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Valorisation of Wool Waste and Chicken Feathers for Medical Textile Applications

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Abstract: ***Waste valorisation is the key to waste minimization. Chicken feathers and wool fabric waste are rich in protein content. Keratin forms a major part of these two materials. However, these keratin rich material are often discarded and finally end up as waste in landfills or incinerated. This research aims to upcycle woolen waste and chicken feathers by selectively extracting keratin from them. This study reports the development of a wound-healing nanofibre patch derived from non-conventional keratin sources like waste wool and chicken feathers. It aims to repurpose these abundant and underutilised materials, taking advantage of their high crude protein content. A three-step process for developing wound healing material is reported: cleaning waste wool and chicken feathers and extracting keratin to make electrospun nanofibre patch. The electrospun keratin patch is incorporated with honey, a natural antiseptic agent for producing desired wound healing properties. The extraction of keratin is initially tested qualitatively using Biuret test. The Scanning Electron Microscopy (SEM) images confirm the successful electrospinning of keratin nanofibres, demonstrating a well-defined and uniform fibrous surface morphology. The FT-IR spectrum confirms the presence of functional groups associated with keratin. Furthermore, the antimicrobial study shows promising results, indicating that the protein-based nanofiber patch supports cell growth activity. These findings suggest that the keratin-based nanofiber patch derived from waste wool and chicken feathers has the potential to facilitate the regeneration of damaged tissue and can aid in the wound-healing process. The findings of these study confirms possible extraction of keratin from wool waste and chicken feathers and its application in medical textile applications.***

Keywords: Antiseptic, Electrospinning, Keratin, Protein-extraction, Wound healing

I. INTRODUCTION

Wound healing is a complex and intricate process that involves the regeneration of damaged tissue to restore the skin's barrier function and protect the body from the external environment [1][23]. A wound is characterised as a disruption in the continuity of the epidermal cell lining of the skin resulting from physical or thermal damage [2].

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Acute wounds are sudden injuries to the skin caused by accidents or surgical procedures [3]. The expected healing time for acute wounds is around 6 to 8 weeks, which can vary based on factors such as the size, depth, and extent of the injury and the presence of cuts, ulcers, or burns. Numerous wound healing products are available to aid healing [4]. Among these products, electrospun nanofiber patches have emerged as a novel approach to wound dressing [5]. These patches are composed of fine fibres with a high surface area-to-volume ratio, allowing for efficient absorption of wound exudate and delivery of therapeutic agents [6]. They provide a protective barrier against bacteria and contaminants while promoting a moist environment that facilitates wound healing. Research efforts have focused on developing innovative wound-healing products to enhance healing [7].

One promising approach involves using keratin protein to provide a source of keratinocytes, which are crucial for initiating the healing cascade [8][26][27]. Keratinocytes play a vital role in reepithelialisation, the process by which the epidermis regenerates to close the wound [9]. By incorporating keratin protein into wound dressings or topical formulations, the delivery of these essential cells can be optimised, potentially accelerating the healing process [10]. In recent days due to its excellent biocompatible and biodegradable qualities as well as its medical applications, keratin has recently grown to be quite popular in several sectors like poultry, textiles, agriculture, cosmetics, and pharmaceuticals [11]. In addition to being utilised to create fibrous composites, keratin is a versatile biopolymer that can be transformed into gels, films, nanoparticles, and microparticles with the right alterations [12].

The characteristic feature of keratin is its high cysteine content compared to other fibrous proteins like elastin and collagen. Many studies were conducted on extraction, purification, and its application. The keratin in chicken feathers, beaks, claws, nails, horns, hooves, human hair, and toes has been extracted and evaluated for various applications [13]. Wool is a substantial additional source of keratin [14].

Wool that contains up to 95% keratin is considered a pure source of intermediate filament proteins. It has gained significance in the biomedical and cosmetic industries [15]. Wool and human hair-derived keratin biomaterials have cell-binding motifs with haemostatic and potential cell binding characteristics [16]. In this study, a medical patch is prepared from keratin derived from waste sources like chicken feathers and wool waste.

II. EXPERIMENTAL

A. Materials

Waste wool and chicken feathers were selected as the two main raw material sources for the extraction of keratin. For this study, waste wool was collected from the Wool Research Association (WRA) and chicken feathers were taken from a local poultry shop. Chemicals like sodium sulfide, sodium hydroxide, acetic acid, ammonium sulfate and polyvinyl alcohol (PVA) were purchased from SDFCL Chemicals Mumbai. Honey from Phondaghat Pharmacy was purchased from a local Ayurvedic medicinal shop. ESPIN NANO PVT LTD made electrospinning machine was used for spinning fibres from polymer solution [24][25].

B. Methods

a. Pretreatment of Waste Wool and Chicken Feathers

The soiled chicken feathers and waste wool have certain acquired impurities. To get rid of dirt, oil, grease, moisture and other impurities present in the procured chicken feathers and waste wool, both these raw materials were initially pretreated. Wool picking was performed to separate fibres from the chunk of wool. It was further soaked in ethanol for 24 hours to remove oily matter and then dried in the oven at 50^0C for 1 day. Similarly, chicken feathers were washed using 3g/l non-ionic wetting agent. The washed feathers were oven-dried for 1 day at 60°C. Out of this, 150g of oven-dried feathers devoid of moisture were disinfected in an autoclave at 121°C at 15 psi pressure. The feathers were also treated in a soxchlet apparatus to remove oily matter with petroleum ether.

b. Keratin Extraction from Waste Wool

The pretreated waste wool was weighed accurately and dissolved in 3.5% Sodium Hydroxide (NaOH). After dissolution, the solution was collected and precipitated with acetic acid until a white residue was obtained. This white residue was collected and dried to make powder. The obtained white powder was dissolved in the 2% NaOH for dialysis. Dialysis was carried out in cellulose membrane tubes. Optimisation for the dialysis process was done by varying the concentration of sodium hydroxide from 3% to 5.5% with a fixed time of 3 hours and a temperature of 30°C.

c. Keratin Extraction from Chicken Feathers

The pretreated chicken feathers were weighed and dissolved in 0.5 M sodium sulfide. The dissolution parameters were varied as follows. The sodium sulfide concentration was varied from 0.5M to 0.8M by keeping the dissolution time 7 hours for 30^0C, respectively. This solution was reprecipitated by using ammonium sulfate (70 g/l). The obtained solid precipitate residue was collected and taken for dialysis. The sample residue was again dissolved into 2% sodium hydroxide and kept for the dialysis for 5 days in cellulose membrane dialysis tubes. Water was changed after every 2 hours in the dialysis tubes.

d. Electrospinning of PVA and Keratin Copolymer Nanofibre Sheet.

In the electrospinning technique, a polymer solution is prepared and subjected to high voltage to create fine fibres. In this study, the polymer solution was prepared using a 10% Polyvinyl Alcohol (PVA) to form a copolymer with extracted keratin. To ensure optimal electrospinning conditions, several parameters were controlled and optimised. The flow rate of the polymer solution was maintained at 0.75ml/hr, providing a consistent and controlled release of the solution from the needle. The distance between the needle and the collector drum, crucial for fibre formation, was set at 10cm. Different combinations of protein-to-polymer ratios, such as 50/50, 60/40, and 70/30, were tested to determine their impact on the electro-spun fibers' characteristics. The polymer solution for electrospinning consisted of 15 ml (dialysed)Keratin solution, 10% PVA and Honey 5%. The parameters of the electrospinning machine were set as follows: Voltage: 20 KV, Drum speed: 250 rpm, Needle to collector distance: 10 cm, Time: 5 to 6 hours.

III. RESULTS

A. Qualitative Test

The Biuret test is a qualitative chemical test to identify the presence of peptide linkages in a given solution [17]. It relies on the reaction between copper ions and peptide bonds, forming a violet-coloured complex. Keratin protein is mainly comprised of peptide linkages [18]. Biuret reagent (1% copper sulfate + 1% potassium hydroxide solution in a 1:1 ratio) was added to both (chicken feathers and waste wool) extracted keratin samples. After 10 mins, both the keratin test solutions showed a violet colour as indicated in Fig.1(a) and Fig.1(b). Thus indicating the presence of peptide linkage in the extracted keratin samples.

B. The Biuret Test

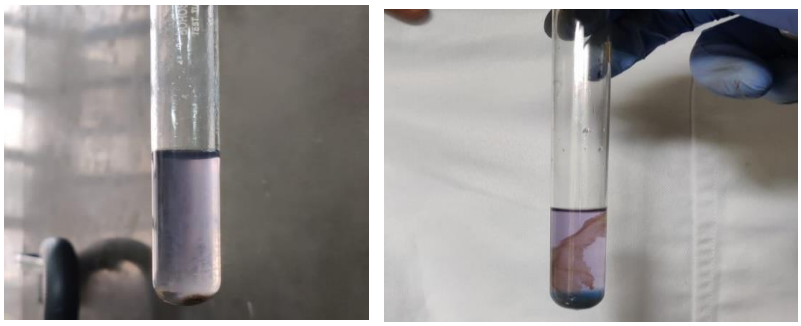

Figure1(a) - Keratin Extracted from Waste Wool 1(b)- Keratin Extracted from Chicken Feathers

C. Extraction Yield

The percentage yield evaluation is a quantitative analysis method used to determine the efficiency of a chemical reaction or extraction process.

In the case of extracting keratin from wool and chicken feathers, the per cent yield test can help evaluate the effectiveness of the extraction process and determine the amount of keratin obtained relative to the theoretical maximum. The process of extracting keratin from wool and chicken feathers involves several steps. Firstly, the raw materials (wool and feathers) are thoroughly cleaned to remove impurities or contaminants. Then, the samples are treated with a suitable solvent, typically an alkaline solution, to break down the protein structure and release the keratin. The resulting solution is filtered to separate the keratin from the residual materials. Finally, the keratin is collected and dried to obtain the final product.

To evaluate the percent yield of extracted keratin after this multistep process, the following formula was used:

Percent Yield = Y% = $(m_0 - m_{dry})/m_0 \times 100$

Where, m_0 : initial weight of material before treatment,

m_{dry} : final weight of material after treatment

D. Keratin Extraction from Waste Wool

Initially, the extraction trials were conducted by varying the duration of the experiment, specifically from 2 hours to 3 hours. During these experiments, the data obtained was used to create a graph representing the relationship between the concentration of NaOH and the corresponding percentage yield.

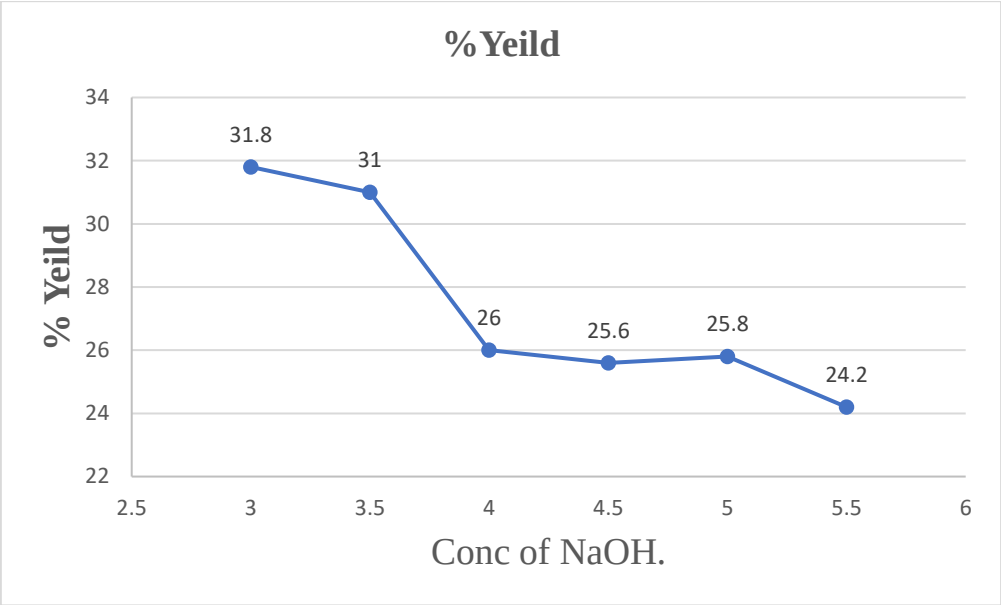

Figure 2- Graph of Keratin Extraction by Wool with 2 hrs Time

As seen in Figure 2, the maximum yield was achieved when NaOH concentration was 3%. This suggests that utilising a 3% concentration of NaOH, coupled with a duration of 2 hours, would result in the highest achievable yields of keratin from waste wool. In another set of trials, the duration of the experiment was kept constant at 3 hours. Various concentrations of NaOH were tested, and the corresponding percentage yields were recorded. These results were then used to create a graph, visually representing the relationship between NaOH concentration and the resulting yield. The graph provided in Figure 3 illustrates the observed outcomes of these experiments.

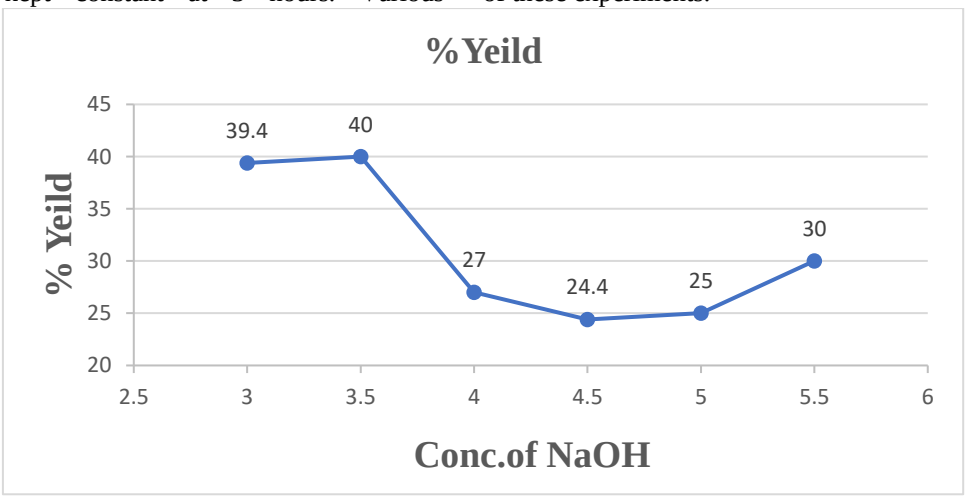

Figure 3 - Graph of Keratin Extraction With by Wool 3 hrs Time

As the concentration of sodium hydroxide increased, the extraction yield decreased. Remarkably, the maximum yield was achieved at a NaOH concentration of 3.5%. This suggests that utilising 3.5% concentration of NaOH, coupled with a duration of 3 hours, would result in the highest achievable yield.

The trials involved altering the duration of the experiment, ranging from 7 to 8 hours. Throughout these experiments, data was collected to construct a graph illustrating the connection between the concentration of Na2S and the resulting percentage yield. The following graph portrays the observed results.

E. Keratin extraction by Chicken Feathers

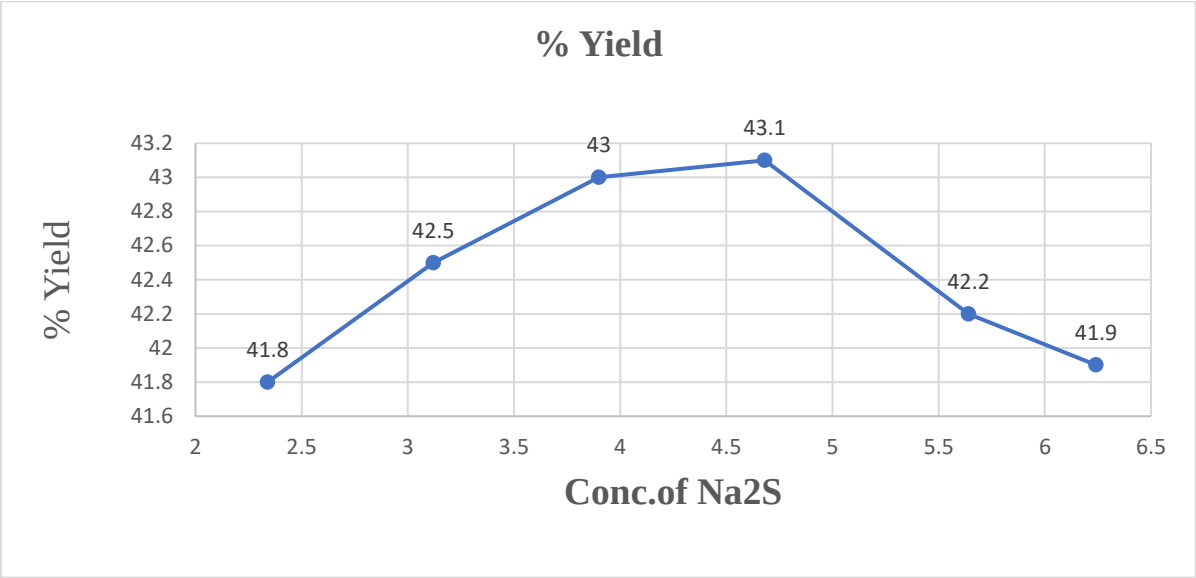

Figure 4 - Percentage Yield Graph of Keratin by Chicken Feathers at 6 hrs

The graph shown in Figure 4 indicates that as the concentration of sodium sulfide increases, the extraction yield initially increases, *and* then it starts to decrease again. Interestingly, the maximum yield is achieved at a Na2S concentration of 3.90%. Based on this information, it can be inferred that utilising a concentration of 3.90% Na2S, along with a duration of 7 hours, would likely result in the highest achievable yields in this particular extraction process.

Another set of trials was conducted to extract keratin from chicken feathers. The experiment had a constant duration of 6 hours. Different concentrations of Na2S were tested during these trials, and the corresponding percentage yields were measured and recorded. Based on these results, a graph was plotted to visually represent the relationship between the concentration of Na2S and the resulting yield. The graphs are shown below, depicting the observed outcomes of these experiments.

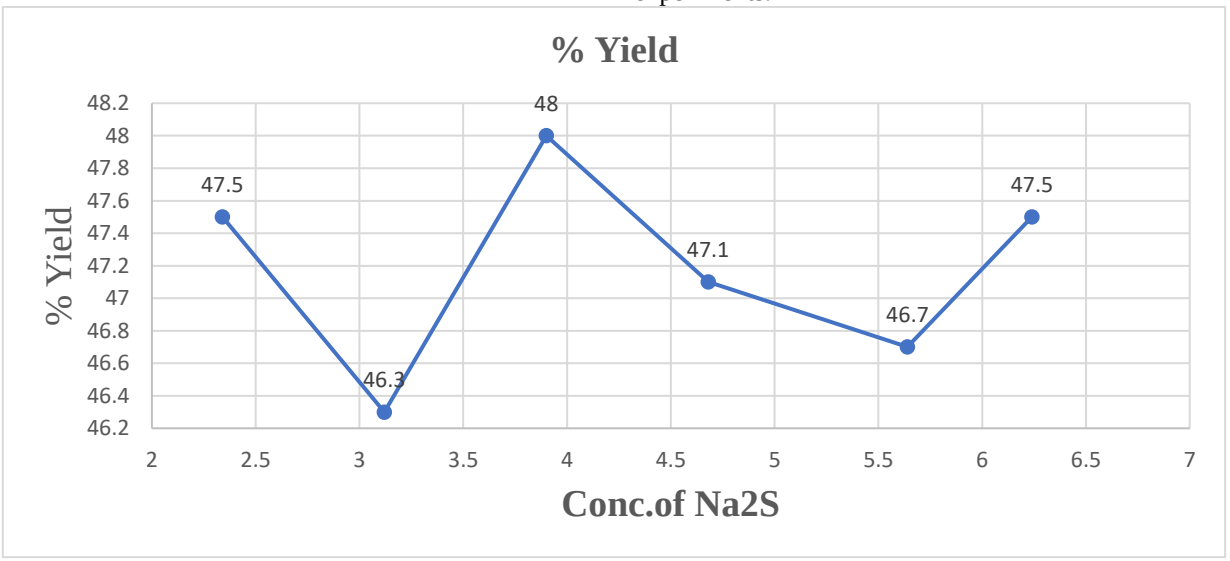

Figure 5 – Percentage Yield Graph of Keratin Extraction from Chicken Feathers after7 hrs

As seen in Figure 5, as the sodium sulfide concentration increased, the extraction yield decreased and then increased. The maximum yield was achieved at a Na2S concentration of 3.90%. This suggests that utilising a 3.90% concentration of Na2S, coupled with a duration of 7 hours, would result in the highest achievable yields.

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FTIR Analysis

FTIR spectra were analysed for samples of the extracted keratin from wool and chicken feathers, electrospun nanofibre patch using Shimadzu IRSPIRIT Fourier Transform Infrared spectrophotometer (FTIR). Figure 6 shows the infrared spectrum of extracted keratin from waste wool.

FTIR for Keratin Extracted from Waste Wool

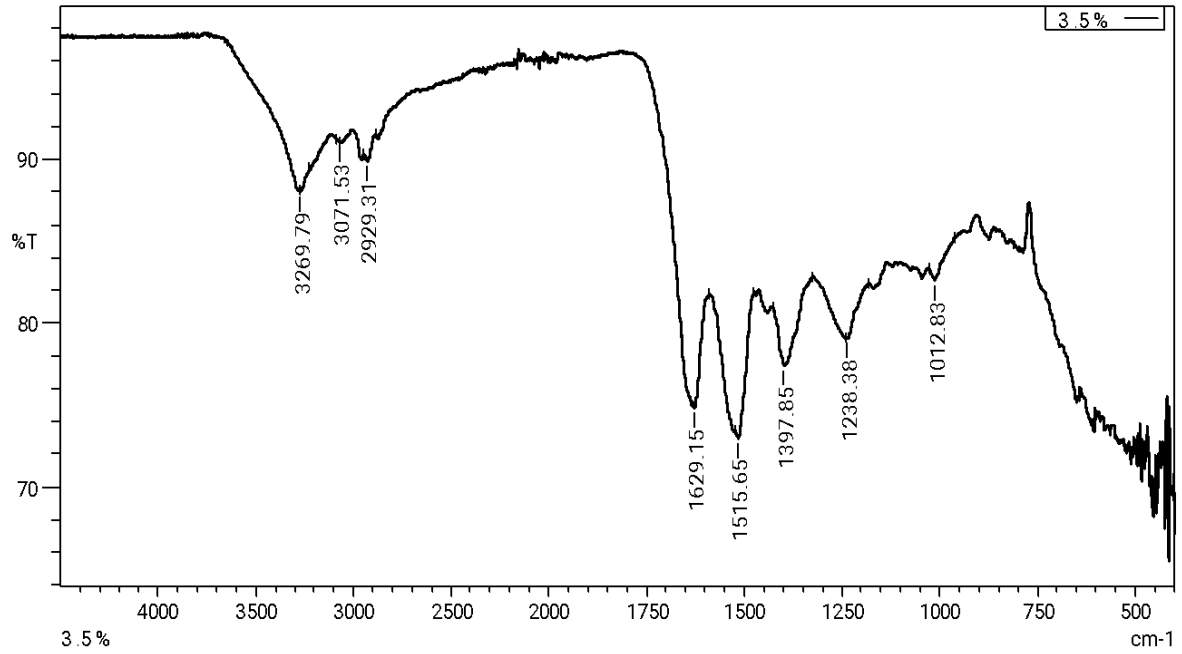


Figure 6 - FTIR Graph of Keratin Extracted from Waste Wool.

The peaks at 3269.79cm^{-1}, 3071.53cm^{-1}, and 2929.31cm^{-1} are assigned to the (C=O) stretching vibrations, indicating typical structural features of the carboxylic group [19]. Similarly, the peak of 1629cm^{-1} absorption is assigned to the (N-H) 1° amines [20]. The peak of 1515.65cm^{-1} is associated with the asymmetric stretch of the nitro (N-O) group. The 1397.85cm^{-1} peak depicts C-C stretching in aromatic rings. The peaks 1238.38cm^{-1} and 1012.83cm^{-1} are assigned to the (CN) stretch seen in aliphatic amines. Hence, based on all the FTIR peaks, the carboxylic group and amine linkages in keratin were present in the tested sample.

FTIR for keratin extracted from chicken feathers

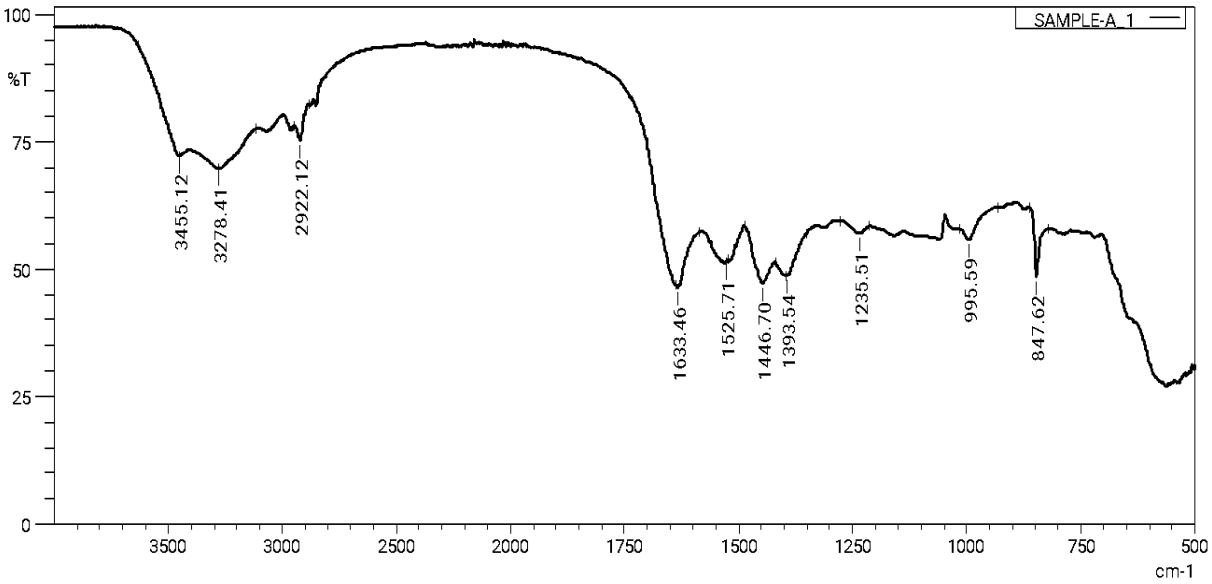


Figure 7 - FTIR Graph of Keratin Extracted from Chicken Feathers

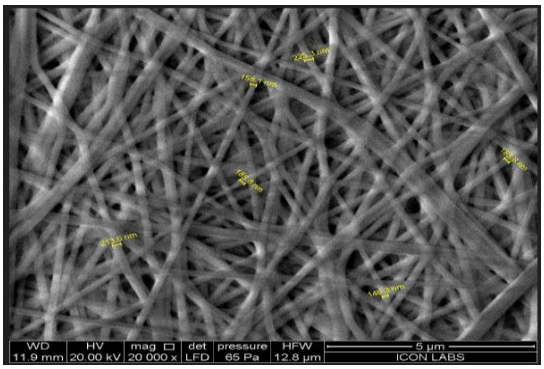
FTIR spectra indicated in Figure 7 shows the infrared spectrum of keratin extracted from chicken feathers. Like the spectrum obtained for keratin derived from waste wool, three peaks were seen at 3455.12cm^{-1}, 3278.41cm^{-1}, 2922.12cm^{-1}.

These confirm the presence of the carboxylic group, primary and secondary amines, respectively. The peak obtained at 1633.46 cm^{-1} indicates absorptions of (N-H) 1* amines. The peak at 1525.71 cm^{-1} indicates the presence of asymmetric stretching in the nitro compound. The peaks 1446.70 cm^{-1} and 1393.54 cm^{-1} show C-C stretching of aromatic compounds. The peaks at 1235.51 cm^{-1} and 995.59 cm^{-1} are associated with the (C-N) stretch in aliphatic amines. All the peaks confirm that the extracted material from waste chicken feathers is keratin.

SEM (Scanning Electron Microscope) Analysis

SEM analysis of PVA (poly-vinyl alcohol) sheet

The surface morphology of the electrospun nanofibre patch was evaluated using Scanning Electron Microscopy (SEM).



Figue 8 (a)

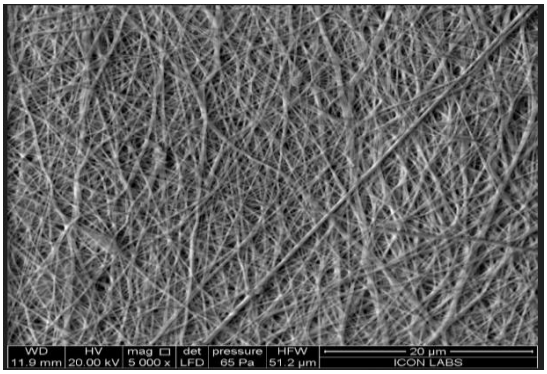


Figure 8 (b)

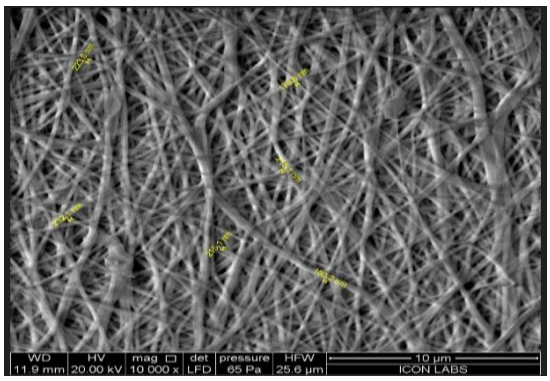


Figure 8 (c)

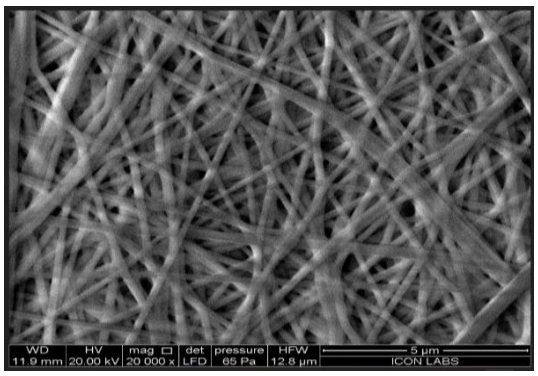
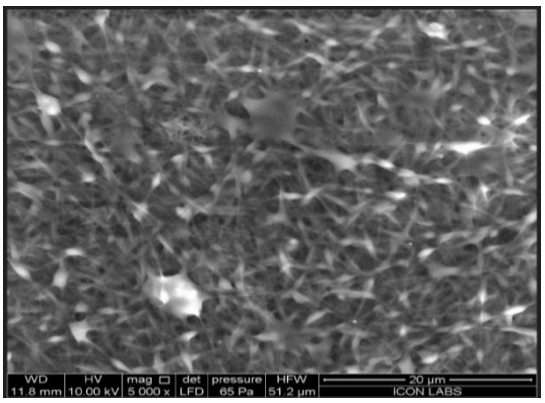


Figure 8 (d)

The above images (Figures - 8a, 8b, 8c, and 8d) represent the surface morphological structure of the electrospun nanofiber patch at different magnifications. The average size of the nanofibers is 185.33nm with 20000x, 10000x, and 5000x magnifications with a lens tab is 5µm to 20µm. here, nanofibers with 185.33nm were formed in sheet format, as shown in the figure.

SEM analysis of PVA-Keratin (chicken feathers) sheet



Figue 9 (a)

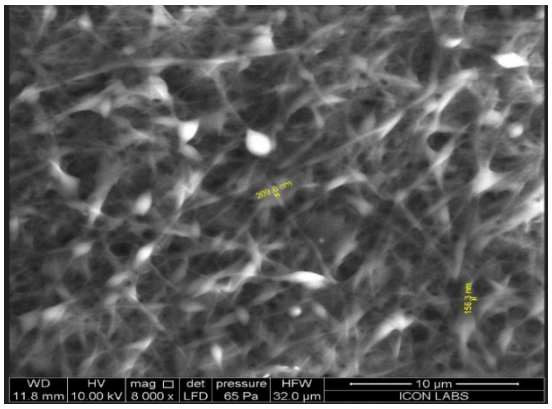


Figure 9 (b)

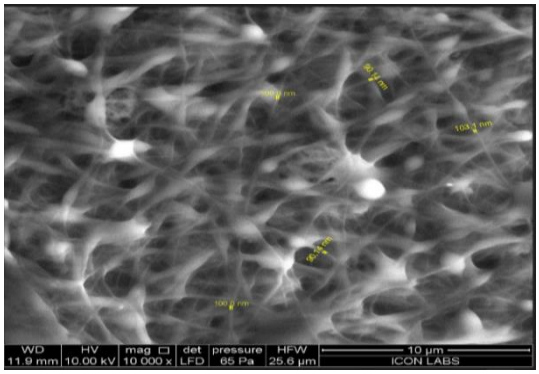


Figure 9 (c)

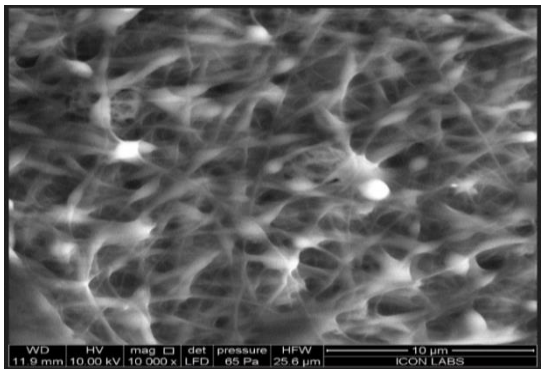


Figure 9 (d)

The images (Figure 9a, 9b, 9c, and 9d) depict the surface morphological structure of a PVA-feather keratin nanofibers sheet at different magnifications. The surface appears smooth and clear, indicating a well-formed structure. The nanofibers exhibit an average size of 96.62nm, as observed under magnifications of 20000x, 10000x, and 5000x, using a lens tab ranging from 5µm to 20µm. Upon closer examination, it is evident that the nanofibers with an average size of 96.62nm are arranged in a sheet-like format. The larger spots in the images can be attributed to the presence of feather keratin and the PVA polymer.

This surface morphology suggests successfully fabricating a PVA and feather keratin nanofiber sheet [21].

SEM analysis of PVA-Keratin (waste wool) sheet.

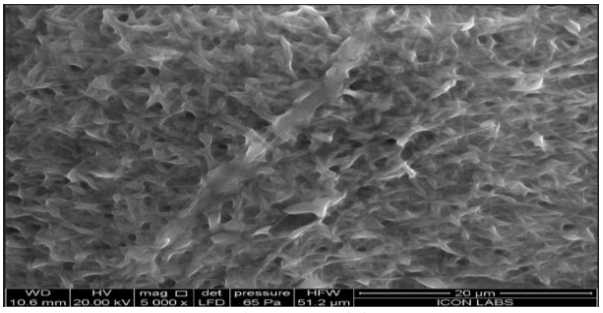


Figure 10 (a)

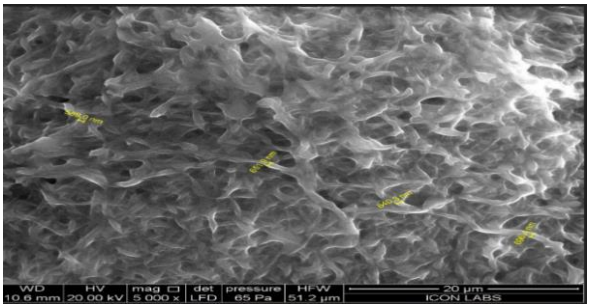


Figure 10 (b)

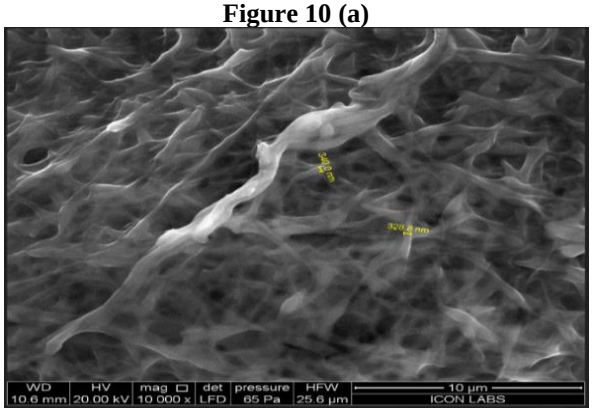


Figure 10 (c)

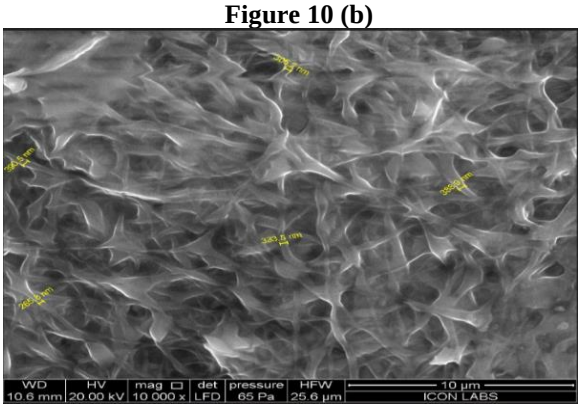


Figure 10 (d)

The images, labelled as Figure 10a, 10b, 10c, and 10d, depict the surface morphological structure of a PVA-wool keratin nanofibers sheet. Each image corresponds to a different magnification level, specifically 20000x, 10000x, and 5000x, utilising a lens tab ranging from 5µm to 20µm. The images collectively reveal a smooth and clear surface of the nanofiber sheet. Upon closer examination, it was determined that the average size of the nanofibers within the sheet is 326.2nm. These nanofibers exhibit a uniform distribution and arrangement, forming a structured sheet.Overall, the images demonstrate the successful formation of PVA-wool keratin nanofiber sheets with a consistent average size of 326.2 nm, showcasing the potential for further exploration and utilisation of these materials in various industries.

F. Antimicrobial Test

The anti-microbial evaluation was conducted as per the AATCC 147 test method [22]. After 24 hours of incubation following the introduction of bacteria, the growth of bacteria was observed, indicating a low area of inhibition in plates containing keratin nanofibers. This suggests the sample lacks antimicrobial activity. The developed samples lack cytotoxicity which can facilitate cell growth, reepithialisation and promote healing of wounds.

IV. DISCUSSION

The present research focuses on exploring alternative products for wound healing, specifically utilising keratin extracted from waste wool and chicken feathers. The extraction process yielded a 40% keratin yield with waste wool and a 48% yield with chicken feathers, providing a sustainable and cost-effective source of keratin. Keratin, a fibrous protein found in various animal tissues, has shown promising properties for wound healing due to its biocompatibility and biodegradability.

To further enhance its utility, the extracted keratin protein was successfully fabricated using polyvinyl alcohol (PVA) as a polymer. This fabrication process allows for the development of nanofibers that can be used as wound dressings or scaffolds for tissue regeneration. To confirm the presence of keratin in the extracted protein, Fourier-transform infrared spectroscopy (FTIR) analysis was conducted. The analysis results confirmed the presence of keratin protein, ensuring the successful extraction and preservation of the desired biomaterial. In addition to confirming keratin's presence, the fabricated nanofibers' surface characteristics were studied using scanning electron microscopy (SEM) analysis.

V. CONCLUSION

The present research focuses on exploring alternative products for wound healing, specifically utilising keratin extracted from waste wool and chicken feathers. The extraction process yielded a 40% yield with waste wool and a 48% yield with chicken feathers, providing a sustainable and cost-effective source of keratin. In conclusion, the extraction and fabrication of keratin-based nanofibers using waste wool and chicken feathers hold significant promise as an alternative product for wound healing. FTIR analysis, SEM analysis, and cytotoxicity studies confirm the extraction of keratin, the surface characteristics of the nanofibers, and their biocompatibility, respectively. This research represents an important step towards developing sustainable and effective wound healing solutions that can potentially benefit millions of individuals worldwide.

SUMMARY

This analysis provides valuable insights into the physical properties of the nanofibers, such as their diameter, alignment, and morphology, which are crucial factors affecting their performance in wound healing applications. The extraction process yielded a 40% yield with waste wool and a 48% yield with chicken feathers. Developing an alternative wound healing product using keratin extracted from waste wool and chicken feathers offers a sustainable solution to address the growing demand for wound care materials and a valuable opportunity to repurpose and recycle waste materials. This research contributes to the field of biomaterials by utilising natural fibrous and textile waste thus reducing environmental impact and offers a potential alternative for wound healing that is both effective and economically viable.

DECALARION STATEMENT

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Conflicts of Interest	No conflicts of interest to the best of our knowledge.
Ethical Approval and Consent to Participate	No, this article does not require ethical approval and consent to participate with evidence.
Availability of Data and Material	Not relevant.
Authors Contributions	All authors having equal contribution for this article.

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