Research Article

FABRICATION AND CHARACTERIZATION OF ALOE VERA AND TEA TREE OIL LOADED ANTIMICROBIAL NANOFIBEROUS DRESSING

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ABSTRACT

Skin infections due to microbes are reported with high spread and reportedly affected one –third of the world population. Connective tissue cells produce collagen and connective tissue fibers to heal wounds of skin and white blood cells produce antibodies to fight against harmful microbes. This study presents the process of synthesis of nanofiberous dressing loaded with Aloe vera and tea tree oil. Aloe Vera and Tea tree oil contain glucomannan and terpinin-4-ol, which help in the production of collagen and kill microbes. The surface morphology of prepared dressing was studied using Scanning Electron Microscope micrographs. The average diameter of nanofibers was measured to be 137.32nm. Fourier transform infrared spectroscopy of nanofiberous dressing confirmed the loading of aloe vera and tea tree oil. Drug release profile was analyzed by UV-vis spectroscopy. It was observed that 100% drug released within 12 minutes. The antimicrobial properties of prepared fibers against Aspergillus Niger (fungi) and Escherichia coli (bacteria) were systematically evaluated. The results showed that aloe vera- tea tree oil composite fibrous dressing was found to be effective for antimicrobial activity.

Key Words: Aloe vera; Tea Tree oil; Skin infections; Nanofiberous

INTRODUCTION

Infectious diseases caused by microorganisms are leading cause of morbidity all around the world. These organisms can grow almost anywhere and multiply rapidly when exposed to moisture and conducive temperature(1). Diseases caused by fungi are severe threat to the human body (2,3) specially in immune-compromised patients. Bacterial cohabitation is a crucial problem during fungal infection, which increases inflammation and hampers therapy(4).As a result, it usually requires antibiotic and antifungal treatment(5) and increases the side effects due to dual therapies. Aloe vera and tea tree oil are traditionally known to help in healing process with the aid of their antimicrobial properties.

Aloe vera is well-known for health benefits(6). It has been utilized for quite a long time for its therapeutic, curative, and skin nourishment properties. Moreover it is biocompatible, biodegradable, and non-toxic (7, 8, 9, 10). The hydro alcoholic part of aloe-vera has antifungal action against numerous kinds of fungi [7]. Aloe-vera has antiseptic property; it has six different antiseptic elements including lupeol, salicylic acid, urea nitrogen, cinnamon acid, phenols, and sulfur. They all have antimicrobial properties against fungi, bacteria and viruses (10, 11). Due to active biological ingredients in aloe-vera integrating it into nanofiber would further improve its effectiveness(12).

Many plants from the aromatic plants family have been investigated for essential oil contents (13). Essential oils are popular these days because of their antibacterial, antifungal, antiviral, emollient, regenerative, and fragrant characteristics(14, 15). Tea tree oil (Melaleuca alternifolia) is mainly used in medicine for its antibacterial, antifungal , anti-allergic, analgesic, and antioxidant activities(16). Terpnen-4-

ol, the primary active component in tea tree oil exhibits antimicrobial properties (13). Luckily, electrospinning is a modern technique that can uphold the bioactivity of these chemical components by converting them into nanofibers (13, 15, 17).

It is a technique for producing high-quality polymer-based nanofibers, in the textile industry, wound dressings, tissue engineering, drug delivery systems and medical coating methods by using a high voltage supply(5, 14). Electrospun nanofibers not only encapsulate bioactive substances but also uphold and increase their therapeutic potential by offering a substantial surface area to volume ratio (15).

Since 1970s, beta-cyclodextrin (β-CD) water-soluble oligosaccharides have been used in formulations to increase the stability (13, 18, 19) and delivery of active chemicals in a variety of applications, including food preservation and drug delivery. β-CD is a conical shaped substance with an outer surface hydrophilic and a lipophilic cavity in the middle (18). This form allows hydrophobic molecules to be encapsulated and increase their water solubility (20). β-CD is proved to be effective for protecting essential oil molecules and functional properties by encapsulating hydrophobic essential oils inside the lipophilic cavity(13).

The study aimed to prepare antifungal and antibacterial nanofiber film, which can be used for medical applications. For this purpose, aloe vera and tea tree oil loaded Polyvanyl Alcohol (PVA) nanofibers were prepared by electrospinning process; due to the hydrophobic nature of tea tree oil, β-CD used to encapsulate tea tree oil into its lipophilic cavity. Functional group testing of aloe vera- tea tree oil nanofibers was analyzed through Fourier Transform Infrared (FTIR), and surface morphology was analyzed by Scanning Electron Microscope (SEM). The drug release profile was observed by UV-vis spectrometry. Antifungal activity against Aspergillus Niger and antibacterial activity against Escherichia coli were analyzed using the shake flask method.

MATERIALS AND METHODS

Materials

Polyvinyl alcohol(CAS-NO:9002-89-5), beta-cyclodextrin(CAS-No:7585-39-9)were purchased from Al-Beruni scientific store Hyderabad. Aloe vera gel was obtained from aloe vera leaf, tea tree essential oil was purchased online.

Method

Preparation of polyvinyl alcohol nanofibers

Polyvinyl alcohol nanofibers were prepared through electrospinning. To prepare pure PVA nanofibers sheet 0.3gm of PVA (10% of total weight) was dissolved into the 2.7ml of distilled water. The solution was stirred on a magnetic stirrer for 3hours. Once the solution became homogenous, the polymer solution was filled into the syringe. A high voltage (14 kV) was applied to the solution containing syringe, due to electric field charged fibers spurt out of syringe. The fibers were collected on aluminum foil at a stationary collector 7inches apart from a polymer solution syringe.

Preparation of aloe vera loaded polyvinyl alcohol nanofibers

To prepare the aloe vera loaded PVA nano fibers sheet same procedure was followed by setting concentration of solution as 8% PVA, 7% AV and 85% solvent distilled water. After preparing a homogenous solution, the fibers were prepared through electrospinning.

Preparation of tea tree oil loaded polyvinyl alcohol nanofibers

To prepare tea tree oil loaded PVA nanofibers, the wall material β-CD used to load essential oil due to its hydrophobic nature. The solution was prepared by taking amounts as 8% PVA, 3% tea tree oil, 3%-

ß-CD and 86% distilled water. Once the solution became homogenous then the fibers were prepared by electrospinning.

Preparation of aloe vera- tea tree oil loaded polyvinyl alcohol nanofibers

To prepare nanofibers, PVA (8%of total weight) was dissolved into distilled water (76% of total weight), aloe-vera gel(10% of total weight) was added and stirred on a magnetic stirrer for 30mins, then 3% of β-CD and 3% of tea tree essential oil were added into the solution. The solution were stirred 30 min further to form homogenous solution, following the protocol of electrospinning nanofibers were prepared at 18kV and collected on grounded aluminum foil plate. Once the process was completed, the nanofiber sheet formed and was kept for drying at room temperature in the drying room. The dried fibers sheets were kept in a zipper bag till further analysis. Figure 1 presents preparation process.

Figure 1. Schematic presentation of aloe vera- tea tree oil loaded polyvinyl alcohol nanofiber preparation

Antibacterial and Antifungal testing

Antimicrobial activity aloe vera nanofibers were investigated against Escherichia coli (Mac Conkey's ager) bacteria and antifungal activity against Asparagus Niger (potato dextrose agar). Common shake flask protocol was used to examine antimicrobial action(1, 21) of aloe vera nanofibers. Asparagus Niger (potato dextrose agar) and Escherichia coli (Mac-Conkey ager) were cultured in nutrient broth for 19 hours at 37 °C (98.6° F), and the broth dilution method was used to examine the prepared solution. Bacterial cells reached to 1 × 10^9cfu/ml. It was adjusted at 3 × 10^5 CFU/ml to 4 ×10^3 CFU/ml by serial dilution with 0.03 mol/L Phosphate Buffer Saline (PBS). After getting the desired growth of bacterial and fungal cells, 50mg of aloe vera-tea tree oil fibers (sample) were added to the conical flask holding 65mL of 0.3mM PBS solution and 5mL of the prepared solution containing bacterial/fungal growth. The solution was then shaken for 18 hours at 37° C on shaking machine. Then the procedure was repeated three times for serial dilution by mixing 1mL of solution from the flask and 9mL of 0.3mM PBS. Finally, 1ml of the solution of bacterial growth with different concentrations was taken and placed onto an agar plate. After 24 h of incubation at 37.8 °C, microbial colonies formed were visually counted on the agar

plates (Figure 2). Furthermore, based on acquired results, the antibacterial and antifungal activities were determined and bacterial /fungal reduction was calculated using the equation below:

$$R = \frac{(B - A)}{B} \times 100$$

Here R is the reduction percentage, B and A is the number of microbe's colonies before and after being treated with aloe vera- tea tree oil nanofibers.

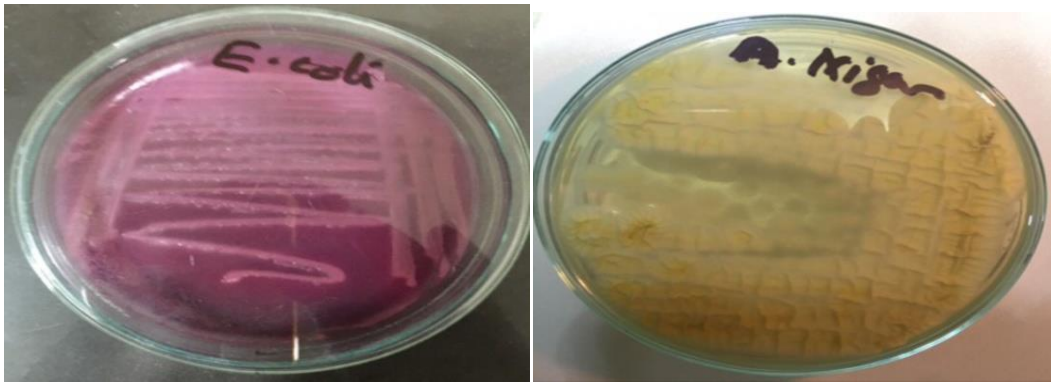

Figure 2 Fresh Culture of Escherichia coli and Fresh Culture of Aspergillus Niger

Characterization

Surface morphology of all nanofibers was analyzed by using SEM. Average diameter of nanofibers was measured by taking 80 different measurements using image J software and represented graphically. Functional groups of materials were analyzed through FTIR (FT-IR spectrometer, PerkinElmer). Drug release behavior was analyzed through UV-vis spectroscopy, and samples were analyzed through (UV/VIS Lambda 365, PerkinElmer).

RESULTS

Chemical Analysis

FTIR spectrum of neat polyvinyl alcohol nanofibers shown in Figure 3 has the characteristic bands at 3419cm-1, 2932cm-1, 1706cm-1, 1419cm-1, 1257cm-1, 883cm-1 is related to the OH stretch, CH stretch, C=O stretch, OH bending ,CO stretch CH bending. FTIR spectrum of aloe vera loaded polyvinyl alcohol nanofibers shown in Figure 4 has the characteristic bands at 3417cm-1, 2929cm-1, 1769cm-1 ,1644cm-1, 1425cm-1, 1250cm-1 ,1064cm-1, 87cm-1 is related to the OH, CH, C=O, C=N, CO, SO, stretching and OH, CH bending. The band of aloe vera appeared at 1644 and1064 in the loaded sheet, which expresses the successful loading of aloe vera. FTIR spectrum of tea tree oil/ β-CD loaded polyvinyl alcohol nanofibers shown in Figure 5 has characteristic peaks at3433cm-1, 2922cm-1, 1737cm-1,1631cm-1, 1428cm-1, 1397cm-1, 1257cm-1, 1100cm-1, 1031cm-1, 875.2 are related to OH, CH, C=O, C=C stretching, O-H bending, C=C, C-O, C-O, CO-O-CO stretching, CH bending and has vibrations between 620-1000 cm-1due to C-O-C aromatic rings of tea tree oil. Peaks at1100 cm-1 and 1397 cm-1 are due to functional group of tea tree oil and peaks. FTIR spectrum of aloe vera- tea tree oil β-CD loaded polyvinyl nanofibers shown in figure 6 has characteristic band as OH stretch-3426cm-1, CH stretch – 2928cm-1,C=O stretch-1742cm-1,C=N stretch- 1646cm-1,O-H bending- 1436cm-1,C=C stretch- 1388cm-1,C-O stretch- 1272cm-

1,C-O stretch- 1101cm-1,S=O stretch- 1060cm-1,CO-O-CO stretch- 970cm-1,CH bending-879and has vibrations between 620-1000cm-1 due to C-O-C aromatic rings of tea tree oil. This shows the proper encapsulation of aloe vera and tea tree oil using β-CD as wall material for tea tree oil in PVA.

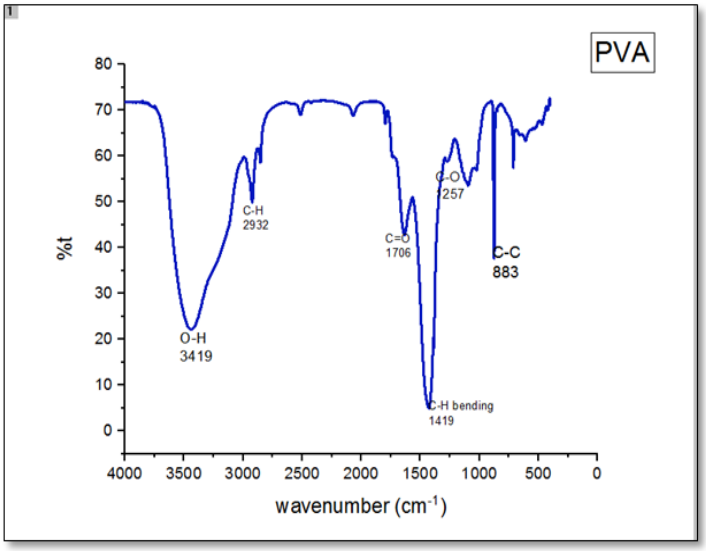

Figure 3. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)

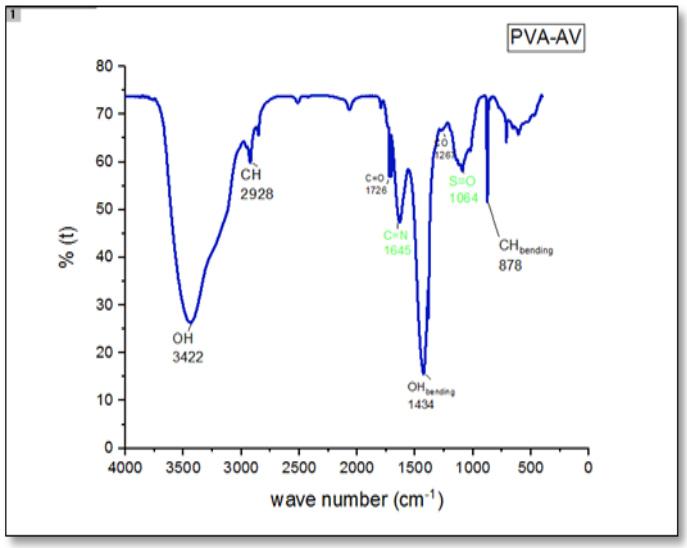

Figure 4. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)-aloe vera (AV)

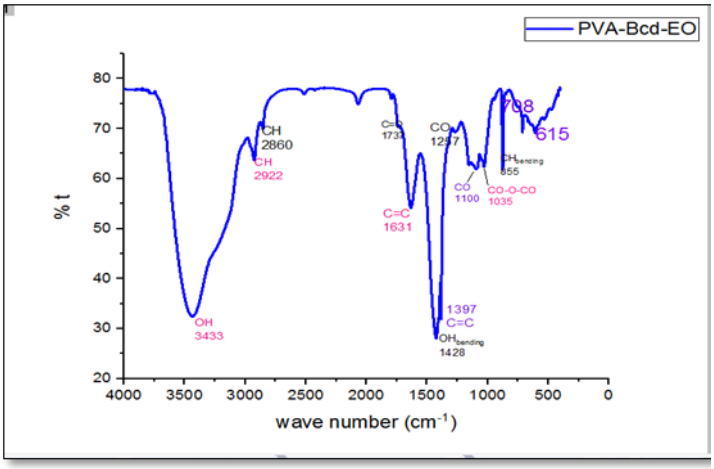

Figure 5. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol (PVA)-β-CD-Tea tree oil(TTO)

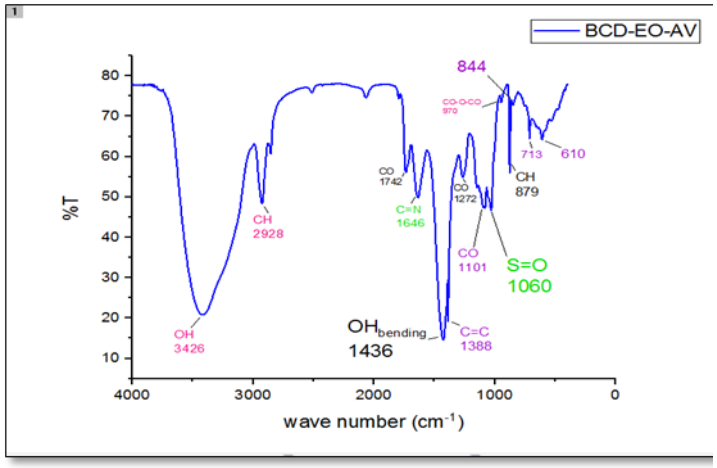

Figure 6. Fourier transform infrared spectroscopy spectrum of Polyvinyl Alcohol(PVA)-Tea Tree Oil(TTO)-Aloe Vera (AV)

Surface morphology of nanofibers

Surface morphology of all these fibers was studied from SEM images. It was observed that fibers were smooth and the average diameter of nanofiber was 200.67nm, 113.53 nm, 272nm, and 137.32nm for aloe vera- polyvinyl alcohol, tea tree oil- polyvinyl alcohol and aloe vera- tea tree- polyvinyl alcohol fibers respectively shown in Figure 7. The average diameter of fibers was calculated by taking 80 distinct measurements from SEM images by image j software.

Release behavior of nanofiber

Drug release profile of aloe vera- tea tree oil nanofibers was observed by UV–vis spectrophotometer. PBS was prepared by dissolving NaCl, KCl, NA2HPO4, KH2PO4 (8gm, 0.2gm, 1.44gm, 0.24gm respectively) in 1-liter of distilled water. Aloe vera- tea tree oil loaded polyvinyl alcohol nanofibers were peeled off from aluminum foil weighted carefully and immersed into PBS solution and stirred slowly. The solution was analyzed under UV-vis spectrometer under multiple time spans. Figure 8 shows the UV-vis spectrum of drug released in PBS solution, it was observed that the drug was completely released within 10 minutes.

Antimicrobial activity

The antimicrobial properties of aloe vera- tea tree oil nanofibers against Escherichia coli (bacteria) and Aspergillus Niger (fungi) were systematically evaluated. Neat polyvinyl alcohol electrospun nanofibers were examined as a control experiment. The bacterial and fungal colonies were incubated in growing medium with aloe vera- tea tree oil nanofibers and antibacterial property were examined by standard shake flask protocol. Aloe vera- tea tree oil nanofibers have played a vital role for antimicrobial activity. Total density of microbial colonies for neat polyvinyl alcohol was high (Figure 9, Figure 10 (A, B)), and has nearly zero reduction in colonies. The highest reduction was observed in bacterial and fungal colonies treated with aloe vera- tea tree nanofibers. The results shows that aloe vera- tea tree oil has 99.9% reduction rate and calculated in Figure 9.These results suggest that neat polyvinyl alcohol did not show any bacterial reduction and addition of aloe vera- tea tree oil fibers were the reason behind the reduction of fungal and bacterial colonies.

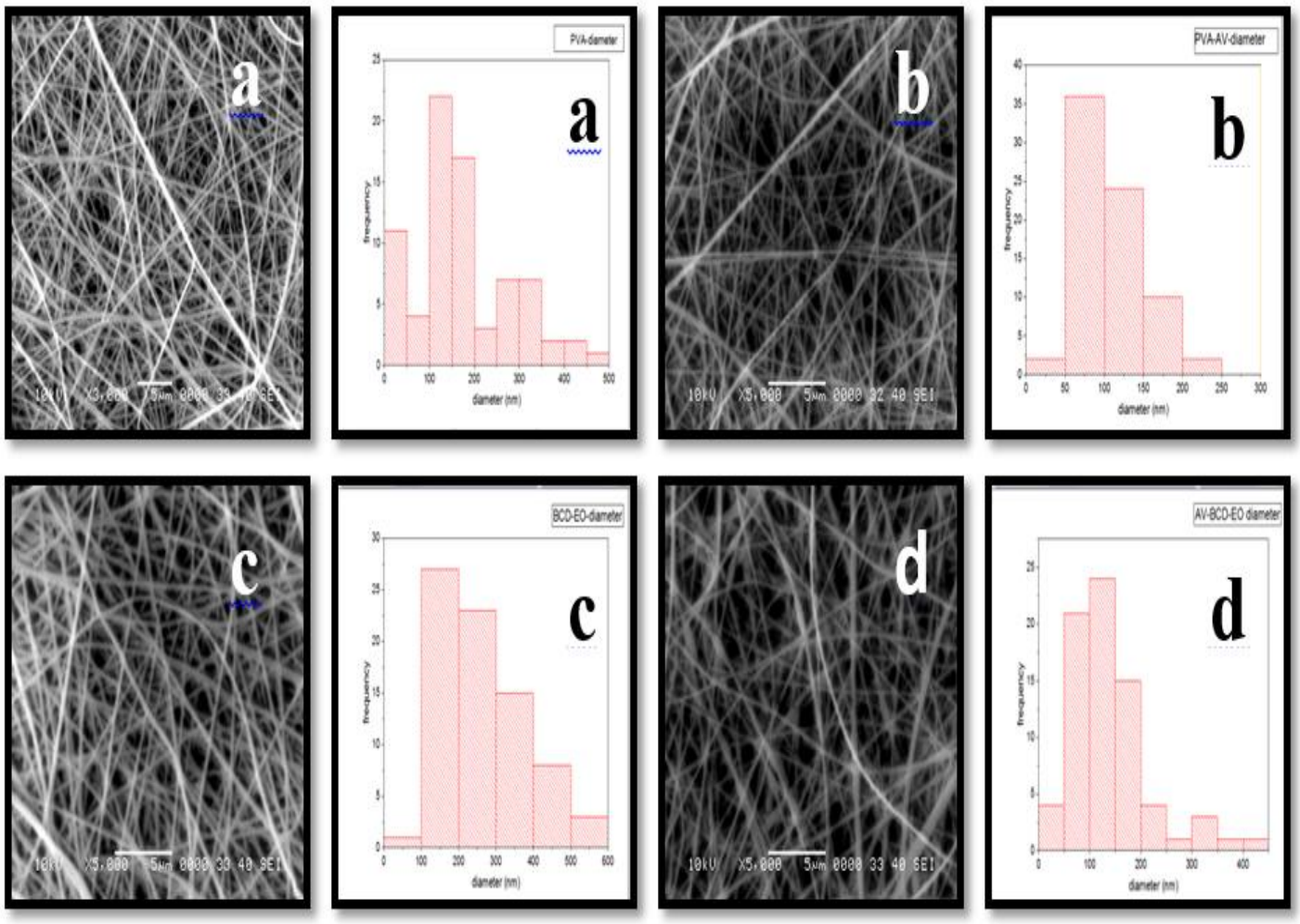

Figure 7. Scanning Electron Microscopics images and graphical representation of (a) Polyvinyl alcohol nanpfiber, (b) Polyvinyl alcohol- Aloe vera nanofiber (c)Polyvinyll alcohol-β-CD-Tea Tree Oil, (d)Polyvinyl Alcohol –β-CD- Tea Tree Oil nanofibers.

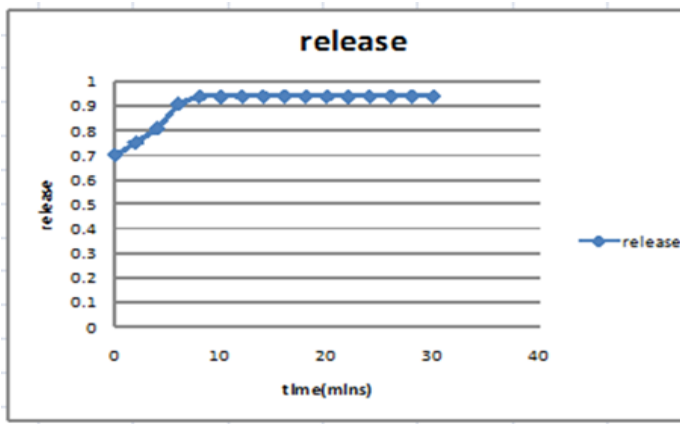

Figure 8. Drug release behavior of nanofibers

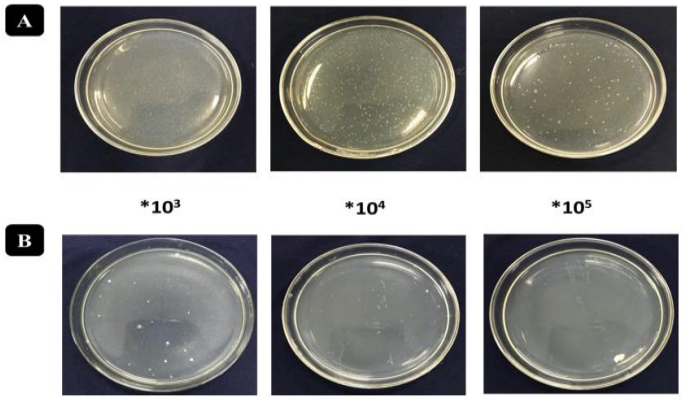

Figure 9. Aspergillus Niger colonies (A) treated with neat polyvinyl alcohol nanofibers (B) treated with aloe vera- tea tree nanofiber

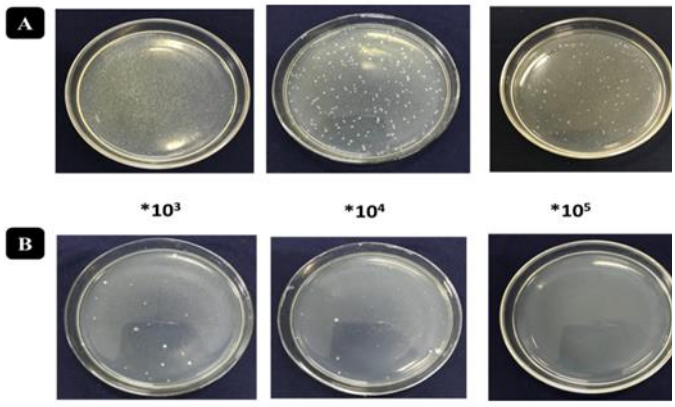

Figure 10. Escherichia coli colonies (A) treated with neat Polyvinyl alcohol nanofibers (B) treated with aloe vera- tea tree oil nanofibers

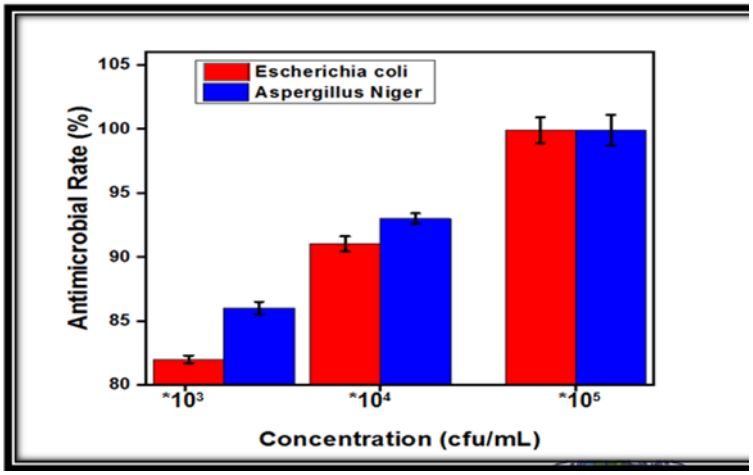

Figure 11. Bacterial and Fungal growth reduction rate

Table 1. Bacterial and Fungal reduction rate with different nanofibers

Sample	Antibacterial percentage		Antifungal percentage	
Nanofibers	**Polyvinyl Alcohol**	**Aloe Vera-Tea Tree Oil**	**Polyvinyl Alcohol**	**Aloe vera- Tea Tree Oil**
1(*10 3)	0	82	0	86
2(*10 4)	0	91	0	93
3(*10 5)	5	99.9	5	99.9

DISCUSSION

Fungal skin infections pose a severe threat to human health. Fewer standard therapies, a dearth of antimycotics for addressing fungal contamination, and the impediment of bacterial cohabitation in the treatment all decreases wound healing effectiveness resulting in raising cost of treatment and morbidity. In addition to these conventional treatments, which mostly consist of gauze dressings, limited ability to load medicament of choice while nanofibers are ideal for use in wound dressings because they are light weight, have tiny diameter, controlled porosity structures, and cover a larger surface area with a small amount of medicine or substance as compared to conventional fibrous dressings as drug carriers. In our study, we prepared antimicrobial nanofibrous scaffolds to treat superficial skin infections caused by pathogenic fungi and bacteria by utilizing the antifungal and antibacterial potency of tea tree oil and aloe vera brought on by phytochemicals. Since Aspergillus Niger and Escherichia coli cause widespread skin infections, the effectiveness of the prepared nanofiberous scaffold offers new possibilities for antifungal and antibacterial wound dressing that will help treat and eradicate these infections. Ogidi, 2021 in this study found phytochemicals and bioactive components in turmeric essential oil and aloe vera gel. It also evaluated the antifungal efficacy and synergistic effects of antifungal creams, turmeric essential oil and aloe vera gel using the agar diffusion disc method. The study came to the conclusion that unique product made with bioactive ingredients from plants and commercially available antifungal

creams will be a possible alternative therapy for treating dermatological infections (22). Several similar studies were conducted in the study in 2017 by Orchard, van Vuuren and reported that for dermatological infections the development of antimicrobial medicines the accessibility of essential oils or plant extracts are an optional therapy. Essential oils aromatic compounds and plant extracts in the formulation of topical antifungal creams to obtain best antifungal efficacy (23). According to Yue et al, tea tree oil compounds were created using the co-precipitation method, and their antifungal effectiveness against botrytis was tuned. It was also found that various types of wall materials affect tea tree oils volatility and oxidation, and that tea tree oil complexes also have antifungal properties. When compared to Fluconazole, itraconazole, and voriconazole, essential oils of oregano, pine, thymus vulgaris (thyme red), melaleuca alternifolia, and their components showed antifungal effectiveness against Cryptococcus neoformans strains. Potential synergistic interactions between two or more antibiotics can decrease the emergence of resistant mutations, boost their potency against pathogens, and function as an effective substitute for traditional therapy for a number of fungal infections. Nazzaro, Fratianni, Coppola, and Feo claimed that elements in essential oils act as antifungal agents (fungistatic and fungicidal) against fungi and disrupt the structure and function of fungal cell membranes by inhibiting extracellular/intracellular enzymes and reducing nuclear material or protein production (24). Khan, et al reported that due to their high surface-to-volume ratio, durable elasticity, and particular strength, nanofibers are essential in the creation of textiles and biomedical products. Due to its smaller diameter, less weight, and higher surface to volume ratio, nanofiber is significantly more important than conventional dressing (25). According to the above mentioned studies aloe vera and tea tree oil have antifungal and anti bacterial properties. In our study tea tree oil and aloe vera are used as therapeutic agents to fabricate effective dressing for skin infections caused by microorganisms. Antifungal activity against Aspergillus Niger and antibacterial activity against Escherichia coli of prepared nanofiberous scaffold were analyzed. Nanofiberous scaffold containing tea tree oil and aloe-vera have shown 99.9% efficiency against bacterial and fungal culture, which is remarkable and will serve great in microorganism's growth reduction. Further large scale clinical trials will be required to prove its effecacy in human subjects.

CONCLUSION

In this study, synthesis route of aloe vera- tea tree oil fibers via electrospinning has been introduced in order to report antibacterial and antifungal properties. The average diameter of nanofibers measured was 137.32nm. Fourier transform infrared spectroscopy of nanofiberous dressing confirmed the loading of aloe vera and tea tree oil.The current study confirmed the antimicrobial effect of aloe vera- tea tree oil nanofibers and it can be used as promising antibacterial and antifungal material for medical application. Large scale clinical trials are recommended to produce high level evidence for use of nanofibers in clinical practice.

ETHICAL CONSIDERATION: The study was approved by local ethical committee, there was no ethical concern identified in this study.
FUNDING SOURCE: This study required no additional funding
CONFLICT OF INTEREST: Authors declare no conflict of Interest

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