Physical, Thermal And Mechanical Characterization Of Aloe-Almond Hybrid Biopolymer Film.

M.Muthulakshmi, T.S. Subha

ABSTRACT: Natural polymers are biocompatible, non toxic and abundant. The natural polymers such as gums and mucilage are preferred over synthetic materials for their characteristics of sustainability, biodegradability and biosafety. Natural gums and mucilages are the polysaccharides or complex of one or more monosaccharides or their derivatives linked in bewildering linkages and structures. The gums has huge and broad application in both food and non-food industries being frequently used as thickening, binding, emulsifying , stabilizing agents and matrices for drug release in pharmaceutical and cosmetic industries. The present study describes the preparation and characterization of polymeric sheets in which almond gum is blunded with *Aloe* was prepared by casting / solvent evaporation technique. The prepared films were characterized by thickness, folding endurance, antibacterial activity, tensile strength, Differential scanning calorimetry, Fourier transform-infrared spectroscopy, Scanning electron microscope, water absorption test and antimicrobial activity. The Results show that the aloe vera enhances the thermal and mechanical properties of the films. It also revealed that the film had appreciable antibacterial activity. The prepared and characterized films were further used for the wound healing studies.

Keywords: Almond, *Aloe*, wound healing, biocompatible

1 INTRODUCTION

Biopolymers have entice considerable interest for the development of drug delivery systems owing to their biocompatibility, nontoxicity, renewability, and mild processing conditions. These polymers are biologically degradable invivo either by enzymatically or non enzymatically, and can be metabolized and excreted via normal physiological pathways. Application of biopolymers in living systems would diminish the immunological reactions and toxicity that occur when a synthetic polymer is used in a host (Monica et al., 2018) [1]. Now-a-days naturally derived biopolymers are considered as smart materials. They furnish a versatile and tunable platform to design the appropriate extracellular matrix that support tissue generation, with less adverse events. In the past decades, fabrication of bioactive materials based on natural polymers, either derives from protein or polysaccharide based, has been tremendously exploited to rig wound healing related problems(Giulia suarato et al., 2018) [2]. Natural polymers are obtained from plants which have high molecular weight; water soluble polymers are made up of monosaccharide unit bonded by glucosidic linkages(Hematyar et al.,2018; Sadozai et al.,2013) [3],[4]. Gummy exudates of natural polymers such as protein, enzyme, fibre and polysaccharide which have been used to contrived the various pharmaceutical products(Sadozai et al.,2013; Maru et al.,2012) [4],[7].Nowadays the formulation developers shows their prime focus on the natural gums especially polysaccharide gums over synthetic polymers because they are easily available, biodegradable and shows least immunogenic and biocompatibility(Ponchel et al.,1998; Bharadwaj et al.,2000) [5],[6].

- *program in Botany, Bharathi women's college, Tamilnadu, PH-6369946712. E-mail: m.lachuayyappan@gmail.com.*
- *TS.Subha is Assistant Professor in Botany, Bharathi Women's College, Tamilnadu, PH-9840946823.E-mail: subhatss@gmail.com.*

1.1 Almond:

Almond gum is acquired from the tree *Prunes amaygdalus* (Family: Rosaceae) which is water soluble gum extrudes from the wounds on almond trees. The composition of almond gum includes aldobionic acid, L-arabinose, D-mannose, L-galactose etc. In addition to this it have the different components like emulsifier, thickener, suspending pharmaceutical, adhesive, glazing agent and stabilizer (Sujitha et al.,2012; Girish et al.,2009) [8],[9]. Gum is a secondary product acquired as a result of metabolic mechanism of plants. Natural gums form a viscous solution either when it absorbs water or water soluble. Natural gums are easily available, economic and useful as a tablet binder (Uzma Faroog et al.,2014) [10]. The injured bark secrets the gum which looks like white or brown colored little rocks or ice stones when it gets dried up(Ruchi et al.,2018) [11]. It is used for various medicinal purposes like to treat diarrohea, stomach ulcers, as weight gainer, natural coolant and also used in pharmaceutical industries(Sarojini et al.,2010; sarojini et al.,2016; Jameer et al.,2017) [12][13][14].

1.2 Aloe

Aloe vera L. is a native plant of south-west Arabian peninsula (Tanwar.,2005) [15]. The studies report that Aloe vera contains over 75 potentially active constituents, including vitamins, minerals,enzymes,sugars, phenolic compounds ,polysaccharides and organic acids(Tanwar.,2005; Bourtoom.,2008) [15],[16]which constitute 0.5%-1.0% of the plant material, while the remaining percentage is water(Bourtoom.,2008) [16]. It has the capacity to rejuvenate, soothen and healing properties(Cheng Pei et al.,2010; Rhim et al.,2009; Kirsi et al.,2007) [17],[19],[20]. Aloe vera plant has gel and latex. Aloe gel is usually clear gel or mucilagenous substance produced by parenchyma cells situated in the central region of the leaf. The gel is mainly consisted of 99% of water and mono- and polysaccharides (25% dry weight of the gel)[18]. Aloe solutions shows greater antimicrobial effect against the pathogens like *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*(Cheng Pei et al.,2010) [17].

2 MATERIALS AND METHOD

All chemicals of AR grade were procured from SRL CHEMICALS, Chennai. Almond gum is obtained from the Khadi bavan, Kandakottam. The Aloe plant was purchased from green house.

2.1. Preparation of Almond solution and Aloe vera extract

Almond solution at a concentration of 1.5 % (w/v) was prepared by dissolving 7.5 g of Almond gum in 500 mL of distilled water, this solution is heated at 90°C with stirring at 200rpm to dissolve Almond gum completely. The almond gum solution is used for the film preparation. The Aloe vera leaves were collected and washed with distilled water to remove the superficial contaminants. The gel was extracted from leaves and subsequently washed and ground. The resulting suspension was filtered by traditional hand filtering technique to remove the larger fragments. The filtrate was further used for film preparation.

2.2. Film preparation

Aloe blunded Almond polymeric films were prepared by mixing the 1.5 % of slurry Almond solution and *Aloe* solution in different proportion like(1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1). Each proportion of both solutions at or just below 40°C with continuous stirring (200 rpm) until a uniform dispersion was obtained. The films were prepared by solvent casting technique with slight modifications [22]. Briefly, air bubble free solutions were cast into the film on the plastic tray and air dried under normal room temperature [15]). Among the proportions only 40%+60% of Almond + Aloe shows a good film production. The films were dried for 72hrs to 96 hrs, then the films were carefully removed from the plastic trays and in desiccators prior to testing.

TABLE 1

DIFFERENT CONCENTRATION OF FILMS

Code of the film	No of parts	
	Almond(%)	*Aloe vera (%)*
F1	10	90
F2	20	80
F3	30	70
F4	40	60
F5	50	50
F6	60	40
F7	70	30
F8	80	20
F9	90	10

2.3. Characterization of film

2.3.1 Thickness

The film thickness was analysed using a micrometer (Model 102-301 Mitutoyo). The thickness of film was calculated at five different places and the mean was calculated.

2.3.2 Folding endurance

The folding endurance of the film was calculated to determine the flexibility of film. The flexibility prediction of film is necessary for easy handling, comfort, secured application of film on wound. It was tested by repeated folding of film at same place till it breaks or folded up to 300 times manually. The value of folding endurance is the number of times the film could be folded without breaking.

2.3.3. Tensile strength

Tensile strength is to measure the capability of film to resist rupture, mechanical pressure, or the force required to break the film. Tensile strength of the film was determined by using the Instron tensile testing machine [Model No 3369/J 7257] under the condition of 23±2°C and 50±4% of RH. It was designated in MPa units.

2.3.4. Swelling index

Water absorption capacity is the important criteria in the case of all biological applications especially in wound healing process. Initially the one inch dry film weight was noted. Then it is placed in 15ml of Phosphate buffered saline (PBS) at room temperature for 30mts [17] .The weight of the film was noted at regular interval of time like 1hr, 2hrs, and 3hrs to 24 hrs. The % of water absorption capacity of film was deliberated by following formula;

$$\% \text{ of Swelling Index} = \frac{\text{Final weight } (W_f) - \text{Initial weight } (W_o)}{\text{Initial weight } (W_o)} \times 100$$

2.3.5. Film morphology

The morphology of the film was analysed by Scanning Electron Microscope (SEM: JSM-52- TOKYO, JAPAN.) and sputtering the samples with gold.

2.3.6. Thermal analysis

The thermal properties of the film were studied by subjecting the film materials for thermo gravimetric analysis and Differential scanning calorimetric analysis

2.3.6.1 Thermo gravimetric analysis [TGA]

The prepared films were subjected to TGA (MODEL Q15, Temperature range: Ambient to 800°C; temperature accuracy: ±1°C) for examining the physical and chemical properties of film materials are measured as a function of increasing range

2.3.6.2. Differential Scanning Calorimetric Analysis [DSC]

The film was subjected to Differential Scanning Calorimetric analysis (Q200 MODEL, Temperature range -90°C to 550°C with RCS90 cooling system, temperature accuracy: ±0.1°C). The analysis to find out the heat flow produced in a sample when subjected to a temperature programme. The function temperature of the substance also detected.

2.4.7. FTIR analysis

Fourier transform infrared spectroscopy (MODEL JASCO 4700; Wavelength range 4000nm to 400nm) was carried out to determine the functional group in the prepared polymeric film i.e aloe loaded gelatin film. The spectra were measured in the frequency range of 4000-400cm^{-1} using JASCO 4700 Fourier transform infrared spectroscopy

2.4.8. X-Ray Diffraction Analysis

It is one of the microstructural analysis methods of identification of crystallinity of polymers, recognition of crystalline phases (polymorphism). XRD pattern of film sample was analysed with a Rigaku X-Ray Diffractometer with CuKa radiation at 30 mA and 40kV.

2.4.9. Antibacterial activity

The prepared films were assessed for antibacterial activity against three different strains with agar plate diffusion method by measuring the zone of growth inhibition of micro organisms according to Cheng-Pei[18]. The polymeric film was cut into square(1cm×1cm) which is placed on Nutrient agar plates and these plates had been previously seeded with 0.1ml of inocula containing obviously 1.5 × 108 CFU/ml of test bacteria *Pseudomonas aeruginosa*(Gram Negative Strain)*, Eicherechia coli*(Gram Negative Strain)*, Staphylococcus aureus*(Gram Positive Strain). The plates were subjected to incubation for 48hrs at 37ºC.The size of the zone of inhibition was measured and the average was taken as inhibition zone.

3 Result And Discussion

Among the different proportion, sheet is formed when the proportion of 60% Almond and 40% Aloe (6:4). At low proportions of Almond gum (F1, F2, F3), the sheets produced are highly sticky. According to Ruben Pereira [21] increasing proportion of Aloe vera increases the film adhesion to the petri dishes, probably due to film hydration. at higher concentration of almond gum (70%, 80%, 90%) containing sheets are very difficult to handle and are brittle. Increasing concentration of almond gum facilitate the film production due to the presence of galactomannans in almond gum that enhance the cohesive strength [20]. The F6 sheets with 60% almond and 40% aloe proportion only had the good film production capacity and good flexibility also. All other sheets except F6 sheet are sticky or brittle in nature. So F6 sheet is only taken for the characterization studies. Higher density of almond gum usually leads to higher mechanical strength for the specific application [20, 21]. The tensile strength of F6 film is 8.25 Mpa units which show in the Table 1.

TABLE 1

MECHANICAL PROPERTIES OF F6 FILM

F6film[Almond+Aloe Concentration]	Thickness(mm)	Folding endurance(Times)	Tensile strength(mpa units)
60%+40%	0.08±0.01	275±4	8.25±0.23

Duration in hours	Percentage of water absorption of Almond + Aloe [F6] film.
1	103%
2	130%
3	160%
4	165%
5	165%
6	165%

According to Tran[22], the higher amount of Aloe vera caused a gradual decrease in mechanical properties and when the Aloe is incorporated with natural material(Almond), the bonding occurred mostly between functional group of them and mechanical strength presented good result at low concentration of Aloe vera. The folding endurance and thickness of the F6 sheet was given in the Table.1.

Fig. 1. *Almond+Aloe F6 Film(60%+40%)*

The film morphology is uniform on both sides. The pure almond sheet is colourless. It is somewhat coloured when aloe is blended with almond solution. Visual examination of the SEM picture [Fig.1(a)] indicated that the films were essentially smooth and slightly rougher surface which have no detection on changes, heterogeneity, or component segregation. The same condition was also explained by Gabreila[23]. The morphology of the film was somewhat rougher surface due to the presence of aloe vera which is also explained by R. Pereira[21]. It is possible to identify aloe vera aggregates dispersed on the surface of the film.

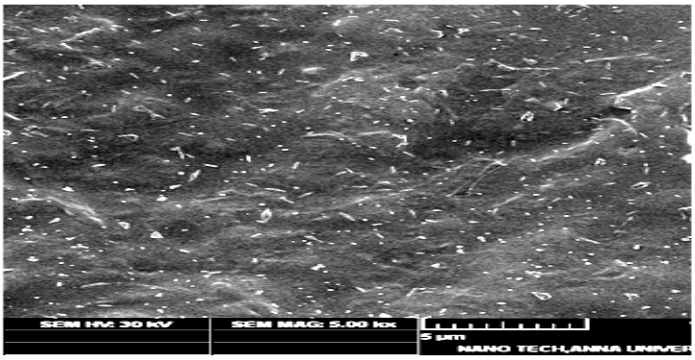

Fig. 1(a). *SEM image of F6 film (60%+40%)*.

TABLE 2

PERCENTAGE OF SWELLING INDEX

The F6 sheet of Almond+Aloe were evaluated for their swelling ability. The swelling ability was measured upto 6hours and the results are given in Table 2. The swelling index is initially 103% and it is gradually increased to 165% at fourth hour. Then there is a constant level maintained from 4th hour to sixth hour. The greater water absorption capacity of sheet is due to the presence of almond gum as reported by Chaithanya [24].

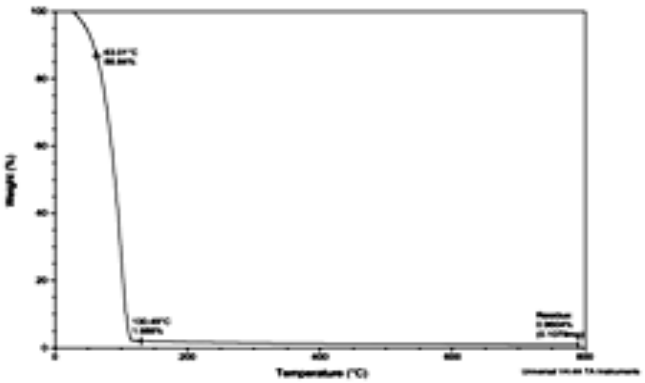

Fig. 2. *Thermogravimetric analysis of F6 sheet*

Figure 2.shows that Thermo Gravimetric analysis of F6 sheet, in which the weight loss of substance occurs from 86.4% to 1.986%. The mass of the sheet polymer starts decreasing at 60°C and totally degraded at 139.49°C. At the end, 0.9604% of residue remained at 800°C. It is designated in weight as 0.1079 mg. Likewise Panneerselvam Jithendra[25] analysed the thermogravimetric study of Aloe blended chitosan sheet and explained about the intermolecular interactions and their influence of thermal properties. In Fig.(2) the plot illustrated endothermic thermal events that occurred during the temperature scan from 50°C-300°C.The peak of F6 sheet of TGA comprises three ranges which exhibit 40°C to 100°C. In this temperature the dehydration starts. The second range of peak ranges from 105°C to 200°C. In this temperature the volatile substances starts to pyrolyse. The 200°C to 800°C covers the third range of peak in which the strong substances pyrolysed.

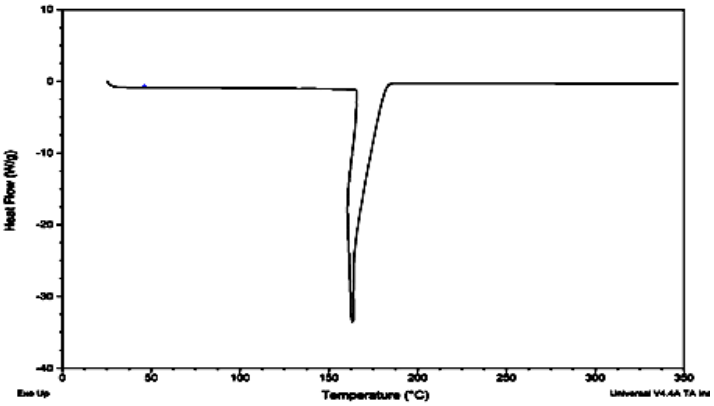

Fig. 3 *Differential scanning calorimetry of F6 Sheet*

The Fig.3 shows differential scanning calorimetry of the polymer sheet exhibit endothermic reaction and its melting point ranges at 160°C. According to Imtiyaz, the pure almond exhibit the sharp, symmetric, melting endotherm at 84.01°C(Imtiyaz et al.,2017) [27]. The pure Aloe vera thermogram exhibit a sharp peak point at 120.17°C(Konsolakis et al.,2015) [28]. In this paper the amount of heat is required to increase the temperature of polymer sheet is 160°C and it is considered as function of a temperature of polymer. The FTIR analysis of F6 sheet[Fig.3] shows that the three peak ranges from 1000-2000 region referred as the finger print region for many organic compounds. This is the single bond

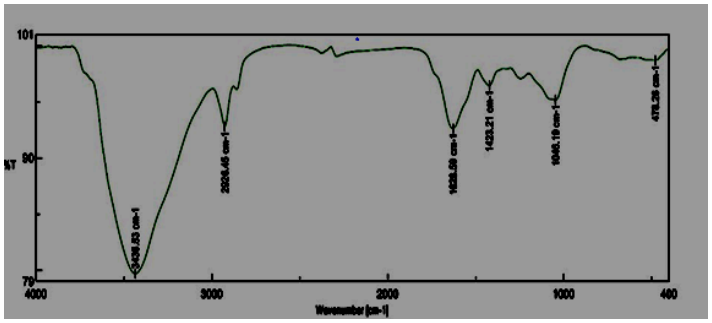

Fig. 4
Fourier Transform Infrared Spectrophotometry of F6 Sheet

region and it is very common to coupling of individual single bonds which have similar force constants and connect similar masses in which C-O, C-C, C-N stretches often couples.At 3000-4000 range of peak shows –OH stretching. There is a C-O,C-C,C-N stretching and –OH stretching at 3000-4000 range. The peak at 2926.45cm^{-1} reveals that alkyl chain of polymer and Peaks were observed in 1628cm^{-1} for C-H bond. The presence of carboxylic groups with O-H stretch region and amide group were determined at 3436cm^{-1}.The 1628.59 cm^{-1}. indicates the presence of aromatic nitro compounds which is the peak characteristic of Aloe Vera. Presence of alkanes at the region of 1423.21cm^{-1}. When we analysed the pure Almond(Imtiyaz et al.,2017)[27] and Aloe(Konsolakis et al.,2015) [28] FTIR, the F6 sheet shows the great molecular interactions between the Aloe and Almond gum.

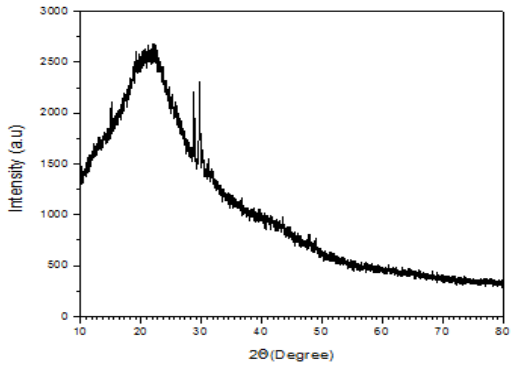

Fig.5 *X-Ray Diffraction analysis of F6 sheet*

The X-Ray diffraction of the F6 sheet[Fig.5] shows the position of peak at 15.025° to 48.829°. The different position of the peaks and its height explained in the table 3. The typical broad peak of carbonaceous structure in the XRD of F6 sheet is due to the amorphous organic matter such as cellulose, hemicellulose and lignin in the almond [28], [29].

TABLE 3
POSITION AND HEIGHT OF THE XRD PEAK OF F6 SHEEET

Position [°2Th]	Height [cts]
15.0253	223.35
19.0222	92.46
28.7165	568.20
29.6710	796.26
31.0815	142.13
47.7983	118.54
48.8290	78.35

The F6 sheet shows a great antibacterial activity[Fig.7(a), 7(b), &7(c)] against *Pseudomonas aeruginosa*(Gram Negative Strain), *Eicherechia coli*(Gram Negative Strain) and *Staphylococcus aureus*(Gram Positive Strain). The zone of inhibition was observed for three bacterial strains which is shown in the table.3. Like wise Bouaziz[30], also proved the antibacterial activity of Almond gum against various bacterial strains. As the same the Aloe also having high magnificient antibacterial activity against the bacterial strains which is explained by Cheng Pei[17]

TABLE 3
F6 SHEET PRODUCE ZONE OF INHIBITION AGAINST DIFFERENT STRAINS OF BACTERIA

Name of organisms	*Zone of inhibition(in mm)*
Salmonella typhii	*12±0.251*
Eicherechia coli	*12± 0.115*
Pseudomonas aeruginosa	*12.2± 0.352*
Staphylococcus aureus	*11.8±0.305*

4 CONCLUSION

Almond films containing Aloe at different proportions were tried and successfully (60% + 40%) of F6 film is produced by Solvent casting method, showing good film-forming properties, thickness, flexibility and folding endurance. Both aloe vera and almond play a vital role in thermal and mechanical properties of film. The FTIR result shows the interaction between aloe and almond molecules. The water absorption capacity of films indicates that this can keep as a wound dressing material. The combined data obtained from different tests allowed a better understanding of Aloe loaded almond film as biomaterial for wound healing as well as its potential to be applied in such different fields as the biomedical, food and pharmaceutical industries.

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