Fabrication and Characterization of Antibacterial and Biodegradable Facial Tissue Papers Using Bio-Based Raw Materials: Effect of Glycerin

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Abstract: Antibacterial facial tissue papers were prepared by solution casting method with chitosan loaded bleached cellulosic pulp. Chitosan, in the film act as an antibacterial agent. Tissue paper films were fabricated by 50% cellulose and 50% chitosan (by weight, dry basis). Mechanical properties of the films were evaluated. It was found that tensile strength (TS) and elongation at break (EB) of the 50% chitosan contain films were 24 MPa and 10.8% respectively. To increase the plasticity of the tissue paper films glycerin was added 0.4 to 2% (by weight). It was found that with the incorporation of 1% glycerin in the tissue paper films the flexibility increased to 50%. Molecular interaction due to the chitosan addition was investigated by Fourier Transform Infra-Red (FTIR) spectroscopy. Water uptake property of glycerin contains films were also evaluated. In the soil medium, the degradation properties of the tissue paper films were carried out. The antibacterial property of the tissue paper was evaluated by disk diffusion method.

Keywords: Bleached cellulosic pulp, Chitosan, Glycerin, Facial tissue, Antibacterial, Biopolymer, Biodegradable.

1. INTRODUCTION

The demand for antibacterial products is increasing day by day because of now a day consumers are very conscious about bacterial infections by dust and pollution. During the last decade, environmental issues are becoming increasingly important and there is an increasing motivation to eliminate bacterial diseases. Various types of microorganisms can have harmful effects on human health. Main skin floras that are present in human skin are *Staphylococcus*, *Streptococcus, Pseudomonas*, *Propionibacterium*, and *Corynebacterium*. Antibacterial facial tissue paper is one of the highly demanding antibacterial products now a day. Facial tissue papers are soft, absorbent, disposable papers that are suitable for external use. 255,360,000,000 disposable facial tissues papers were used by Americans a year. By 2021 it was expected that the growth of global demand for tissue paper is expected to 4% every year with Latin America accounting for 15% of the growth, China just over 40%, Western Europe (11–12%), and the rest of the world. Several different renewable materials have been studied for antibacterial facial tissue paper applications, but only a few are on the market today. The most common renewable tissue paper materials are cellulose-based. Cellulose and its derivations are widely applied in chemical engineering, medicine, and foodstuff for its renewable and biodegradable properties [1-7].

Cellulose is a natural linear carbohydrate polymer chain consisting of D-glucopyranose units joined together by β-1,4-glycosidic linkages. Each repeating unit contains three hydroxyls (-OH) groups. These hydroxyl groups have the ability to form hydrogen bonds. It is the most abundant organic polymer which is the main constituent of plants and can be found in wood, cotton and other sources. Cellulose has many uses such as emulsifier, stabilizer, dispersing agent, thickener, and gelling agent. Cellulosic materials have good mechanical properties because of this cellulosic fibers were widely used as a reinforcing phase in polymeric matrix composites. They are lightweight, biodegradable and renewable. With this renewable and biodegradable properties, cellulose and its derivatives are widely used in paper and textile industries, foodstuff, and medicine [8-11].

In commercial antibacterial facial tissue paper, synthetic chemicals such as Triclosan, Polyvinylamine, 2-phenylphenol, sodium-2-phenylphenolate, Polyethyleneimine are widely used as an antibacterial agent which are not environment-friendly. Whereas chitosan is a natural antibacterial agent which is nontoxic, biodegradable, biofunctional, biocompatible and has antimicrobial characteristics. Chitosan is an important derivative produced by deacetylation of chitin, chitin is the second most abundant polymer which is obtained generally from prawn or crab shell

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wastes by deproteinization and demineralization process. Chitosan is an amino polysaccharide, comprises unbranched chain of β (1 → 4) 2-amino-2-deoxy-D-glucan residue, and is one of the most attractive materials because of its many unique properties. The biocompatibility and nontoxicity of chitosan films permit its use for water treatment, food, cosmetics, pharmaceuticals and biomedical applications for instance membranes dialysis, contact lenses, dressings, and the encapsulation of mammal cells, including cell cultures. The presence of active groups such as amides and hydroxyl groups in chitosan molecules allows for easy chemical modification and chitosan can be frequently blended with other polymers for cross-linking to improve their functional properties. Cellulose and chitosan have sufficiently similar primary structures to facilitate the formation of homogeneous composite films which combine the physicochemical properties of chitosan with the excellent mechanical properties of cellulose fibers. Chitosan–cellulose combinations are of particular interest many authors have been studied the structure of these mixtures and found evidence of interactions at the interfacial region between the two polymers. One practical application of chitosan loaded cellulose composites is their processing into films with high strength and good biocompatibility and biodegradability [12-22].

Glycerin (1, 2, 3-propanetriol or glycerol) is an organic molecule isolated by heating fats in the presence of ash (to produce soap) is an industrial chemical with tons of applications. Usually, glycerin is produced as a by-product of the hydrolysis of fats in soap and other related materials. It can be produced either by microbial fermentation or by chemical synthesis from petrochemical feedstock. Glycerin being one of the most preferred and most studied plasticizers which reduced the intermolecular forces, soften the rigidity of the film structure and increase the mobility of the biopolymeric chains. Glycerin is a simple alcohol with many uses in the pulp and paper, pharmaceutical, cosmetic, paint, leather, automotive, food and textile industries or as a feedstock for the production of various chemicals [23-28].

The objective of this research was to fabricate antibacterial facial tissue papers using cellulosic pulp and chitosan. To increase the softness and plasticity of the tissue paper glycerin was added as a plasticizer. The films were cast via solution casting method and the mechanical properties were estimated. Molecular interactions due to the incorporation of chitosan were supported by Fourier transform infra-red (FTIR) spectroscopy. Water uptake and antibacterial behavior of the films were studied.

2. MATERIALS AND METHOD

2.1. Materials

The cellulosic pulp was obtained from the white printed paper collected locally (Bashundhara Paper, 80 GSM, and 297×210 mm, A4 size). Dry chitosan powder from shrimp shell was obtained from chitosan lab of Institute of Radiation & Polymer Technology (IRPT), Atomic Energy Research Establishment (AERE), Dhaka, Bangladesh. Laboratory grade glycerin was used. Glacial acetic acid was purchased from Scharlau, analytical grade, ACS, Reag, Ph. Eur.The chemical structure of cellulose, chitosan and glycerin are given in Figure **1**.

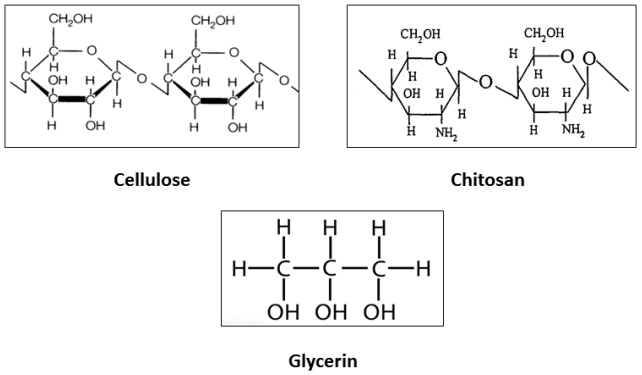

Figure 1: Chemical structure of cellulose, chitosan, and glycerin.

2.2. Preparation of Biodegradable Films by Solution Casting

1% (weight %) cellulosic pulp suspension was prepared by dissolving white printing paper (cellulose) in de-ionized water with constant blending using kitchen blender (Jaipan family mate) and kept for 7days in an airtight bottle. After 7 days, pure cellulosic pulp based films were prepared by casting the cellulose suspension onto silicon-coated glass mold and allowed to dry for 24 hours, at room temperature (30ºC) and at 65% relative humidity. Dried films were peeled off manually using a spatula and stored in the desiccator contained blue silica beads prior to characterization. 1% chitosan solution (w/w) was prepared using 2% (w/w) acetic acid solution. Pure chitosan films were prepared by solution casting method at the same parameters mentioned above**.** Then cellulosic pulp suspension and chitosan solution mixed together at different proportion by using a magnetic stirrer and

antibacterial tissue paper films were cast at same parameters via solution casting method. To increase the plasticity 0.4-2% (by weight) glycerin was added with the 50:50 chitosan/cellulosic pulp based formulation. The thickness of films was maintained to 100 µm by adding the same amount of raw materials.

2.3. Measurement of the Mechanical Properties

Mechanical properties of the films were investigated by the Universal Testing Machine (Hounsfield series, model, INSTRON 1011, UK) with a crosshead speed of 10mm/min. The dimensions of the test specimen were: 80mm×15mm×0.01 mm. The experiment was carried out according to the ASTM D 638-01.

2.4. Water Uptake Test

Water uptake test of the films was carried out in deionized water at room temperature (30°C). The test was carried out up to 60 min. Samples were taken into glass beakers containing 50 ml of deionized water. After 60 minutes, samples were taken out from the beaker and wiped out properly and then reweighed. The water uptake was measured by a weight difference methodology. The equation for water uptake was as follows: Water uptake (%) = $(W_{wet}- W_{dry})/W_{dry} \times 100$, where W_{wet} indicated the weight of the film after immersion in water and W_{dry} represented the weight of the film before immersion.

2.5. Characterization Using Fourier Transform Infra-Red (FTIR) Spectroscopy

The FTIR spectra of the films were recorded using a Spectrum two spectrophotometer (Perkin-Elmer) equipped with an attenuated total reflectance (ATR) device for solids analysis and a high linearity lithium tantalite (HLLT) detector. Spectra were analyzed using the Spectrum 10^{TM} software. Films were stored at room temperature for 72 minutes in a desiccator containing blue silica beads. Films were then placed onto a zinc selenide crystal, and the analysis was performed within the spectral region of 450- 4000 cm^{-1}. After attenuation of total reflectance and baseline correction, spectra were normalized with a limit ordinate of 1.5 absorbance units. Resulting FTIR spectra were compared in order to evaluate the effects of cellulose filling in the chitosan-based films, based on the intensity and shift of vibrational bands.

2.6. Degradation Test of the Films

The degradation test of the films in soil medium was studied. For this purpose, films were weighed individually and buried in a garden for 1–7 days. After these periods, samples were withdrawn carefully and degradation of the samples was determined visually.

2.7. Microbiological Analysis

The antimicrobial activity was characterized by disk diffusion method. Bacterial samples were collected from skins of five different people by using sterile cotton buds. Dilution was done in sterile distilled water and after that 100µl of the bacterial sample was spread onto different agar media and placed into an incubator at 37^0 C for 24 h for bacterial growth. The single bacterial colony was isolated by streaking method (Figure **2**). Single bacterial colonies were further cultured in nutrient broth medium at 37^0 C for 24 h. Then chitosan, prepared tissue paper films, and commercial facial tissue paper were cut into a disk with a diameter of 4mm and then sterilized under ultraviolet rays for 30 min. 100µl of bacterial inoculation was spread in sterile nutrient agar plate with a sterile glass rod. The disks were put on the agar plate and in absence of light, bacterial culture was carried out at 37^0C for 24 h. Then, the agar plates were examined and the zone of inhibition was calculated manually.

Figure 2: Bacterial single colony by streaking method.

2.8. Statistical Analysis

Duncan's multiple range tests were used to perform statistical analysis of all results, using PASW Statistics Base 18 software (SPSS Inc., Chicago, IL, USA). For each measurement, three samples in each replicate were tested. Differences between means comparison between each treatment were based on Duncan's multiple-range tests ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Mechanical Properties of the Films

The average tensile strength (TS) and elongation at break (EB) of the prepared cellulosic pulp based films

were found to be 3 MPa and 2.4% respectively. To determine the optimum composition of film chitosan contain in the films was varied from 5-50%. It was found that the TS and EB values of cellulose-based films were improved gradually with the addition of chitosan. For 5, 10, 20, 30 addition of chitosan in cellulosic pulp-based films, the TS and EB values of the films were found to be 6, 8, 11, 13, and 16 MPa and 3.5, 5.6, 6, 7% respectively.

The TS and EB of films containing 50% chitosan and 50% cellulosic pulp were observed to be 24 MPa and 10.8%. It is revealed that chitosan has been attributed as a reinforcing agent in cellulosic pulp-based biodegradable films and caused a significant rise in mechanical properties of cellulosic pulp-based films. Mechanical property improvement in all different studies (TS and EB) is rarely observed which is achieved in the cellulosic pulp-based films upon addition of chitosan [29]. The film containing 50% chitosan and 50% cellulosic pulp was considered as the optimum because at this proportion film showed more uniform structure and good mechanical properties. Glycerin was added to the optimized cellulosic pulp/chitosan (50:50) films to investigate the effectiveness of glycerin as a plasticizer.

The effect of glycerin on the tensile strength (TS) values of chitosan/cellulosic pulp (50:50) based films were studied and the results are presented in Figure **3**. It was found that when glycerin was added in the films, the TS values decreased simultaneously. Addition of 0.4, 0.6, 0.8, 1, and 2% glycerin in cellulose/chitosan films, the TS values decreased to 20, 18, 16, 12, and 8 MPa respectively. Glycerin can reduce intermolecular forces in cellulose or chitosan and thus decreased the strength of the films significantly.

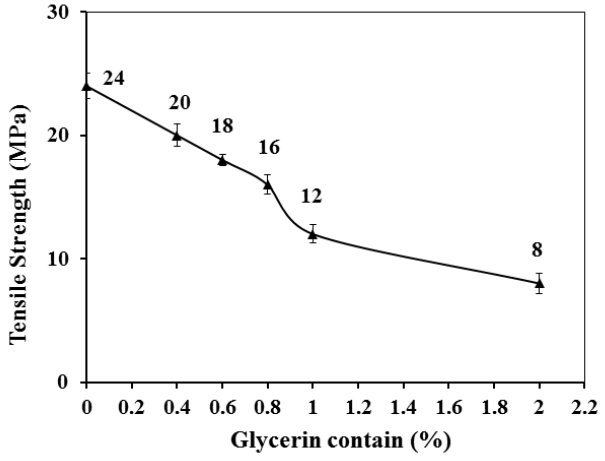

Figure 3: Effect of glycerin on the tensile strength (MPa) of 50:50 chitosan/cellulosic pulp-based film.

Figure **4** showed the effect of glycerol on the elongation at break (EB) values of the films. Loading of 0.4, 0.6, 0.8, 1, and 2 % glycerin, the EB values of the chitosan/cellulosic pulp based films was found to be 11, 11.5, 11.8, 12, and 12.5% respectively. Flexibility and plasticity of the films are essential parameters that are considered during the application of those films as facial tissue papers. These two phenomena are related to the elongation at break of the films. The results indicated that addition of 1% glycerin in 50:50 chitosan/cellulosic pulp-based films, the flexibility increased by 50%. But in the case of 2% glycerin content films, the films got wetted by absorbing moisture from the atmosphere. From this investigation, this is clear that glycerin acted as a plasticizer for chitosan/cellulosic pulp-based facial tissue paper.

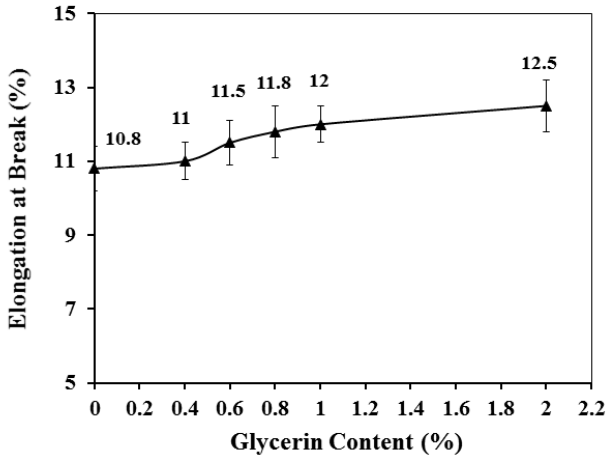

Figure 4: Effect of glycerin on the elongation at break (%) of 50:50 chitosan/cellulosic pulp-based film.

3.2. Water Uptake of the Films

In Figure **5** the water uptake of (a) chitosan, (b) cellulosic pulp/chitosan (50:50), and (c) 1% glycerin contain films are presented.

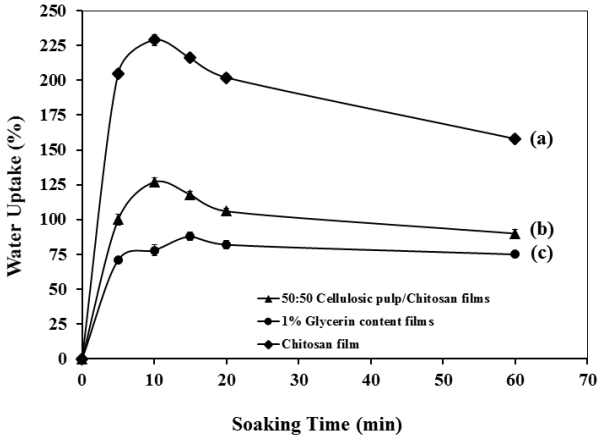

Figure 5: Water uptake (%) of (a) chitosan film, (b) 50:50 cellulosic pulp/chitosan film, and (c) 1% Glycerin contain film.

The water uptake of 50:50 chitosan/cellulosic pulp based films after 10 min immersion in water were found to be 127% and chitosan films were 229% from their respective mass. But 1% glycerin contained films absorbed 78% water after 10 min immersion in water and after 15 min it reached a plateau at 88% of water and then became almost static. After 60 min of immersion, water uptake was gradually decreased.1% glycerin contained films exhibited lower water uptake values than those of the chitosan loaded films.

3.3. Characterization by Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of cellulosic pulp film, chitosan film and 50:50 chitosan loaded cellulosic film were presented in Figure **6**. The FTIR analysis was carried out to investigate the molecular interactions in chitosan and cellulosic pulp based films. The position of the peaks of chitosan film and cellulose film spectrum is similar to those described by the different author [30-33]. Very strong absorption at 3348cm^{-1} belongs to the -OH stretch gives considerable information concerning the hydrogen bonds in cellulosic films and the bands at 2921 cm^{-1} corresponding to the C–H stretching (Figure **6a**). The absorption at 1635cm^{-1} is principally associated with the adsorbed water since the cellulose has a strong affinity for water. The weak absorptions at 1415 cm^{-1}, assigned to asymmetric -CH_2 bending vibration. The strong absorption at 1025 cm^{-1} attributes to the C–O–C stretch. The chitosan spectrum (Figure **6b**) reveals a band at 3343 cm^{-1} characteristics of the –OH and – NH_2 groups stretching vibration and at 1071 cm^{-1} for C-N stretching. The absorption peaks of the chitosan films are mainly assignable to the fingerprint peak absorption of chitosan of amide II and N–H bending vibration appeared at the 1650 cm^{-1} and 1555 cm–^{1}, respectively. Cellulose and chitosan films shared the similar functional group of hydroxyl (OH) stretching vibration, alkane C–H stretching vibration, and C–O stretching vibration from polysaccharide polymers.

Some differences were observed in the spectrum (Figure **6c**) after addition of chitosan in the cellulosic pulp-based film. When two or more substances are mixed, physical blends versus chemical interactions are reflected by changes in characteristic spectral peaks. After incorporation of chitosan in cellulose amide II peaks shifted slightly to higher frequencies and peaks became sharp. Hydroxyl groups of cellulose were shifted to 3349 cm^{-1}. This was attributed to the presence of -OH stretching from chitosan functional groups in the cellulose films. Furthermore, the strong peak absorption of -OH bending bound of water in cellulose molecules (1635 cm^{-1}) was observed to reduce and shifted to 1641 cm^{-1}. The corresponding peaks were suggested to be the overlapping peak and interaction between -OH bending of water from cellulose and chitosan [34, 35]. But in the case of glycerin containing film, all the peaks in the FTIR spectrum that are present in chitosan/cellulose films were shifted slightly. But no new peaks were observed

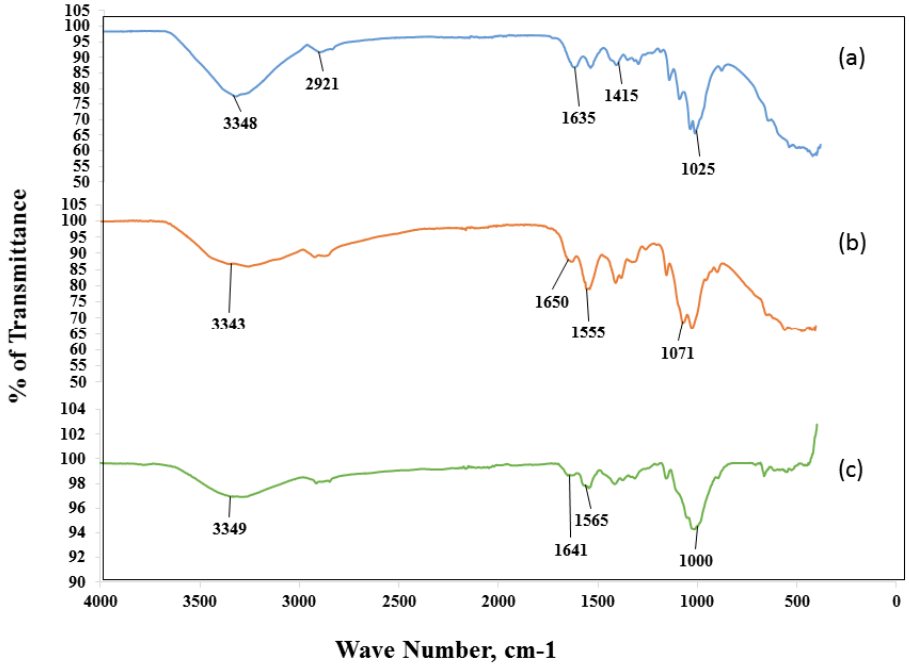

Figure 6: FT-IR spectrum of (a) cellulosic pulp film, (b) chitosan film, (c) cellulose/chitosan (50:50) based film.

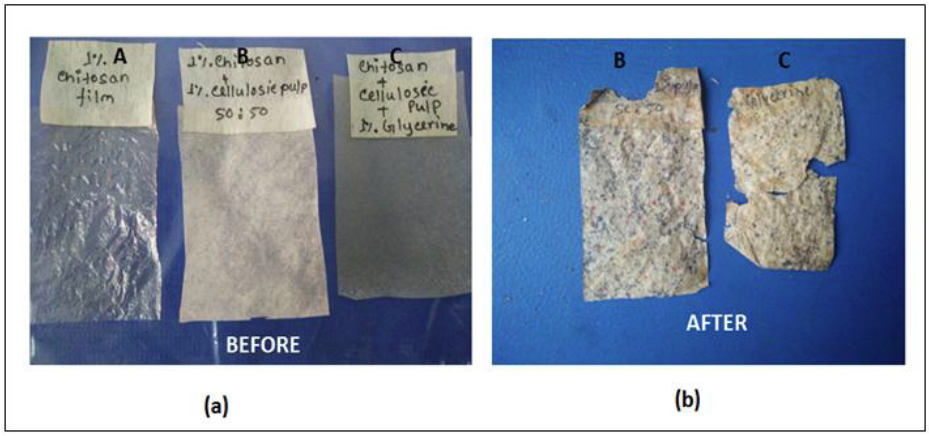

Figure 7: Digital image of degradation test (**a**) Before buried in soil (**b**) Withdrawn from soil after 7days where A- 1% chitosan film, B- 50:50 Chitosan/cellulosic pulp-based film, C- 1% glycerin contain film.

as glycerin is the very stable chemical. Glycerin was only acted as a plasticizer and did not change the structural integrity of the chitosan/cellulose films.

3.4. Biodegradation Test

The degradation test of pure chitosan film, 50/50 chitosan-cellulosic pulp based films and glycerin contained films were carried for 7 days at the ambient condition at soil medium. Figure **7** illustrated the digital image of chitosan film, chitosan/ cellulosic pulp-based film (50:50) and glycerin contained films (**a**) before buried into the soil (**b**) withdrawn from soil after 7 days. After 7 days, it was found that the chitosan-based film was completely degraded and mixed with soil and the chitosan/cellulosic pulp films and glycerin contained films were partially degraded.

3.5. Microbiological Test

Chitosan had strong antibacterial activity against bacteria and the tissue paper films showed the same. The antibacterial test was carried out with glycerin contained chitosan/cellulosic pulp based films and “no growth” was observed around the disk Figure **8**. “No growth” is referred to as the zone of inhibition. The zone of inhibition was measured in mm. This zone of inhibition was compared to a standard interpretation chart used to categorize the antibacterial activity as susceptible, intermediately susceptible and resistant. Zone of inhibition of most of the samples was 6 mm and falls in the range of 6-16 mm which is termed as “resistant” according to the zone interpretation chart. Table **1** illustrated the zone of inhibition of prepared tissue paper films in different bacterial strain collected from human skin. The bacterial concentration used for the test is much higher than human skin or air. On the other hand, commercial facial tissue paper had no antibacterial activity and bacteria grow rapidly on its matrix.

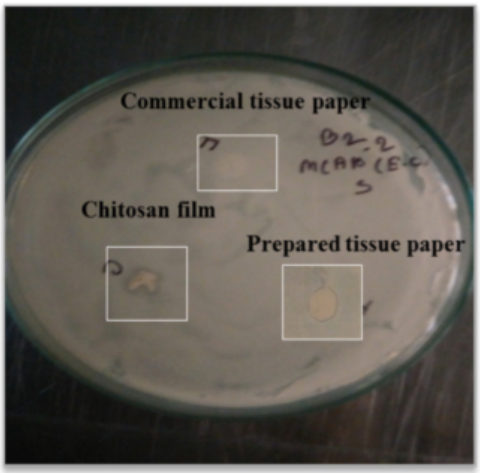

Figure 8: Antibacterial effect: zone of inhibition of commercial tissue paper, chitosan films and prepared tissue paper.

Table 1: Zone of Inhibition of Prepared Antibacterial Tissue Paper with Different Bacterial Strain

	ZONE of INHIBITION, mm
BACTERIAL STRAIN	Prepared tissue paper
1	6
2	6
3	6.5
4	6
5	6
6	6

4. CONCLUSIONS

In summary, bio-based facial tissue was successfully fabricated. Glycerin increased the

plasticity and softness of the tissue paper. Chitosan was found as a good reinforcing and antibacterial agent. Structural changes were proved due to chitosan addition in the cellulosic films. The fabricated facial tissue paper had a good appearance, softness, antibacterial and biodegradable property. The collectivity of the above investigation indicate that prepared films can be performed as an antibacterial facial tissue paper.

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