0C until used for further analyses.

2.1.2 Papaya leaves extraction

Papaya leaves were extracted according to the method described by [8]. Fresh leaves of *Carica papaya* were cleaned with distilled water, alcohol, and then again with distilled water in order to keep away from dirt materials adhered to the leaves. Subsequently, the leaves were crushed and extracted directly by screw press without adding any solvent. Leaves extract was collected in a clean bottle, filtered and stored under refrigeration for further analysis.

2.2 Characterization of the Extracts

2.2.1 Antimicrobial activity of the extracts

The antimicrobial activity of *Aloe debrana* extract (ADE) and papaya leaves extract (PLE) against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans* and *Fusarium xylarioides* were evaluated. The antimicrobial activity test for *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albicans* was employed by disc diffusion method as described by [9]. Mueller Hinton agar plates were prepared by pouring 65 ml of molten media into 15 cm diameter sterile Petri plates. The plates were allowed to solidify for 5 minutes and inoculums suspension was swabbed uniformly using sterile cotton swab. The inoculums were allowed to dry for 5 minutes and the different extracts were loaded on 5 mm diameter sterile discs (30µl/disk). The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^0C for 24 hours. Disks that were loaded with distilled water were used as a control. The same procedure was followed for the *Candida albicans*, except that potato dextrose agar plates were used and incubated at 27^0C for 72 hours. The diameters of zone of inhibitions in mm were recorded using transparent ruler after incubation. The experiments were performed in triplicates and average diameters of zone of inhibitions were recorded.

The antifungal activity of the extracts on *Fusarium xylarioides* were determined by poisoned food technique according [10]. Five-day old fungal cultures were punched aseptically with a sterile cork borer of 5 mm diameter. The fungal discs were then put on the gelled potato dextrose agar plate. The agar plates were prepared by impregnating 3 ml plant extract into 60 ml potato dextrose agar at a temperature of 45 - 50^0C (poisoned plate) and 3ml distilled water into 60ml agar (non poisoned plate) as a control. The plates were then incubated at temperature of 27^0C for fungi for three days. The colony diameter was recorded by measuring the two opposite circumference of the colony growth. Percentage inhibition of mycelia growth was evaluated by comparing the colony diameter of poisoned plate with plant extract and non-poisoned plate with distilled water and calculated using the formula described by [10].

$$\%\ \text{Mycelial inhibition} = \frac{MGc - MGt}{MGc} x100 \qquad \text{Eq.(1)}$$

Where: MG_c Mycelia growth for control
MG_t Mycelia growth for treated

2.2.2 The effect of incorporation of papaya leaves extract into *Aloe debrana* extract on antimicrobial activity

The effect of incorporation of PLE on the antimicrobial activity of ADE was evaluated by varying the amount of PLE to be added into ADE from (0-50%) with 10% interval. The antimicrobial activity was performed using the methods explained above in order to achieve the appropriate film forming ratio by considering the antimicrobial activity.

2.2.3 Colour and transparency of the extracts

The colour of the extracts was analyzed by visual inspection. Spectrophotometer (6405 UV/Vis spectrophotometer, UK) was used to measure the transparency of extracts by light-transmittance or absorbance using the Beer-Lambert's law relating the amount of light absorbed or transmitted by a material to the nature of the light absorbing material. The transparency, as of percentage transmittance at 600nm was calculated from the absorbance measured by the spectrophotometer using the following equation:

$$A = 2 - \log_{10} \%T \qquad Eq.(2)$$

Where: A: Absorbance
T: Percentage transmittance

2.2.4 Chemical composition of the extracts

The contents of moisture, ash, crude fat, and crude protein of the extracts were determined according to Association of Official Analytical Chemists, AOAC [11] official methods 925.10, 923.03, 933.05, 920.87; respectively. The content of total carbohydrates was obtained by difference.

2.2.5 Film-forming ability of *Aloe debrana* Extract

The film-forming ability of *Aloe debrana* extract was evaluated according to the method described by [12]. The extracts was heated at 50^0C for 30 minutes and poured onto rectangular plastic material. The extract was then allowed to dry at room temperature and peeled off for observation.

2.3 Packaging Film Development

Aloe debrana extract, papaya leaves extract, gelatine and glycerol were used for the development of the packaging films. The ratio of *Aloe debrana* extract to papaya leaves extract was set at 7:3. This amount was adjusted based on the effect of incorporation of papaya leaves extract into *Aloe debrana* extract on antimicrobial activity results (subsection 3.1.4). Consequently, 70% *Aloe debrana* extract and 30% of papaya leaves extract (v/v) standard solution was prepared. For stabilization of the aloe gel, (2.0 g/l) ascorbic acid and (4.5 g/l) citric acid were added. Films were developed by varying gelatine and glycerol concentrations on 100 ml of the prepared standard solution based on the experimental design specified in Table (1).

Table 1. Experimental design for preparation of packaging films using various concentrations of gelatine and glycerol on 100 ml of standard solution

Gelatine (g)	Glycerol (g)		
	0	**0.5**	**1**
1	$P_{0,1}$	$P_{0.5,1}$	$P_{1,1}$
2	$P_{0,2}$	$P_{0.5,2}$	$P_{1,2}$

Where: $P_{0,1}$ = no glycerol+1g gelatin; $P_{0,2}$ = no glycerol +2g gelatin; $P_{0.5,1}$= 0.5g glycerol +1g gelatin; $P_{0.5,2}$= 0.5g glycerol+2g gelatin; $P_{1,1}$= 1g glycerol+1g gelatin and $P_{1,2}$= 1g glycerol+2g gelatin

Preliminary test was conducted to identify the amount of glycerol to be added into the standard solution. According to the observations from the preliminary test, the ranges glycerol concentration was adjusted between zero and one gram per hundred grams of the standard solution. The gelatine proportion to be added in to the prepared standard solution was taken from the study of [6]. The films were prepared by the procedures adapted from [12]. Initially gelatine was hydrated with the standard solution (70% *Aloe debrana* extract with 30% papaya leaf extract) at room temperature, and solubilised later in a water bath at 50^0C. After complete solubilisation glycerol was added and the solution was kept in water bath under slight agitation for 30 min. Finally, the film-forming solution was conveniently applied on rectangular (15 cm x 20 cm) plastic tray and dried by using tray dryer (TAURO B105EC, N11/2006). Films were peeled off when dried and stored in desiccator for further analysis.

2.4 Evaluation of Aloe Based Packaging Films

2.4.1 Moisture content and thickness of packaging films

The moisture contents of composite films were determined by Moisture Analyzer (MB45, Switzerland). A digital micrometer was used to measure the films thickness.

2.4.2 Transparency of aloe based packaging films

The transparencies of the films were determined using spectrophotometer (6405 UV/Vis spectrophotometer, UK). The film samples were cut into rectangles and placed in the internal side of the spectrophotometer cell. Transparency as percentage of transmittance of films was determined at 600nm as described by [13].

2.4.3 Solubility of aloe based packaging films

The films solubility's (FS) were determined according to the techniques described by [14] Films strips with dimension of (2cmx2cm) were immersed in distilled water (50 ml) for 24 hours with slow mechanical stirring using shaker (ExcellaE24, incubator shaker) at room temperature. Samples were then removed from the solution by filtration and dried in electrical oven (105^0C for 24 hours). The film solubility was calculated from weight difference by the following equation.

$$(\%)FS = \frac{W_1 - W_2}{W_1} \times 100 \qquad \text{Eq.(3)}$$

Where: W_1 is the initial weight of the film and
W_2 is final weight of the film after immersion

2.4.4 Swelling properties of aloe based packaging films

Water absorption capacities of films were determined according to [15] by soaking them in phosphate buffered saline (PBS) at room temperature. Films strips with dimension of (2cmx2cm) were weighed and placed in the PBS media for 30 minutes. The wet weights of the films were determined by first blotting the surface of the composite films with filter paper to remove excess water, and the films weighed immediately. The percentage of water absorption (Wsw) in the medium was calculated from the equation:

$$(\%)\text{Wsw} = \frac{W_f - W_o}{W_o} \times 100 \qquad \text{Eq. (4)}$$

Where: W_f represents the final weight of the film after 30 minutes of absorption and W_0 is the initial weight of the film.

2.4.5 Antimicrobial activity of aloe based packaging films

Testing of the antimicrobial activity of the films was carried out using the agar diffusion method according to [6]. The edible films were cut into squares (l cm x l cm) and were placed on Mueller Hinton agar plates and potato dextrose agar plates. These plates had been previously seeded with 0.1 ml of inocula containing approximately 1.5 x 108 CFU/ml and 104 - 105 CFU ml-1 of test bacteria and fungus; respectively. The plates were then incubated at 37^0C for 48 hours and at 27^oC for 72 hours for bacterial and fungal cultures; respectively. The plates were visually examined for zones of inhibition around the film discs, and the size of the zone area was measured and the average was taken as the inhibition zone. Similar dimension of thick synthetic plastic was used as a control. Observations were made of the area of the inhibitory zone surrounding film discs including contact area of film with agar surface.

2.4.6 Mechanical properties of aloe based packaging films

Tensile measurements for tensile strength (TS) and elongation at break (EB) of films were determined by using the method described by [6] with a tensile testing machine (Computer–electro-hydraulic universal testing machine, model WAW600, China). The initial grip separation was 50 mm and the crosshead speed was set at 30 mm $minute^{-1}$. For each test run, the dimensions (thickness and width) of the film strip were input to the coupled personal computer; and TS and EB were automatically calculated by the computer software installed in the computer by the manufacture of tensile testing machine.

2.5 Experimental Design and Statistical Analysis

The experiments were performed with three experimental stages. In stage 1, the antimicrobial activity, colour and transparency of *Aloe debrana* and papaya leaves extracts were studied. In stage 2, experiments were conducted to evaluate the effect of incorporation of papaya leaves extract into *Aloe debrana* extract on antimicrobial activity, colour and transparency. In this section, appropriate proportions of *Aloe debrana* to papaya leaves extract were adjusted. In the final experiment the effect of various concentrations of gelatine and glycerol on effective packaging film development were evaluated. Data obtained from experiments were analyzed by one way ANOVA (Analysis of Variance) using JMP statistical analysis software version 5.0. Significance was accepted at 0.05 level of probability

(P<0.05). Mean separation was performed by "Each Pair Student's t-test" for multiple comparisons of means.

3. RESULTS AND DISCUSSION

3.1 Characteristics of the Extracts

3.1.1 Antimicrobial activity of the extracts

The combined use of aloe and papaya extracts in the film-forming solution was an attempt to give a wide antimicrobial spectrum that could inhibit the growth of several food spoilage and poisonous microorganisms. A packaging material with a wide antimicrobial spectrum would be necessary and desirable for universal use to improve the storage stability of a variety of foods.

Most foods serve as good growth medium for many different microorganisms. Considering the variety of foods and the methods used for processing, it is apparent that practically all kinds of microorganisms are potential contaminants. Therefore, the principal food spoilage microorganisms are mixed cultures; including Gram (+) bacteria, Gram (-) bacteria, molds and yeasts. Taking this in to consideration it was tried to take the test cultures from all group of microorganisms; bacteria, yeast and fungus. The ability to inhibit all types of organisms is favorable to be an effective antimicrobial food packaging [16].

This study has showed that aloe and papaya extracts have antimicrobial activity against bacterial and fungal cultures (Table 2). This implies that the extracts contain active compounds that have antimicrobial properties. The results of the antimicrobial activity of the extracts against *Escherichia coli, Salmonella typhi*, *Staphylococcus aureus, Candida albicans,* and *Fsarium xylarioides* are presented in Table (2).

Table 2. Antimicrobial activity of the extracts against *E. coli, S. typhi, S. aureus, C. albicans* and *F. xylarioides*

Samples	Inhibition zone (mm)				Mycellia Inhibition (%)
	E. coli	*S. typhi*	*S. aureus*	*C. albicans*	*F. xylarioides*
Aloe debrana extract	11.96 ± 0.50^{a}	9.66±0.28^{b}	10.36±0.32^{ab}	9.16±0.35^{a}	36.66±1.52^{b}
Papaya leaves extract	10.66± 0.28^{b}	12.16±0.36^{a}	11.26±0.46^{a}	7.90±0.36^{b}	56.00±2.04^{a}
Control	ND	ND	ND	ND	ND

All values are means of triplicates ± standard deviation

$^{a-b}$ *Means with different superscript letters within a column are significantly different (p < 0.05).*

ND = Not detected

From the result in Table 2, *Aloe debrana* extract and papaya leaves extract have shown different antimicrobial activity. The highest inhibition zone (12.16mm) was recorded by papaya leaves extract on *S. typhi.* Generally *E. coli* and *C. albicans* were more sensitive to aloe extract where as *S. typhi*, *S. aureus* and *F. xylarioides* were more sensitive to papaya leaves extract (Table 2). Inhibitory effects obtained in this research study are higher than those reported by Saks et al. [17] who found antifungal activity of the pulp against:

Penicillium digitatum, Penicillium expansum, Botrytis cinerea, and Alternaria alternate. The results also indicated the need of addition of papaya leaves extract on aloe gel extract to make a film-forming solution posses wide antimicrobial spectrum.

3.1.2 Film-forming ability of *Aloe debrana* extract

The result of the study showed that it was not successful to produce films from the aloe extract alone. The films were highly brittle with poor physical appearance. [6] also reported that films made of aloe gel powder were brittle and not easy to handle. The moisture content of the aloe leaves gel is usually greater than 98% and the mixed polysaccharides including glucomannan, galactan, arabinan and pectic substances make up the most parts of the gel solid matter [18]. The relatively low solid content and brittle characteristic of mixed polysaccharide film make the aloe leaves gel itself technically infeasible for using as the supporting base material for edible films.

Combination of different biomaterials to form composite or blend is a useful solution to enhance the mechanical and/or functional properties of bioactive packaging materials [18]. To overcome the lack of film-forming capability of the aloe leaves gel, it is advisable to incorporate other suitable film-forming materials with the aloe gel to form edible films. Moreover, composite edible coatings or films can combine the advantages of each component. Gelatine has been successfully used to form films that are transparent, flexible, water-resistant, and impermeable to oxygen. Similarly gelatine was used as a film-forming material in this study. Protein and polysaccharide are both hydrophilic biopolymers and have been combined to form composite edible films [18,19]. Thus, it is reasonable for gelatine and the aloe leaves gel to be formulated together to form composite films or coatings.

3.1.3 Colour and transparency of aloe and papaya leaves extract

The color of papaya leaves extract was red brownish, while aloe extracts was translucent white. The transparencies of these extracts are given in Table 3.

Table 3. Transparencies of *Aloe debrana* gel extract and papaya leaves extract

Extracts	Transparency (% transmittance)
Aloe debrana extract	63.83 ± 0.16^{a}
Papaya leaves extract	0.86 ± 0.05^{b}

All values are means of triplicates ± standard deviation

[a-b] Means with different superscript letters within a column are significantly different (p <0.05)

The transmittance of *Aloe debrana* (64%) was very much greater than the transmittance of papaya leaves extract (0.8). *Aloe debrana* gel is better to produce more transparent packaging films. Papaya leaves extract is almost opaque with transparency value (0.8%); it absorbs 99.2 % of the light directed to it. Different pigments like phenols are responsible for larger absorbance.

Colours of the film-forming solution were affected by the incorporation of papaya leaves extract into the *Aloe debrana* extract. The effect of papaya leaves extract on the colour of the film-forming solution is illustrated in Fig. 1.

Fig. 1. Various concentration of extracts

Where: d=Aloe debrana extract, p= papaya leaves extract
dp1, dp2, dp3 and dp4 are 10%, 20%, 30% and 40% papaya leaves extract in Aloe debrana extract; respectively.

Addition of papaya leaves extract affected the appearance of the film-forming solution in colour. The film-forming solution without papaya leaves extract appeared clear and translucent. The film-forming solution with increasing level of papaya leaves extract was became light brown.

3.1.4 The Effect of incorporation of papaya leaves extract into *Aloe debrana* extract on the antimicrobial activity

It was quite easy to produce the packaging film from aloe gel without incorporation of papaya leaves extract. But the film will not have wide antimicrobial spectrum. The amount of the papaya extract has significance influence on physicochemical and mechanical properties of the packaging film. The packaging film with low papaya extract has attractive features. Therefore it was found important to study the amount of papaya leaves extract to be added in to the *Aloe debrana* extract for significance antimicrobial activity enhancement, with little influence on other properties. The effect of papaya leaves extract on the antimicrobial activity of the *Aloe debrana* extract is given in Table 4.

Table 4. The effect of incorporation of papaya leaves extract into *Aloe debrana* extract on antimicrobial activity

	Inhibition zone (mm)				Mycelia Inhibition (%)
PLE (%)	*E. coli*	*S. typhi*	*S. aureus*	*C. albicans*	*F.xylarioides*
0	11.96±0.50^{a}	9.66±0.28^{d}	9.70±0.26^{c}	9.30±0.43^{b}	36.66±1.52^{d}
10	12.06±0.30^{a}	11.40±0.40^{c}	11.13±0.30^{b}	9.40±0.20^{b}	40.66±1.43^{c}
20	12.33±0.29^{a}	12.73±0.11^{b}	12.56±0.26^{a}	9.83±0.05^{a}	51.33±3.05^{b}
30	12.43±0.15^{a}	12.96±0.20^{b}	12.50±0.26^{a}	9.86±0.11^{a}	54.00±2.00^{ab}
40	12.43±0.25^{a}	13.43±0.21^{a}	12.63±0.28^{a}	9.90±0.10^{a}	55.33±1.52^{a}
50	12.52±0.30^{a}	13.60±0.20^{a}	12.60±0.20^{a}	9.93±0.23^{a}	56.00±1.80^{a}

All values are means of triplicates ± standard deviation
$^{a-d}$ Means with different superscript letters within a column are significantly different ($p < 0.05$)

In this study, antimicrobial activity of *Aloe debrana* gel extract was significantly increased with increasing level of incorporation of papaya leaves extract. But the influence on *E. coli* was not significance. Generally increasing the papaya leaves extract proportion beyond 30% did not brought significant effect on the antimicrobial activity of the solution (Table 4).

Therefore, it is appropriate to use about 30% papaya leaves extract in the film-forming solution so that the mechanical and physicochemical properties of the packaging film will not be much affected.

3.1.5 Chemical composition of *Aloe debrana* extract and papaya leaves extract

All of the properties of the packaging film are the result of the constituents present in the film. The compositions of *Aloe debrana* extract and papaya leaves extract were evaluated. The results are given in Table 5.

Table 5. Chemical composition of *Aloe debrana* extract and papaya leaves extract in dry weight basis

Sample	Moisture (%)	Total Carbohydrates (%)	Protein (%)	Fat (%)	Ash(%)
ADE*	97.27±0.08	1.45±0.02	0.04±0.01	0.62±0.01	0.63±0.03
PLJ**	98.64±0.06	0.47±0.04	0.23±0.03	0.39±0.02	0.27±0.01

All values are means of duplicates ± standard deviation

Where: ADE = Aloe debrana extract, PLJ** = Mechanically obtained papaya leaves extract*

The moisture content of the ADE was 97.27 ± 0.08 %. The result was similar with other report in the literature [18]. The contents of crude protein, ash, fat and carbohydrate were 0.04±0.02%, 0.63±0.03%, 0.62±0.01% and 1.45±0.02%; respectively. With the exception of crude fat and protein content, the results were similar to previously published results by Femenia et al [18]. The difference in cultivar or climate could be the reasons for the inconsistence. Although the aloe leaves gel has been used as edible coating after blending with water [5], the results of this study reveal that the moisture of aloe leaves gel is 97.27± 0.08% and it is difficult to form edible film by itself. However, it is feasible to use aloe in combination with other film-forming materials to formulate composite films.

The moisture content of papaya leaves extract was 98.64 ± 0.06% which is greater than the moisture content of *Aloe debrana* extract. The incorporation of papaya leaves extract in aloe gel could further increase the moisture content of the film-forming solution. This also indicates that there should be film-forming material which can construct the body of the film. Gelatine was used as a film-forming material to compensate the weakness. The amount of gelatine in the film-forming solution was adjusted based on the dry mass of the film-forming solution. The contents of crude protein, total carbohydrates, ash, crude fat were 0.23 ± 0.03%, 0.47 ± 0.04%, 0.27 ± 0.01%, and 0.39 ± 0.02%; respectively.

3.2 Evaluation of Aloe Based Packaging Films

3.2.1 Moisture content and thickness

The moisture contents and thickness of the packaging films are shown in Table 6. Among the six different treatments, only three were effective to build a standalone film. The first two treatments $P_{0,1}$ and $P_{0,2}$ resulted in brittle films which are difficult to handle. On the other hand, the fifth treatment ($P_{1,1}$) was unable to give stand alone film. Due to the fact, it was very difficult to separate the film ($P_{1,1}$) from the support.

Table 6. Moisture content in dry basis and thickness of the packaging films

Treatments	Moisture (%)	Thickness (mm)
$P_{0,1}$	8.20 ± 0.33^{e}	0.23 ± 0.07^{d}
$P_{0,2}$	7.15 ± 0.29^{f}	0.42 ± 0.03^{c}
$P_{0.5,1}$	10.19 ± 0.39^{c}	0.34 ± 0.12^{cd}
$P_{0.5,2}$	9.26 ± 0.21^{d}	0.54 ± 0.04^{b}
$P_{1,1}$	12.83 ± 0.36^{a}	0.40 ± 0.08^{c}
$P_{1,2}$	11.25 ± 0.58^{b}	0.68 ± 0.13^{a}

All values for moisture content are means of triplicates ± standard deviation; All values for thickness are means of five measurements ± standard deviation.

[a-f] Means with different superscript letters within a column are significantly different (P<0.05).

Where: $P_{0,1}$= no glycerol+1g gelatine, $P_{0,2}$ = no glycerol +2g gelatine, $P_{0.5,1}$= 0.5g glycerol +1g gelatine, $P_{0.5,2}$= 0.5g glycerol+2g gelatine, $P_{1,1}$= 1g glycerol+1g gelatine, $P_{1,2}$= 1g glycerol+2g gelatin

Films with composition 0.5g glycerol and 1g gelatine in the film forming solution ($P_{0.5,1}$), 0.5g glycerol and 2g gelatine in the film forming solution ($P_{0.5,2}$), and 1g glycerol and 2g gelatine in the film-forming solution ($P_{1,2}$) were the only treatments that produce stand alone plastic films to which most of the analyses performed. The moisture content of the packaging films significantly increased (P<0.05) as the percentage of glycerol in the film-forming solution increased and decreased (P<0.05) as the percentage of gelatine increased. The lowest moisture content of 7.15 % was obtained in $P_{0,2}$, while the highest moisture content of 12.83% was detected in $P_{1,1}$ (Table 6) . The moisture content of $P_{0,1}$ (8.20%) is greater than the moisture content of $P_{0,2}$ (7.15%). It is because that the major component of aloe gel powder is mixed polysaccharides including glucomannan, galactan, arabinan and pectic substances [18], which exert higher affinity to water molecules for more hydroxyl groups existing in the polysaccharide structure than in gelatine structure.

[19] reported that higher content of hydrophilic hydroxyl groups of polysaccharide increased the water absorbability of gelatine/polysaccharide blend films. The moisture content of edible films depends on the hydrophilic group density of the film. That is why $P_{1,1}$ with the highest proportion of glycerol has the highest moisture content(12.83%) since glycerol is more hydrophilic than gelatine and aloe gel. Compared to previous study conducted by [6], the films have higher moisture content. The difference in moisture content could be related to the difference in composition of the films. In this study papaya leaves extract and glycerol were added, in addition to gelatine and aloe gel. The increase in moisture content could also be related to the hydrophillicity of glycerol and papaya leaves extract. $P_{1,2}$ has the highest thickness (0.68mm), and $P_{0,1}$ has the lowest thickness(0.23mm). Addition of both gelatine and glycerol has significantly ($p < 0.05$) influenced the thickness of the films. It is obvious that the film dry matter increases by the addition of these solutes which result in larger thickness.

3.2.2 Colour and transparency

Visually, all the films developed from papaya leaves extract and *Aloe debrana* extract were light brown as shown in Fig. 2. Fig. 3 demonstrates the colour of the packaging films developed by excluding papaya leaves extract, to see the influence of papaya leaves extract on the colour of the composite films.

Fig. 2. Packaging films developed from *Aloe debrana* extract, gelatine and glycerol in the presence of papaya leaves extract

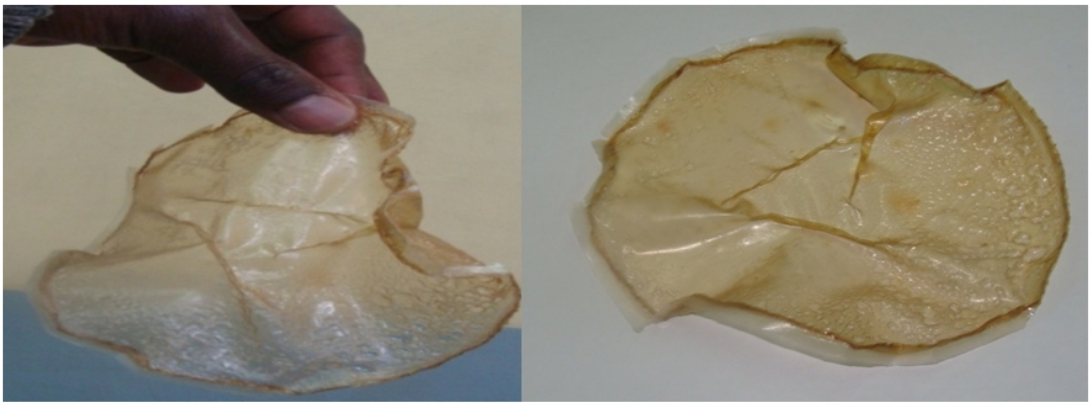

Fig. 3. Packaging films developed from *Aloe debrana* extract, gelatine and glycerol without papaya leaves extract

Addition of papaya leaves extract affected the colour of the packaging films. This was due to the red brownish colour of papaya leaves extract. The colour of the composite film developed by excluding papaya leaves extract was white and translucent as illustrated in Fig. 3.

Transparency is also one of the common optical properties of light permeable materials. It was difficult to analyze the transparencies of films with Treatment codes ($P_{0,1}$, $P_{0,2}$ and $P_{1,1}$) because of their fragile nature. The transparencies of the three standalone films are given in Table 7.

Table 7. Transparencies of the packaging films

Treatments	Transparency (% transmittance)
$P_{0.5,1}$	32.67 ± 0.57^{b}
$P_{0.5,2}$	28.00 ± 0.40^{c}
$P_{1,2}$	36.67 ± 0.62^{a}

All values are means of triplicates ± standard deviation

[a-c] Means with different superscript letters within a column are significantly different ($p < 0.05$)

Where: $P_{0.5,1}$= 0.5g glycerol +1g gelatine, $P_{0.5,2}$= 0.5g glycerol+2g gelatine, $P_{1,2}$= 1g glycerol+2g gelatin

Transparency was significantly affected by the concentration of glycerol and gelatine. %T of film slightly increased with increasing glycerol content $P_{1,2}$ was more transparent than the other films. Generally these packaging films may not be used as see-through packaging or coating materials, since their transparency is affected by the papaya leaves extract.

3.2.3 Solubility of aloe based packaging films

Solubility in water is an important property of edible films, since potential applications may require water insolubility to enhance product integrity and water resistance. However, in some cases water solubility of the film before consumption of the product might be beneficial [20]. The film solubility of tested samples is presented in Table 8.

Table 8. Solubility of the packaging films

Treatments	Film solubility (%)
$P_{0,1}$	50.82 ± 0.32^{e}
$P_{0,2}$	44.57 ± 0.25^{f}
$P_{0.5,1}$	70.37 ± 0.55^{c}
$P_{0.5,2}$	61.18 ± 0.40^{d}
$P_{1,1}$	90.49 ± 0.65^{a}
$P_{1,2}$	85.70 ± 0.62^{b}

All values are means of triplicates ±standard deviation

[a-f] Means in the same column with different superscript letters are significantly different ($P < 0.05$)

Where: $P_{0,1}$= no glycerol+1g gelatine, $P_{0,2}$ = no glycerol +2g gelatine, $P_{0.5,1}$= 0.5g glycerol +1g gelatine, $P_{0.5,2}$= 0.5g glycerol+2g gelatine, $P_{1,1}$= 1g glycerol+1g gelatine, $P_{1,2}$= 1g glycerol+2g gelatin

Generally, the results indicate that films were highly soluble. The highest solubility was 90.49% of film composed of 1g glycerol and 1g gelatine ($P_{1,1}$) while the lowest solubility was 44.57% of film with composition of 0g glycerol and 2g gelatine ($P_{0,2}$). Films with even more solubility have been reported earlier. Correspondingly, [21] reported 97.98% film solubility for whey protein isolate films. Film solubility increased significantly (P<0.05) as the content of glycerol increased and decreased significantly (P<0.05) as the content of gelatine increased. [22] reported that, in general, hydrophilic plasticizers, such as glycerol, enhance water solubility. It is probably because increasing the plasticizer content in the film increased the water-soluble dry content. The relationship between water-soluble dry matter and hydrophilic plasticizer content is linear [23].

The decrease in film solubility as gelatine content increased is due to the low hydrophilic nature of gelatine compared to aloe gel and glycerol. Addition of more gelatine increases the insoluble portion in the film. Gelatine itself is soluble but the degree of solubility compared to

aloe gel and glycerol is low. Furthermore, the increase of film solubility might be related to the hydrophillicity of papaya leaves extract. [24] reported that an increase in phayom wood extract in edible films led to an increase in film solubility. It could be hastily concluded that papaya leaves extract enhance film solubility in water. This water solubility behavior could not be generalized, and understanding the film solubility remains a complex subject.

3.2.4 Swelling property of aloe based packaging films

Aloe polysaccharides, gelatine and glycerol, all being hydrophilic polymers, show high affinity towards water, hence, upon hydration, aloe based packaging films absorb water and swell and/or solublise. All films showed swelling behavior, but it was not possible to take swelling data since the films did not retain their integrity and showed high degree of dissolving in water. The reason behind the soluble nature of aloe based films was the high hydrophilic nature of the components in the film. Such types of films are good candidates to develop edible films and packaging materials which require complete solubility upon hydration. To develop water resistant films incorporation of hydrophobic components could be mandatory.

3.2.5 Antimicrobial activity of aloe based packaging films

Antimicrobial activity of films was evaluated using the agar diffusion method. The inhibitory activity was measured based on the clear zone surrounding the square film strips. Measurement of clear zone area has included area of film strips. Antimicrobial activity of the composite films on *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Candida albicans* and *Fusarium xylarioides* are given in Table 9.

Table 9. Antimicrobial activity of the packaging films on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida albicans* and *Fusarium xylarioides*

Treatments	Average area of inhibitory zone (cm^2)				
	Salmonella typhi	***Escherichia coli***	***Staphylococcus aureus***	***Candida albicans***	***Fusarium xylarioides***
$P_{0,1}$	6.01 ± 0.22^{b}	4.34 ± 0.12^{a}	5.15 ± 0.71^{a}	4.40 ± 0.20^{ab}	6.20 ± 0.12^{a}
$P_{0,2}$	5.83 ± 0.14^{b}	4.27 ± 0.13^{a}	5.61 ± 0.37^{a}	4.20 ± 0.20^{b}	6.01 ± 0.43^{a}
$P_{0.5,1}$	6.24 ± 0.24^{ab}	4.48 ± 0.33^{a}	5.77 ± 0.81^{a}	4.51 ± 0.08^{ab}	6.38 ± 0.13^{a}
$P_{0.5,2}$	6.09 ± 0.37^{ab}	4.62 ± 0.22^{a}	5.22 ± 0.56^{a}	4.48 ± 0.32^{ab}	6.35 ± 0.66^{a}
$P_{1,1}$	6.52 ± 0.28^{a}	4.63 ± 0.20^{a}	5.53 ± 0.61^{a}	4.61 ± 0.22^{a}	6.39 ± 0.11^{a}
$P_{1,2}$	6.23 ± 0.24^{ab}	4.40 ± 0.20^{a}	5.95 ± 0.27^{a}	4.47 ± 0.24^{ab}	6.25 ± 0.24^{a}
Control	ND	ND	ND	ND	ND

All values are means of triplicates ±standard deviation

$^{a-b}$ Means in the same column with different superscript letters are significantly different ($P < 0.05$).

ND = Not detected

Where: $P_{0,1}$= no glycerol+1g gelatine, $P_{0,2}$ = no glycerol +2g gelatine, $P_{0.5,1}$= 0.5g glycerol +1g gelatine, $P_{0.5,2}$= 0.5g glycerol+2g gelatine, $P_{1,1}$= 1g glycerol+1g gelatin and $P_{1,2}$= 1g glycerol+2g gelatin

All the packaging films inhibited the growth of all the test microorganisms used. The greatest zone of inhibition (6.52 cm^2) was observed at $P_{1,1}$ against *S. typhi*, and the least zone of inhibition (4.20 cm^2) was observed at $P_{0,2}$ against *C.albicans*. All the packaging films have almost similar antimicrobial activity since they contain the same amount of *Aloe debrana* extract (ADE) and papaya leaves extract (PLE). Significance differences between the

antimicrobial activity of the packaging films were observed specially on *S. typhi* and *C.albicans.* This difference may be resulted from the interference of gelatine and glycerol on the antimicrobial activity of ADE and PLE against *S. typhi* and *C.albicans.* The other reason could be the difference in solubility of the packaging films. More soluble films could release the antimicrobial component quickly and large diameter around the films. The antimicrobial component releasing rate of each film requires careful investigation.

In this study different test organisms have showed different sensitivity to similar antimicrobial films. Film with treatment $P_{0,1}$ has 6.01±0.22mm inhibition zone on *S. typhi* but it has 4.34±0.12mm inhibition zone on *E. coli* (Table 9). [24] reported that some organisms including *E. coli* and *P. fluorescens* are capable of growing on tannins as a source of carbon. The difference in sensitivity may also be associated with difference in cell wall structure and function.

The packaging films developed in this study have showed better antimicrobial activity compared to the packaging films developed by [6] from *Aloe barbade*nsis and gelatine. The packaging films developed by this study inhibited *E. coli* and *S. aureus* 4.63 cm^2 and 5.53 cm^2 whereas the packaging films developed by [6] had showed 3.93cm^2 and4.32cm^2 ; respectively. This difference may be resulted from one or all of the following reasons. The first one is that the presence of papaya leaves extract in the films developed in this study has increased their antimicrobial activity. The second reason may be the difference between the aloe species, since [6] developed films from *Aloe barbade*nsis; and the films in this study were developed from *Aloe debrana* (endemic aloe species to Ethiopia). In addition to this, seasonal and geographical variation might also bring significance differences in antimicrobial activity of the aloes' used. Moreover, the film forming conditions may also result in different antimicrobial activity. This study indicates that the possibility to develop antimicrobial edible films which has active ingredients against a broad spectrum of microorganisms.

3.2.6 Mechanical properties of aloe based packaging films

The mechanical properties including tensile strength and elongation at break of the packaging films are shown in Table 10. Due to the fragile nature of the films ($P_{0,1}$, $P_{0,2}$, $P_{1,1}$) evaluation of the mechanical properties were not conducted. It was found difficult to grasp the films by tensile testing machine.

Table 10. Mechanical properties of aloe based packaging films

Treatments	Tensile strength (MPa)	Elongation at break (%)
$P_{0.5,1}$	20 ± 0.00^{c}	89 ± 15.55^{b}
$P_{0.5,2}$	65 ± 7.07^{a}	170 ± 14.14^{a}
$P_{1,2}$	45 ± 7.07^{b}	180 ± 0.00^{a}

All values are means of duplicates ±standard deviation

[a-c] Means in the same column with different superscript letters are significantly different (P < 0.05).

Where: $P_{0.5,1}$= 0.5g glycerol +1g gelatine, $P_{0.5,2}$= 0.5g glycerol+2g gelatine, $P_{1,2}$= 1g glycerol+2g gelatin

Film $P_{0.5,2}$ has maximum tensile strength (65 MPa) followed by film $P_{1,2}$ (45 MPa) and film $P_{0.5,1}$ (20 MPa). The results showed that the TS of aloe based packaging films were significantly (P<0.05) affected by the addition of gelatine and glycerol. The proper amount of gelatin and glycerol in the film-forming solution was crucial to develop functional films.

The tensile strength of composite films increased from 20 to 65 MPa, as the percentage of gelatine increased from 1% ($P_{0.5,1}$) to 2 % ($P_{0.5,2}$). However, glycerol produced an opposite effect on the tensile strength. The effect of plasticizer on reduction of tensile strength is well known and its explanation was reported by some researchers [22]. Plasticizers weaken the intermolecular forces between the chains of adjacent macromolecules, increasing the free volume and causing a reduction of mechanical resistant [25].

Elongation at break was significantly (P<0.05) increased by increasing gelatine content in the film forming solution. [6] reported similar results on the effect of gelatine on tensile strength and elongation at break (EB) of composite films. Maximum elongation was 180% at $P_{1,2}$ and the minimum elongation was 89% at $P_{0.5,1.}$ Similar results were reported by Junianto et al [26] from Tilapia's skin gelatine edible films with addition of plasticizer sorbitol at 10% of gelatine and 5% of sorbitol concentration. The increase in EB was noticeable when gelatine content increased ($p < 0.05$). But the effect of glycerol was not as such significant ($p > 0.05$). Such effect of glycerol on elongation at break was reported by Sazedul et al [27], when gelatines with 0.80 and 1.20% degree of hydrolysis were used. Considering the results of mechanical properties, the obtained films have showed interesting results to be used as a food packaging material.

4. CONCLUSION

Development of packaging films from aloe, papaya, and gelatine using glycerol as plasticizer was studied for the first time in the Ethiopian context. This study signified that aloe, gelatine and papaya leaf can be used to formulate edible films. The film-forming ability of the aloe gel was highly improved by the addition of gelatine and glycerol. Adding up of more plasticizer (glycerol) made the films more hydrophilic, sticky and difficult to handle. Hence, films formed without glycerol were highly brittle. The colours of the packaging films developed were light brown; it was highly influenced by the colour of papaya leaf extract. All aloe based packaging films exhibited high solubility in water and, hence, has potential for developing edible packaging material intended for easy solubility.

The antimicrobial system formed by aloe based packaging films, showed better antimicrobial activity on Gram (+) bacteria, Gram (-) bacteria and fungus. The antimicrobial property of the film-forming solution has been improved by the addition of papaya leaf extract. The packaging film developed has wide antimicrobial spectrum. *S. typhi* with inhibition zone 6.52cm^2 was the most inhibited microorganism by the developed packaging films. The developed packaging films have exhibited good mechanical properties. Maximum tensile strength (65MPa) was observed on treatment $P_{0.5,2}$. The packaging films were highly stretchable with maximum elongation at break of 180% of treatment $P_{1,2}$.Tensile strength and elongation at break were significantly increased by the addition of gelatine. On the contrary glycerol has reduction effect on the tensile strength of the composite films.

Based on the research findings, aloe based packaging films can be used as a packaging material for exporting perishable and high value crops such as Ethiopian coffee and spices. The packaging films can contribute in food security via postharvest loss management and promote the exploitation of underutilized resources in African context. Advance and extensive research activities are required on qualitative and quantitative analyses of the antimicrobial substances in aloe and papaya leaf extracts; barrier properties of films, optimization of the film formation process and efficiency of aloe based packaging films on different food stuffs during storage. Investigations on migration kinetics of the antimicrobial components from the film to the food also require great attention.

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CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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