DEVELOPMENT AND CHARACTERISATION OF EDIBLE FILMS BASED ON THE MUCILAGE OF *HIBISCUS ROSA-SINENSIS* LINN. (MALVACEAE)

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ABSTRACT

A novel combination of film forming suspension based on *Hibiscus rosa-sinensis* leaf mucilage has been standardized. The film forming suspension was subjected to wet solvent processing method to prepare edible biodegradable films.The developed films were subjected to physico-chemical characterisation, microstructure and *in vitro* bioactivity studies. The prepared films showed ideal properties for most of them, including folding endurance, tensile strength, per cent elongation, water vapour barrier properties, light transmittance and moisture content. The films were found to be transparent which is best for product visibility. FTIR spectral studies indicated increased interactive stability in the mucilage based film. The film forming suspensions were also found to exhibit antibacterial activity. Thus, it could be concluded that the developed films have ideal properties to be used as a potential packaging material in the postharvest preservation of fruits and vegetables.

Keywords: *Hibiscus rosa-sinensis*, mucilage, Edible biodegradable films, physico-chemical characterisation, microstructure study and antibacterial activity

1. Introduction

A great effort to improve food quality while reducing packaging waste has become a major focus in recent times. This has encouraged the exploration of new bio-based packaging materials, such as edible and biodegradable films from renewable resources.

An edible film is a thin layer of sheet that can be used to cover food components and can be consumed along with the food product (Šuput, Lazić, Popović & Hromiš, 2015). In addition, they may fulfil other functions like acting as carriers for targeted food additives as antimicrobial agents, antioxidants, flavourings and colouring agents (Kowalczyk, 2016; Mellinas et al., 2016). Edible films may also be used to inhibit moisture, oxygen or carbon dioxide migration and to improve the mechanical integrity or handling characteristics of the food (Shankar, Jaiswal & Rhim, 2016).

Edible films are biodegradable owing to the fact that most of its components are of biological origin. Edible coatings may be composed of polysaccharides, proteins, lipids or a blend of these compounds (Hassan,

Chatha, Hussain, Zia & Akhtar, 2017). The present report describes the utility of *Hibiscus rosasinensis* mucilage based composite as ingredients in the development of biodegradable edible films.

Mucilages are heteropolysaccharide complexes of plant origin with ideal bioactive and physicochemical characteristics (Vignesh & Bindu, 2018). Presence of mucilage is a characteristic feature of the family Malvaceae. *Hibiscus rosa-sinensis* Linn.,family Malvaceae, is a tropical species with wide distribution. Leaves, roots and flowers reportedly possess food, cosmetic and medical value in Chinese herbology.

Hibiscus mucilage has been exploited mostly by pharmaceutical industry and little research was carried out regarding its application in food industry. The present study focuses on the development of a biodegradable edible film loaded with *Hibiscus* mucilage and its characterisation.

2. Materials and methods:

2.1. Materials

The basic components used to prepare edible film were gelatin, chitosan, cassava starch, glycerol, acetic acid and *Hibiscus rosa-sinensis* mucilage. The mucilage was prepared from the leaves of *Hibiscus rosa-sinensis* Linn. collected from a local home garden in Thiruvananthapuram. The fresh leaves of *Hibiscus rosa-sinensis* were collected, washed with water to remove dirt and debris, and shade dried for 20 days. The dried leaves were ground to a fine powder and the leaf powder was stored in air tight containers.

2.2. Mucilage extraction procedure

The method of extraction of mucilage was adopted from Vignesh and Bindu (2018). The powdered material was soaked in water for 5 hours at 50ºC. To remove the marc from the solution, it was filtered through an eight layered muslin cloth. Finally, the mucilage was precipitated by adding acetone to the filtrate and oven dried for further analyses.

2.3. Preparation of film forming suspension

For the preparation of film forming suspensions of required quality, the individual components (1% solutions) were tested for their film forming ability and organoleptic characteristics. Then, numerous combination trials involving suitable proportion of the components were carried out to produce films and finally a combination involving a mixture containing gelatin, chitosan, cassava starch and *Hibiscus* mucilage in 1:0.5:0.5:0.5 ratio was considered ideal, taking into account the criteria of sensorial parameters and ease of preparation.

For experimental purpose, two film forming suspensions were prepared. They are NB (Normal Blend) suspension and MB (Mucilage Blend) suspension. The NB suspension was used to prepare control films, while MB included *Hibiscus* mucilage. The NB suspension was prepared by mixing gelatin (1g in 100 ml distilled water), chitosan (0.5 g in 100 ml of 5% acetic acid), cassava starch (0.5 g in 100ml distilled water)

and made up to 500 ml using distilled water along with 0.5ml of glycerol as plasticiser whereas MB suspension included *Hibiscus* mucilage (0.5g in 100ml distilled water) in addition to NB components.

2.4. Development of the film

The NB and MB suspensions were cast on suitable casting trays. A definite volume of the suspension was poured into the tray just sufficient enough to spread on the surface. Casting trays used include glass petri dishes of 9 cm diameter, rectangular steel trays (40 × 30 cm) and rectangular plastic trays of larger (25.5 × 15.5 cm) and smaller dimensions (15.5 ×10.5 cm).Then, the casting trays were kept under ambient conditions for setting *via* evaporational drying. The rate of setting and film formation was also checked by keeping the tray with film suspension in a hot air oven at 50˚C. After ensuring complete setting, the film was peeled out from the casting tray. Both NB and MB films were developed by casting the respective suspensions on casting trays and stored in a chamber conditioned at 25ºC and 50% relative humidity for 24 hrs prior to testing.

2.5. Physico-chemical characterisation

2.5.1. Appearance

All the prepared films were checked for their physical appearance and also for the presence of air bubbles. The physical appearance of various formulations was determined by visual inspection under black and white background.

2.5.2. Thickness

The thickness of the films were measured by means of electronic vernier callipers. Five random locations were selected along the length and breadth of each film. The values were taken for ten such film samples. The average thickness was determined.

2.5.3. Solubility

Film pieces of about 0.1 g were weighed and immersed in 30 ml distilled water and stored at room temperature for 24 hrs and was then filtered using dessicated pre-weighed filter paper. The filter paper having undissolved portions of film, was dried for 24 hrs at 50°C in a hot air oven, to determine the weight of insoluble dry matter. The soluble dry matter was calculated by subtracting the weight of insoluble dry matter from the weight of initial dry matter. The measurements represent an average of three replications for each film type.

2.5.4. Surface pH

The film to be tested was placed in a Petri dish and moistened with 0.5 ml of distilled water for 1 min. The pH was noted after bringing the electrode of the pH meter in contact with the surface of the formulation and allowing equilibration for 1 min. The average of three determinations for each film was carried out.

2.5.5. Moisture content

The film to be tested were taken in a petridish and its initial weight was recorded. Then, it was kept in the hot air oven at 50˚C until constant weight was recorded. The moisture content was determined by subtracting the initial weight by final weight of the film. The determinations were done in triplicate.

2.5.6. Water vapour barrier properties

The water vapour barrier properties of each film was determined according to ASTM standard test method (ASTM, 2003).Films were cut, adjusted and sealed on glass cup containing dried silica gel with an exposed area (A) of 15.9 cm diameter. Sealed glass cups were weighed and placed in a desiccator with distilled water in a chamber conditioned at 24 ± 0.5°C. The cups were weighed periodically until steady state was reached. The water vapour transmission rate or WVT (g hr^{-1} m^{-2}) was calculated using Equation 1. The water vapour permeability (WVP) transferred through the film and absorbed by the silica gel was calculated using Equation 2.

$$WVT = \frac{w}{A \times t} \quad (1)$$

$$WVP = \frac{WVT \times L}{\Delta P} \quad (2)$$

Where, w is the weight gain of the cell (g) and A is the area of the exposed film, t is the definite time once steady state was reached, L is the film thickness (mm) and ΔP is the partial water vapour pressure difference (KPa) across the two sides of the film.

2.5.7. Light transmittance and transparency value

The light transmittance of films was measured at the ultraviolet and visible ranges (200–700 nm) using a UV–Vis spectrophotometer. The transparency value of film was calculated using the Equation 3:

$$\text{Transparency value} = \frac{-\log T600}{x} \quad (3)$$

Where,T_{600} is the fractional transmittance at 600 nm and *x* is the film thickness (mm). The greater transparency value represents the lower transparency of film *ie*, greater opacity (Prodpan *et al*.,2017; Yao *et al*., 2017).

2.5.8. Folding endurance test

The test involves the repeated folding of the film at the same place until the film breaks. The number of the times the film is folded without breaking is computed as the folding endurance value.

2.5.9. Mechanical properties

This mechanical property was evaluated using the Kalpak universal testing instrument (Model SR NO 130701) with a 100kgf load cell. Film strips in a special dimension (10 × 2 cm) and free from air bubbles

or physical imperfections were held between two clamps. During measurement, the strips were pulled by the top clamps at a rate of 1 mm/min; the force and elongation were measured when the film broke. Results from film samples, which broke at and not between the clamps, were not included in the calculations. Two mechanical properties, namely, tensile strength and percentage elongation at break were computed for the evaluation of the film. Measurements were run in triplicate for each film.

2.5.9.1. Tensile strength is the maximum stress applied to a point at which the film specimen breaks and can be computed from the peak load (Equation 4).

$$\text{Tensile stress (TS)} = \frac{Applied\ force}{cross\ sectional\ area} = \frac{m \times g}{b \times t} \quad (4)$$

Where,TS = tensile stress in 980 dynes/cm^2 , m = mass in grams, g = acceleration due to gravity (980 dynes/cm^2), b = breadth of strip in centimeters, t = thickness of strip in centimeters.

2.5.9.2. Percent elongation: When stress is applied, a strip sample stretches and this is referred to as strain. Strain is basically the deformation of strip divided by original dimension of the sample (Equation 5).

$$\text{Strain (\%E)} = \frac{total\ elongation}{original\ length} \times 100 = \frac{L - L0}{L0} \times 100 \quad (5)$$

Where,L = length after force was applied, L0 = original length

2.5.10. Microstructure study

For microstructure study of surface characteristics, scanning electron microscopy was used. Film pieces of very small dimensions were cut and mounted directly on graphite tape and sputter coated with gold in fine coater to make them conductive, analysed and photomicrographed by SEM. A categorical scale was used to qualify films with the following levels: (1) smooth, (2) slightly roughness, (3) intermediate roughness, (4) intense roughness, (5) slightly lumpiness, (6) intermediate lumpiness, and (7) intense lumpiness (Lira-Vargas *et al*, 2014).

2.5.11. FTIR Spectroscopic study

Fourier transform infrared (FTIR) spectral data was taken on Agilent Cary 630 FTIR spectrometer that is based on ATR (Attenuated Total Reflection) method. This was done to find out whether there is any new chemical interaction that affects stability in MB films compared to NB films due to the incorporation of *Hibiscus* mucilage.

2.6. Statistical analyses

The seven quantitative parameters were tested in triplicate. The values were expressed as mean ± standard deviation (SD). Differences between the properties of two types of films (NB and MB films) were detected by analysis of variance (ANOVA). The statistical analysis was performed using SPSS statistical software (version: IBM Statistics 22).

2.7. Antibacterial activity of the film suspensions

Antibacterial activity of the NB and MB film suspensions were tested using Agar well diffusion method. Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *Klebsiella pneumoniae* (a gram negative bacteria) and *Streptococcus pyrogens* (a gram positive bacteria) (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and samples of 100 µl concentrations were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin (standard antibacterial agent, concentration: 1µg / ml) was used as a positive control.

3. Results and Discussion

3.1. Preparation of film forming suspensions

The films produced using individual components were analysed for their film forming ability and organoleptic features. When chitosan alone was used, the films were yellowish in colour and translucent to light. Moreover, the surface texture was found to be rough. With cassava starch, films were stiff though translucent and brittle. Gelatin films were smooth and transparent but was difficult to peel and gummy in nature.

With a suitable combination trial using the suspension containing gelatin, chitosan, cassava starch and *Hibiscus* mucilage in 1:0.5:0.5:0.5 ratio, much better films were obtained which was nearly transparent and had a smooth surface.

3.2. Development of the films

Films of desired dimensions were developed using steel as well as plastic trays. It was easier to peel the films from steel and plastic trays but was found difficult when cast on glass petri dishes. Plastic trays were identified to be the best substratum for film preparation and removal.

3.3. Physico-chemical characterisation

3.3.1. Appearance

Both NB and MB films were found to be transparent, without air bubbles and resembled fine quality plastic sheets used as packaging materials. Development of transparent packaging materials which allow product visibility is a universal trend and is of primary importance from a consumer point of view (Simmonds & Spence, 2017). Natural colours can be added while preparation to suit or enhance market demand.

3.3.2. Thickness

Both NB and MB films developed had a thickness range of 40-60 microns making up an average thickness of 50 microns. Ideally, thin films are preferred on a commercial scale since it does not add much weight to the finished product.

3.3.3. Solubility

Solubility was found to be higher for the of MB films compared to NB films. This was expected, considering the hydrophilic nature of mucilaginous hydrocolloids in MB films compared to NB films. Generally, high solubility would indicate lower water resistance; however for applications such as packaging wrap, the high solubility is an indicator of biodegradability which could be an advantage (Tajik et al., 2013).

3.3.4. Surface pH

Only slight pH difference was noticed between MB films (5.13) and NB films (4.77). Surface pH of 5.1 is not known to cause any irritation to mucosal lining of oral cavity as it is in range with the pH of many common fruits and vegetables (Anon., 1962).

3.3.5. Moisture content

The moisture content of MB film was 7.86% higher than NB film. This higher moisture content may be accounted for by the presence of *Hibiscus* mucilage in MB films. Mucilage, being a hydrocolloid has a higher moisture retention property, which is beneficial when such films are used in coatings and as wrappers as its moisture content keeps the coated fruits fresh for a longer period.

3.3.6. Water vapour barrier properties

MB films have lower water vapour transmission rate (WVT) and hence, lower water vapour permeability (WVP) than NB films (table1).The high swelling index of Hibiscus mucilage combined with polymeric cross-linking makes it difficult for the liberation of water to the environment, thus reducing WVP (Bhatia, 2016; Farris *et al*., 2011). This accounts for the lower WVT and WVP of MB films.

3.3.7. Folding endurance test

Folding endurance test gives an idea about the flexibility of the films. The results indicated that the films did not break even after repeated folding at the same place for more than 100 times. The NB and MB films retained their integrity. The addition of mucilage did not deteriorate the endurance property of the film but maintained its flexibility.

3.3.8. Mechanical properties

The tensile strength and percentage elongation of NB and MB films were recorded as shown in table 1.The incorporation of mucilage into edible films increased the tensile strength and percent elongation values, providing greater strength and flexibility for MB films in comparison with NB films. Thus, MB films have more elasticity than NB films .This is in conformation with the earlier reports by Muñoz, Aguilera, Rodriguez-Turienzo, Cobos& Diaz (2012) that the films made with the highest ratio of polysaccharides were more resistant and extend more than those with the lowest amount of polysaccharide.

Table 1: Solubility, Surface pH, Moisture content, Water vapour barrier properties and Mechanical properties of the NB and MB films

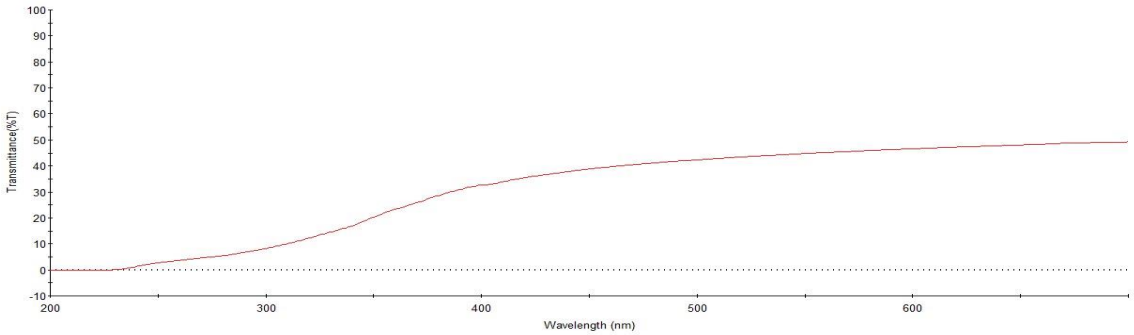
Parameters	NB Film	MB Film	F VALUE df (n-1) = 1
Solubility (%)	64.50 ± 0.46	76.93 ± 0.15	1999.10***
Surface pH	4.77 ± 0.06	5.13 ± 0.06	60.50**
Moisture content (%)	13.54 ± 0.47	21.40 ± 0.13	786.55***
WVT (g mm/Kpahr m^2)	34.81 ± 0.60	25.77 ± 0.76	261.44***
WVP (g/hr m^2)	0.60 ± 0.01	0.44 ± 0.01	7360.99***
Tensile Strength (N/mm^2)	1.54 ± 0.03	4.06 ± 0.04	262.56***
Elongation at break (%)	97.37 ± 0.04	104.67 ± 0.03	63375.26***

** F value significant at p< 0.01 level; *** highly significant at p< 0.001 level

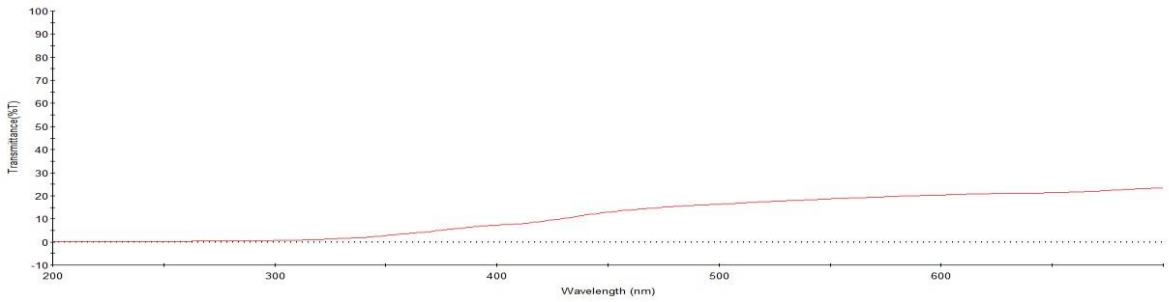
3.3.9. Light transmittance and transparency value

Transmission of UV and visible light in the wavelength range of 200–700 nm of NB and MB films are shown in Graphs 1 and their transparency values were shown in table 2.

A



B



Graph 1: Percentage Light Transmittance of NB film (A) and MB film (B)

Table 2: Light transmittance and transparency value of the films

Film type	T_{600} (%)	Transparency value
NB film	46.5881	- 33.3655
MB film	20.3407	- 26.1673

The transmission of UV light was not observed at 200 nm for both NB and MB films. However, MB films had much lower light transmittance at wavelengths lower than 300 nm, as compared to NB film. It was suggested that the incorporation of *Hibiscus* mucilage into the film could enhance the barrier property against UV light. Lower transmission values (2.83 to 23.42%) were observed for MB films compared to NB film (20.26 to 49.26%) in the visible range. Small particle size of *Hibiscus* mucilage and formation of more extensive crosslinking in film matrix may be the reason for reduced UV and visible light transmittance as opined earlier (Galus and Kadzińska, 2016). Further, polysaccharides in films are suggested to cause light absorption and scattering to different degrees. (Nasatto et al., 2015).

3.3.10. Microstructure study

Figure 1 shows the surface topology of NB and MB films. MB films were analysed and categorised under “slightly roughness” while NB films were analysed and categorised under “intense roughness” based on the categorical scale adopted by Lira-Vargas *et al* (2014).

The roughness in surface morphology of NB films were well evident by the irregular, near globular and flake like aggregates and numerous spinous surface projections which gave the surface an irregularly striated appearance. This might be due to the fact that the electrostatic repulsion between similarly charged particles may not be strong enough to prevent the formation of aggregates. The rather smooth homogenous nature of MB films may be due to the smaller particle size and polysaccharide composition of mucilage that helped fill the intermolecular spaces and enabled extensive intermolecular cross linking (Banerjee and Bhattacharya, 2012)

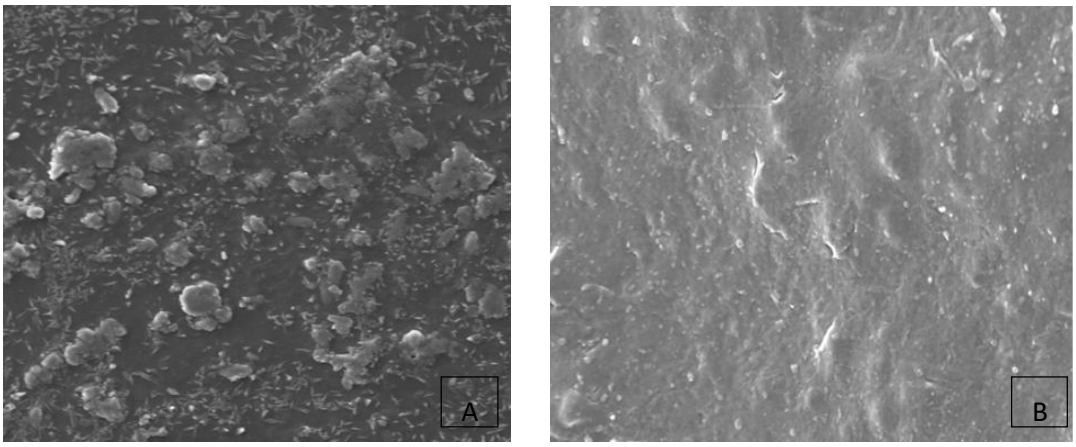
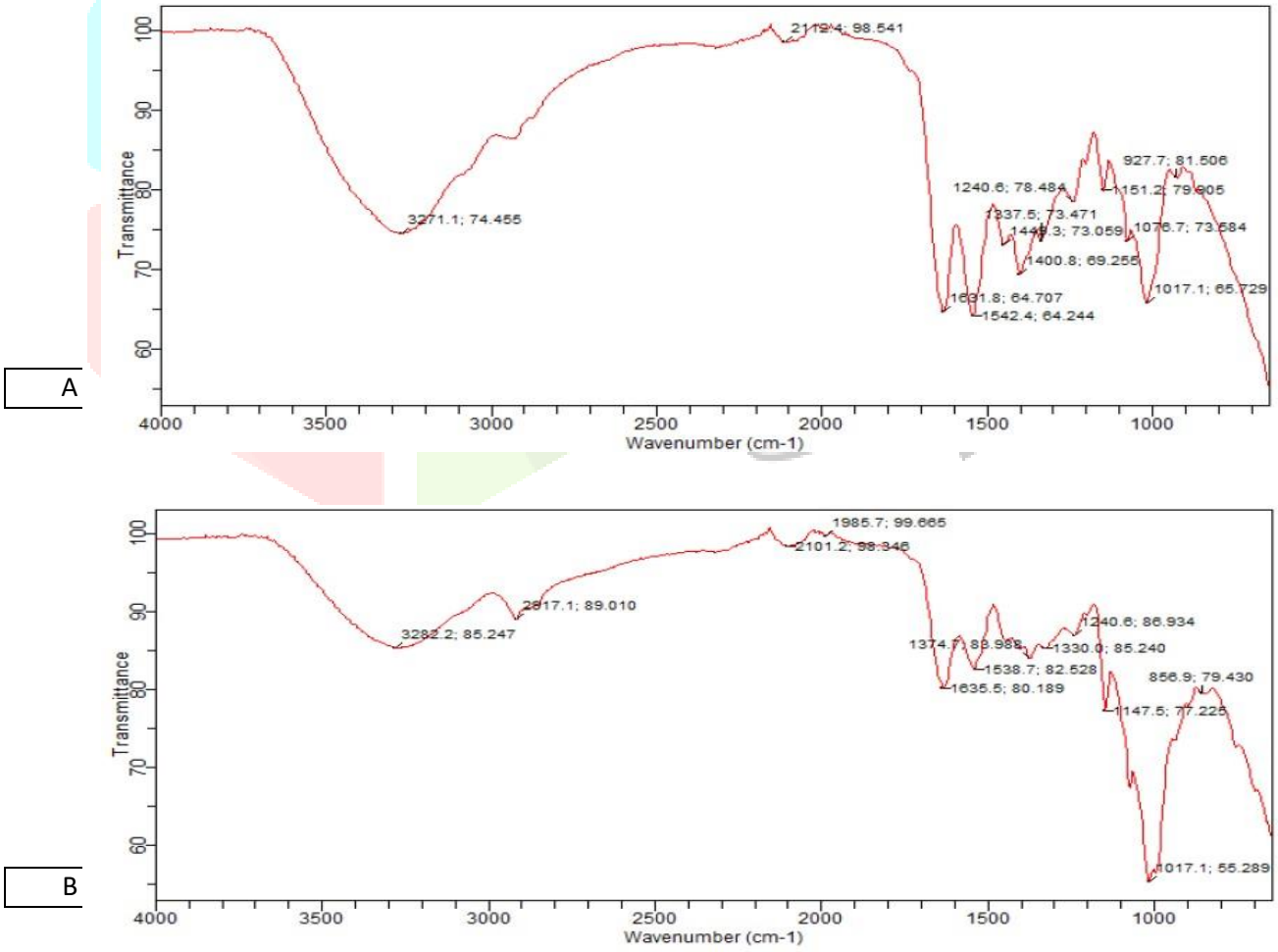


Figure 1 Surface topology. NB films (A) ; MB films (B)

3.3.11. FTIR Spectroscopic studies

FTIR spectroscopy was used to characterise the functional groups and interactions in NB and MB films. The FTIR spectra of NB and MB films developed are presented in the graph 2.



Graph 2: FTIR Spectrum of NB film (A) and MB film (B)

Table 3: Peak frequencies and functional groups in the FTIR spectra of NB and MB films

NB FILM		MB FILM	
Peak frequency (cm^{-1})	**Functional group**	**Peak frequency (cm^{-1})**	**Functional group**
3271.1	Alcoholic/phenolic O-H stretch	3282.2	Alcoholic/phenolic O-H stretch
2821.1	Alkyl C-H stretch	2817.1	Alkyl C-H stretch
2112.4	Alkynyl C=C stretch	2101.2	Alkynyl C=C stretch
1740.1	Carbonyl C=O stretch	1744.3	Carbonyl C=O stretch
1631.8	Amide C-O stretch	1635.5	Amide C-O stretch
1542.4	Amide N-H bending	1531.7	Amide N-H bending
1400.8	C-H bend	1374.7	C-H bend
1240.6	C-O bend aromatic	1240.6	C-O bend aromatic
1017.1	C-O stretch	1017.1	C-O stretch

In the spectrum, the broad band from 3271.1 - 3282.2 cm^{-1} was the OH stretching. The band at 2817.1-2821.1 cm^{-1} was C–H stretching. The bands from 1631.8- 1635.5 and 1531.7-1542.4 cm^{-1} were the C=O stretching (amide I) and NH bending (amide II), respectively. The peak near 1740 cm^{-1} suggested the presence of a carbonyl group in the starch and chitosan films.

The chemical interactions are reflected by changes in the peaks of characteristic spectra after physical blending of two or more substances (Zhong and Xia, 2008). In the spectrum of chitosan/cassava starch/gelatine/ mucilage composite film, the amino peak of NB films shifted from 1542.4 to 1531.7 cm^{-1} with the addition of *Hibiscus* mucilage, and the OH peak found in NB films shifted from 3271.1 to 3282.2 cm^{-1} in MB films (Graph 2). This result indicated that interactions were present between the hydroxyl groups of *Hibiscus* mucilage and the amino groups of gelatin and chitosan.

3.4. Statistical analyses

A significant difference in values were observed between the parameters studies for determining the properties of NB and MB films. The F values of parameters studied for the NB and MB films are shown in the table 1.

3.5. Antibacterial activity of the film suspensions

The diffusion test results of the film forming solutions are shown in Figure 2. The zone of inhibition values showed that both *Klebsiella pneumoniae* and *Streptococcus pyrogens* exhibited sensitivity to both the film forming solutions and MB suspension shows an increased antimicrobial action compared to NB suspension (Table 4).

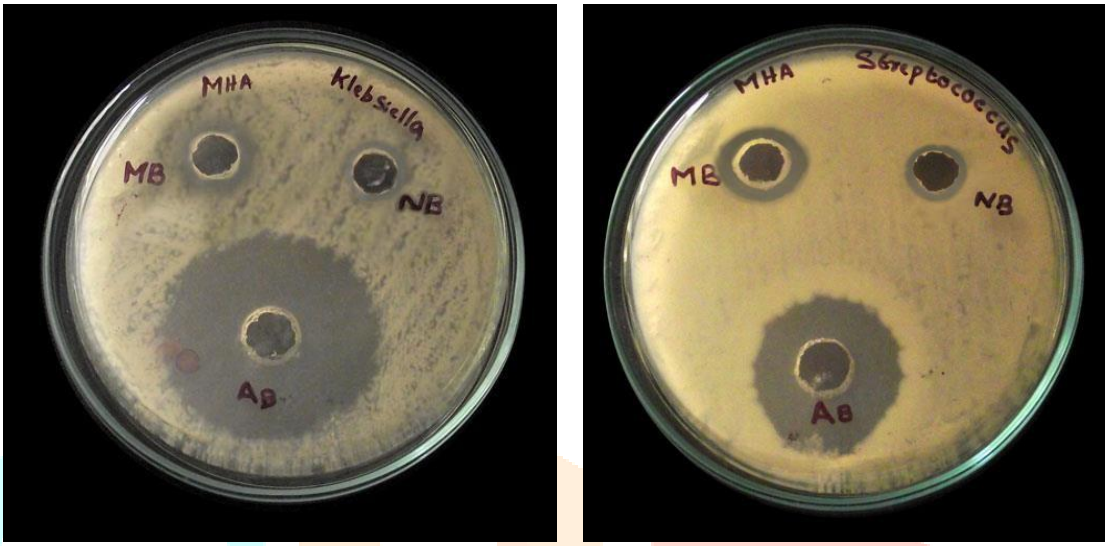


Fig 2: Antibacterial activity of the film suspensions against *Klebsiella pneumoniae* (A) and *Streptococcus pyrogens* (B).

Table 4: Antibacterial activity of the NB and MB film suspensions against *Klebsiella pneumoniae* and *Streptococcus pyrogens*

Suspension (100 µl)	**Zone of inhibition (cm)**	
	Klebsiella pneumoniae	***Streptococcus pyrogens***
Streptomycin (1µg /ml)	3.9	3.0
NB	1.3	1.2
MB	1.8	2.0

Antimicrobial activity of the NB suspension could be attributed to the antimicrobial nature of individual components. The antimicrobial activities of Gelatin and Chitosan were ascribed to the presence of the positively charged amino groups which interact with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Ma et al., 2016; Saloko, Darmadji, Setiaji & Pranoto, 2014;Pereda, Ponce, Marcovich, Ruseckaite & Martucci, 2011). An increased antimicrobial action of MB suspension compared to NB could be attributed to a synergistic action of chitosan, gelatin and the isolated Hibiscus mucilage

4. Conclusion

The present study is concerned with testing the suitability of *Hibiscus rosa-sinensis* leaf mucilage in the development of an edible biodegradable film. The mucilage in MB films improved the film's mechanical and functional properties. Even though, high solubility of MB films indicated low water resistance, its biodegradability is advantageous. A slightly acidic surface pH (5.13) noted is not known to cause any irritation to mucosal lining. Better water vapour barrier properties noticed help lower water vapour transmission rate. A significant advantage noticed was the UV barrier property in the range of 200- 350 nm as evident from the transparency studies. Stabilising nature was also evident through surface topology studies, based on SEM analyses. Besides functional advantages, they offer better tensile strength, percent elongation and folding endurance. FTIR spectral studies indicated additional interactions between the alcoholic and amide groups in MB films which accounts for better stability. Statistical analyses involving one way ANOVA indicated that the recorded values were statistically significant. Thus, it could be concluded that the mucilage of *Hibiscus rosa-sinensis* represents a new functional ingredient with huge potential for the food, cosmetic and pharmaceutical industries to be used as edible film forming agents.

5. Acknowledgement

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6. Conflict of interest

The authors report no conflicts of interest.

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