INTRODUCTION

Plants have been an important source of medicine for thousands of years. Antimicrobial activity in plant extract is attributed to the presence of phytochemicals such as phenolics, steroids, saponins, alkaloids and terpenoids. The therapeutic properties of plants have been evaluated by many studies all over the world and most of them have antimicrobial activity. Carbohydrates derived from some of the plants (such as Aloe) exhibit diverse biological activities. Aloe vera is being a natural bioactive compound, is widely studied for biomedical applications. It is a cactus-like perennial, drought resistant, succulent plant belonging to the Liliacae family of which there are over 360 known species. It has short stem with thick green leaves like structures that grow from central point. Aloe vera has modified thick fleshy leaves, it not only has cell wall carbohydrates such as cellulose and hemicelluloses but also storage carbohydrates such as acetylated mannans the polysaccharides found in the inner storage carbohydrates such as acetylated mannans, the carbohydrates such as cellulose and hemicelluloses but also succulent plant belonging to the Liliacae family of which there are over 360 known species. The green, triangle leaves are up to 0.5 m long and can weigh up to 1.5 kg when mature. They contain gel, with 98% water, it appears to help the pores of the skin open and receive moisture and nutrients of the plants. It is present in the arid regions of India and is believed to be effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation due to radiation injury, wound healing, enteric pathogens of Pseudomonas aeruginosa, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella spp., Escherichia coli and Streptococcus epidemidis and fungal pathogens of Aspergillus niger and Aspergillus flavus. The prepared pristine Pluronic and Pluronic blended Aloe vera films were examined by Fourier transform infrared (FTIR) spectroscopy, UV-Visible spectroscopy. From the antimicrobial activity data, it can be concluded that the Pluronic blended Aloe vera in aqueous media has proved excellent antimicrobial activity against selected antimicrobial pathogens. The transparency of the film and antimicrobial activity gets increased when Aloe vera is mixed with Pluronic. We believe that the eco-friendly non-toxic film might be useful for future biomedical and surface coating application.

RESEARCH ARTICLE

EFFECT OF ALOE VERA/COPOLYMER PLURONIC F127 BLENDS ON THE ANTIMICROBIAL ACTIVITY

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ABSTRACT

The synthesized hydrophobic film using Pluronic blended Aloe vera behaves as good antimicrobial material against selected bacterial pathogens of Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella spp., Escherichia coli and Streptococcus epidemidis and fungal pathogens of Aspergillus niger and Aspergillus flavus. The prepared pristine Pluronic and Pluronic blended Aloe vera films were examined by Fourier transform infrared (FTIR) spectroscopy, UV-Visible spectroscopy. From the antimicrobial activity data, it can be concluded that the Pluronic blended Aloe vera in aqueous media has proved excellent antimicrobial activity against selected antimicrobial pathogens. The transparency of the film and antimicrobial activity gets increased when Aloe vera is mixed with Pluronic. We believe that the eco-friendly non-toxic film might be useful for future biomedical and surface coating application.

INTRODUCTION

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study demonstrated that alginate/gelation hybrid hydrogel incorporated with niosomal AV could be suggested as a promising candidate for hydrogel-based wound dressing applications (10).

Polymers are substances of high molecular weight having repeating monomer units. They are widely used in pharmaceutical systems as suspending, adjuvants, adhesives, emulsifying agents and coating material for controlled and site specific drug delivery systems. Pluronic or Poloxomers are copolymers largely used for hydrogel preparation. Pluronic is a block copolymer that consists of hydrophilic polyethylene oxide (PEO) and hydrophobic polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature (11) and it is arranged in a triblock structure (EO)x-(PO)y-(EO)x, where x= 95-105 and y= 54-60 and chemical formula is [HO-(CH-CH2-O)6-(CH3-CHO)70], It has a molecular weight about 9,840-14,600 (12). PluronicF127 is proven to protect cells and promote drug delivery and it has been shown to increase wound healing rates by regulating the inflammation and growth factor synthesis (13). The unique thermo reversible and drug release characteristics of Pluronic F127 show great promise for its application as a pharmaceutical vehicle for drug delivery (14).

Cory and Ryan have described low-index porous alumina thin films deposited from aqueous solutions containing flat-Al13 clusters and the nonionic block copolymer Pluronic F127 and investigated the chemical, optical, and structural properties of the films and their formation by Fourier transform infrared spectroscopy, temperature programmed desorption, spectroscopic ellipsometry, transmission electron microscopy, atomic force microscopy, and X-ray diffraction (15). Research articles have highlighted of Aloe vera and Pluronic usage separately in biomedical applications as well as the surface coating applications. In the present research work, the effect of antimicrobial property, structural and optical properties of pristine Pluronic and Pluronic blended Aloe vera have been discussed.

Experimental Procedure

MATERIALS

The triblock copolymer Pluronic F127 [HO (C2H4O) 106 (C3H6O) 70 -(C2H4O) 106HO], Deionised water, Ethanol were purchased from Sigma Aldrich, and Aloe vera gel powder was prepared in laboratory.

Microorganisms and Medium

The prepared solutions were experimented with six bacterial strains and two fungal strains to examine the microbicidal effectualness. Disc diffusion method was used to evaluate the microbicidal activity of the prepared solutions against five gram negative bacterial strains (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Shigella spp., and Proteus vulgaris), gram positive bacterial strain (Streptococcus epidermidis) and fungal strains (Aspergillus flavur, Aspergillus niger) in Mueller-Hinton agar plates. The bacterial and fungal culture was seeded on nutrient agar and Rose Bengal plates. The wells were bored with 8mm borer in seeded agar and then 50µl of all prepared solutions were added in each well. Soon after, the plates were then kept at 10°C for 30 min. After it normalized to room temperature, the plates were incubated at 37°C for 24 hrs. After incubation period is completed, the zone of inhibition was measured and recorded. Standard antibiotic disc of Ciprofloxacin was used as the reference drug for antibacterial assay and Nystatin was used for antifungal activity study and all the data are expressed as Mean ± S.D (Standard Deviation).

Preparation of Aloe vera gel powder solution

The fresh and matured plants of Aloe vera were collected from local area of Coimbatore, Nilgiris biosphere. The leaves of Aloe vera were dissected and washed properly with cold running tap water and also washed with de ionized water to remove the external pollutants. Edges of the leaves were cut, and their internal gel was separated in a container. The separated gels were dried using Hot Air Oven at 38 degree Celsius for two days and powderized using Mortar and Pestle.

The constant appropriated weight of Aloe vera gel powder is taken and dissolved in both DI water and ethanol separately. The solution is stirred well in Ultrasonicator for one hour followed by aging for at least one day before blending with Pluronic solution and filtered using whatmann filter paper no.1. The prepared Aloe vera gel powder solution of aqueous and ethanolic extract is taken for the further process. The aqueous extract of Aloe vera gel powder solution is named as Sol A.

Synthesis of pristine Pluronic and Pluronic blended Aloe vera gel powder solution

The preparation process of pristine Pluronic F127 and Pluronic F127 blended Aloe vera solution was shown schematically in Fig 1. The solutions have been prepared using sol-gel method. Initially 1.2g of PF127 is added to 10 ml of Deionised water and the solution is kept in ultrasonicator for an hour. Thus pristine Pluronic F127-Sol B is obtained. Sol C is aqueous PF127-1.2g blended with Aloe vera gel powder aqueous solution. Sol D is ethanolic PF127-1.2g blended with Aloe vera gel powder ethanolic solution. The precursor solutions were aged for five hours at room temperature before spin coating. The Sol B and Sol C were selected for spin coating based on antimicrobial test report and deposited on microscopic glass slides of 1mm thickness using spin coating technology at the spin speed of 1500 rpm for 80 s. The as prepared films were annealed at the ramp rate of 3°C/min in the Muffle furnace.

Fig (1) Schematic representation of Pluronic F127 blended Aloe vera solution preparation
RESULTS AND DISCUSSION

The Prepared films were subjected for functional group analysis using SHIMADZU-FTIR Spectroscopy, UV-Visible transmission spectra was recorded by JASCO, and Antimicrobial activity was determined by agar well disk diffusion method.

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of pristine Pluronic and Pluronic blended Aloe vera obtained by sol-gel process and spin coating are recorded at room temperature in the wavelength range of 400-4000 cm$^{-1}$ is shown in Fig 2a and 2b. The FTIR spectra of pristine Pluronic observed at 2877 cm$^{-1}$ represents -CH$_2$ symmetric $^{16}$. The feature observed at 1743 cm$^{-1}$ in both the films might correspond to the presence of Pluronic F127. The band occurring at 1427 cm$^{-1}$ and 948 cm$^{-1}$ occurs due to the asymmetric -COO$^-$ and =C-H bending alkanes $^{17}$. The vibration band at 3741 cm$^{-1}$ related to the stretching vibration of hydroxyl group. The wave number 1350 cm$^{-1}$ corresponds to the plane O-H bond. The FTIR spectrum of Pluronic blended Aloe vera revealed the presence of flavanoids and amino acids $^{18}$. The peak at 1280 cm$^{-1}$ and 1242 cm$^{-1}$ represents -CH$_2$-twist $^{19}$. The characteristic peak at 2924 cm$^{-1}$ is due to the C-H stretching $^{20}$.

UV-Visible Analysis

Fig 3a and 3b shows the UV-Visible transmittance spectrum of Pristine Pluronic and Pluronic blended Aloe vera film in the range of 400-800 nm. The cutoff wavelength for both Pristine Pluronic and Pluronic blended Aloe vera was observed at 385 nm. The percentage of optical transmission in the entire visible region has increased to 75% as in (Fig 3b) for Pluronic blended Aloe vera while 54% was exhibited by pristine Pluronic film. This increased percentage of transparency is highly essential for Biomedical and surface coating application. This observation is in accordance with a previous study by Diaz and Reales (2017). They tested transmittance of edible films of Aloe vera and cassava starch using optical fibers trifurcated. The report showed high transparency of the edible film and it is applicable for various types of food products $^{21}$.

Photograph of transparent pristine Pluronic and Pluronic blended Aloe vera films are shown in Fig 4.
Antimicrobial Effect

The prepared four different solutions (Sol A, Sol B, Sol C and Sol D) have exhibited microbicidal activity when explored against eight infectious micro organisms on Mueller Hinton agar plates. The measured diameter of zone of inhibition is shown in Table 2. The zone of inhibition was increased with blending of Aloe vera into Pluronic. Out of these four extracts, aqueous extract of Pluronic blended Aloe vera exhibited the most potent antimicrobial effect.

In the case of gram negative bacterial strains E.coli and P.aeruginosa, the antibacterial efficacy is same for pristine Pluronic. Whereas pure Aloe vera, aqueous and ethanolic extract of Pluronic blended Aloe vera has high zone of inhibition for P.aeruginosa when compared to E.coli (22-25). Similar antimicrobial activity of Pluronic containing AgNPs was reported by Meiwan Chen (2010) for the treatment of drug-resistant bacterial strains (E.coli and P.aeruginosa) without generating further drug-resistance (26). In another study, El-Aassar (2016) reported the antimicrobial activity of Pluronic based TiO₂ Nanoparticles against (E.coli, P.aeruginosa) bacteria and opined that nanoparticles could be promising for enhanced wound dressing, skin infection treatments and tissue regeneration (27).

The inhibition zone diameters of Shigella spp., and P.vulgaris were larger than those of S.epidermidis. This implies that Pluronic had better antimicrobial effects against Gram-negative (Shigella spp., and P. vulgaris) than Gram-positive (S. epidermidis) bacteria. This may due to the single thin peptidoglycan layer of Gram-negative bacteria. The peptidoglycan is covalently attached to lipoprotein molecules which project into the outer membrane (28).

For Klebsiella pneumonia, pure Aloe vera and pristine Pluronic showed similar mild growth inhibitory effect. But the blending of Aloe vera into Pluronic increased the antibacterial activity. This observation is in accordance with a previous study by Yadav (2016). They tested antibacterial activity of Aloe vera with AgNPs against K. pneumonia, S. typhi, P. mirabilis, S. typhi, S. marcescens, E. coli, P. aeruginosa, E. faecalis, S. flexneri and S. aureus. The antibacterial report showed moderate zone of inhibition for K. pneumonia and it is applicable for the wound healing and other medical applications (29). The observed lower efficacy of the Pluronic against K.pneumonia may be due to the differences in the membrane structure. Therefore if the antibacterial effect of Pluronic is associated with the peptidoglycan layer, it will be easier and more specific to use Pluronic as an antimicrobial agent against K.pneumonia (26).

Similarly, the results of antifungal activity demonstrated that all the test samples have potent antifungal activity against Aspergillus species tested. The Pluronic blended Aloe vera against Aspergillus niger showed more effect as a susceptible inhibition species (35mm diameter). This antifungal activity of Aloe vera was confirmed by the study of Uda and Harzana shaari (2018) who elucidated the antimicrobial activities of Aloe vera, Citrus hystrix, Sabah snake grass and Zingiber officinale against Pyricularia oryzae that causes rice blast disease in Paddy plants (30).

<table>
<thead>
<tr>
<th>Micro organisms</th>
<th>Zone of Inhibition (mm)</th>
<th>Control (Ciprofloxacin)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sol A</td>
<td>Sol B</td>
</tr>
<tr>
<td>Streptococcus epidermidis</td>
<td>13.3± 0.60</td>
<td>14.02 ± 0.35</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>13.1±0.51</td>
<td>13.98 ± 0.28</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>17.0±0.33</td>
<td>17.92 ± 0.53</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>13.9±0.37</td>
<td>14.26 ± 0.53</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>15.8±0.58</td>
<td>16.96 ± 0.20</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>13.8±0.40</td>
<td>15.86 ± 0.33</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>28.0±0.22</td>
<td>31.02 ± 0.21</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>22.8±0.63</td>
<td>24.24 ± 0.32</td>
</tr>
</tbody>
</table>


CONCLUSION

Aloe vera gel is composed of several polysaccharides and it has the ability to promote wound healing as well as to treat skin burns. The pristine Pluronic and blending of Aloe vera into Pluronic were analyzed by FTIR, UV and Antimicrobial Study. The synthesized pristine Pluronic and Pluronic blended Aloe vera have proved for the antimicrobial activity. The aqueous solution of Pluronic blended Aloe vera has higher antimicrobial characteristic when compared to pure Aloe vera, pristine Pluronic and ethanolic extract of Pluronic blended Aloe vera solution. It has been concluded that, the physical and structural properties of the pristine Pluronic film were increased because of the blending of Aloe vera with copolymer Pluronic F127. We believe that the development of the eco-friendly and non-toxic film might be promising for the antimicrobial coating for future biomedical applications.

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*Declaration of conflict of Interest: None*

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