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Title: **Antimicrobial Activity of Spunlace Nonwovens 100% Bleached Cotton Fabric, Against Selected Pathogenic Microorganisms**

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Antimicrobial Activity of Spunlace Nonwovens 100% Bleached Cotton Fabric, Against Selected Pathogenic Microorganisms

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ABSTRACT

The development of antibacterial textiles has attracted an increasing attention due to growing demands for hygienic and protective materials in medical and cosmetic applications. Nonwoven fabrics are widely used in hygiene and medical applications where antibacterial performance is critical for controlling microbial contamination. Unlike previous studies that investigated mechanically antimicrobial coating and chemically treating the fabrics for disinfection, this study evaluated the intrinsic antimicrobial property against some specific pathogenic microbes in industrially produced bleached nonwoven fabrics. Therefore, the objective of this study was to evaluate the antimicrobial activity of the nonwoven fabrics using standard antimicrobial testing method against selected bacterial and fungal strains. In this study, 100% bleached cotton Spunlace nonwovens fabric was produced by continuous carding and hydroentanglement without binders. For this purpose, purified processed water and hydrogen peroxide (H_2O_2) (1–3 %) were used solely as a hygiene aid, with Benzalkonium Chloride (BKC, 0.3 %) as a positive antimicrobial control. The resulting uniform, defect-free fabrics provided a controlled platform for systematic evaluation of inherent and comparative antibacterial performance. American Type Culture Collection (ATCC) test pathogens, *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), and *Aspergillus niger* (ATCC 16404) were subjected to the antimicrobial properties of the developed fabrics. Sample B treated with 3% H_2O_2 inhibited the visible growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. While, BKC was very active forming 18.8mm zone of inhibition against *Salmonella typhimurium* and 30.05mm against *Staphylococcus aureus.* The antimicrobial activity observed suggested that the nonwoven fabrics may have potential applications in healthcare and hygiene. The results underscored the antimicrobial retaining and disseminating properties of the fabrics. Furthermore, the results highlighted

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the possible use of BKC for the development of fabrics having potential applicability in healthcare.

GRAPHICAL ABSTRACT

Keywords: antimicrobial, non-woven fabrics, *Salmonella*, *Staphylococcus aureus*

Highlights

- 3% H_2O_2 showed greater antimicrobial activity than 1% H_2O_2.
- BKC achieved the highest antimicrobial activity under test conditions.
- Bleached spunlace cotton inhibited selected clinically important pathogens.

1.INTRODUCTION

A wide range of healthcare applications has increasingly incorporated recyclable antimicrobial wound dressings and biomedical fabrics. Key performance parameters for such materials include prolonged antimicrobial activity, stability, and rapid disinfection capability, all of which enhance resistance against microbial contamination [1]. The medical sector including carpets, bedding, wearable accessories, and other auxiliary items faces persistent challenges related to bacterial contamination of textile materials. This may lead towards severe infections among patients and healthcare workers [2].

Hospital wearable textiles, such as lab coats and protective garments, serve as primary barriers against microbial exposure. However, these articles often lack sufficient and durable antimicrobial properties [1–5]. Consequently, there is a growing need to develop cost-effective, biodegradable, reusable, recyclable, low-toxicity, durable, and environmentally friendly antimicrobial fabrics and wound dressings [3]. A potential method is to introduce stable N-halamine compounds into the textile substrates as active biocides. Nonwoven fabrics made from 100% bleached cotton comber noils have to go through multiple steps (web formation, consolidation, water management, drying, winding). Each step provides an opportunity for microbial control or to add antimicrobial attributes to the fabric. The bioburden is inherently reduced when using bleached cotton fibers due to their reduced natural wax content. They create an appropriate surface for the subsequent antimicrobial modification of the fibers either by pre-treatment or by a topical application of an antimicrobial agent when forming the web using a high performing web-forming system, such as a Spinnbau system (Dilo Spinnbau GmbH, Germany) [6–9]. The critical control points for the web consolidation by hydroentanglement with a Fleissner AquaJet system are the large amounts of processed water that come into direct contact with the fiber matrix. At this stage, antimicrobial effects can be obtained either in a short-term period by regulating the dosage of oxidative agents, such as hydrogen peroxide (H_2O_2), which are applied for process hygiene or in a more permanent way by including an antimicrobial agent into the water jets, where it will be deposited at the fiber level [10–12]. An integrated reverse osmosis system (Idrosistem) further promotes efficient water management. It provides highly purified water, thus reducing the risk of bacterial and fungal contamination while enabling active control of any antimicrobial substances added to the water system [6]. The subsequent thermal drying was carried out at about 120 °C. It offers another microbial reduction method besides chemicals, to reduce the remaining moisture and stabilize the textile structure [12–14]. Lastly, precision winding under controlled tension reduces the contamination that can occur after the process. It also helps to maintain the product cleanliness during storage and handling [8]. Overall, these processing steps from fiber selection to carding, hydroentanglement, water purification, drying, and winding, represent both inherent and induced routes of achieving controlled antimicrobial properties in binder-free Spunlace cotton for hygiene and non-sterile medical applications [9]. The Spunlace (hydroentanglement) technology is used because it preserves the inherent properties of cotton fibers by producing nonwoven textiles without the use of chemical binders. Due to the high-pressure water jet tangling of fibers, Spunlace fabrics have greater softness, flexibility, absorbency, breathability, and mechanical strength when compared to other nonwoven manufacturing processes. Based on these qualities, Spunlace nonwoven cotton is especially well-suited for medical dressings, hygiene products, wet wipes, and other healthcare applications where microbiological control, comfort, and cleanliness are critically important. The nonwoven fabric produced by Ihsan Sons Pvt. Ltd. was tested against standard bacterial and fungal pathogens (*Staphylococcus* aureus, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Aspergillus niger*) by the agar diffusion method [10] in this study. The remaining level of H_2O_2 was measured and compared, and the antimicrobial activity of BKC was evaluated as an alternative antimicrobial. While the cotton textiles treated with antimicrobials have been studied by numerous researchers, the industrially-manufactured bleached Spunlace nonwoven cotton textile's antimicrobial effectiveness is not well-documented. The aim of this study was to explore the inherent antimicrobial properties of bleached Spunlace nonwoven cotton fabric and to compare its performance in the presence of BKC as compared to H_2O_2 [11].

2.MATERIALS AND METHODS

2.1 Development of Nonwoven Fabrics

The comber noils from 100% bleached cotton were converted into nonwoven fabrics by a parallel carding technology and hydroentanglement process [26]. The fibers were opened, individualized, and oriented with a high-performance Spinnbau carding system to form a uniform web with controlled basis weight and fiber orientation [12]. The web was consolidated by a Fleissner Aquajet hydroentanglement line using "staged" high pressure water jets to mechanically entangle the fibers without the addition of functional finishes or chemical binders [13]. To guarantee the purity of processed water and reduce the microbial variation, processed water was provided using reverse osmosis system [14]. If standard sample production is used, the H_2O_2 is added only as a process hygiene agent and the concentration used is 1% and 3% in separate batches. However, this does not provide any residual antimicrobial effect to the final fabric (Fig. 1) [15]. To compare the results, another fabric was fabricated using the same processing conditions but replacing the (H_2O_2) with the positive antimicrobial control, BKC at 0.3% [16]. All the fabric samples were then vacuum dewatered and thermally dried at about 120 °C after hydroentanglement. The dried fabrics were then wounded under precision tension on a precision Bastian winder to create uniform and free of fault rolls for systematic evaluation of their antimicrobial properties (Table 1) [17].

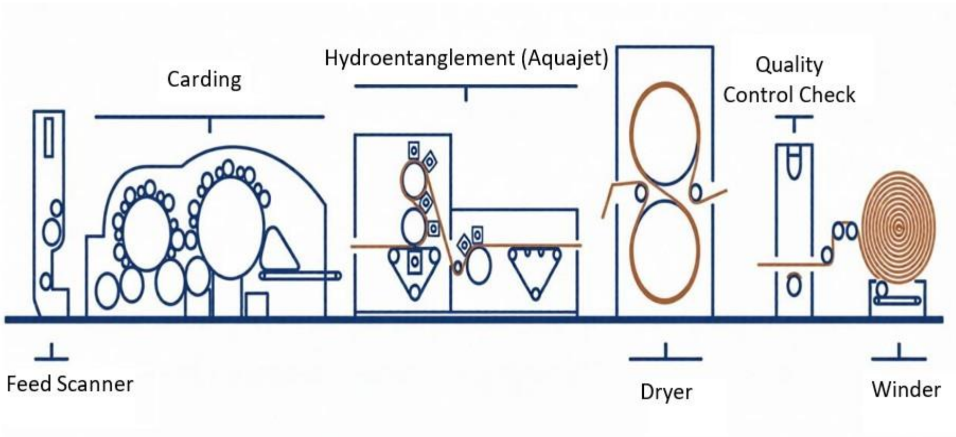

Figure 1. Schematic Representation of the Spunlace (Hydroentanglement).

The technique used in the production of 100% bleached cotton fabric. The process includes feed scanning, carding, hydroentanglement (aqua jet), drying, quality control inspection, and winding to produce the finished nonwoven fabric.

Table 1. Manufacturing Parameters for the Production of Spunlace Nonwoven Fabric

Process Stage	Parameters	Operating Range / Specification	Primary Functional Role
Carding (Spinnbau)	Feed material	100 % bleached cotton comber Noils	Cellulosic fiber source
	Feed Speed	1.45-1.55 m min^{-1}	

Process Stage	Parameters	Operating Range / Specification	Primary Functional Role
	Pre-opener	250–310 m min^{-1}	Fiber opening and alignment
	Main Cylinder Speed	800–880 m min^{-1}	Fiber opening and alignment
	Worker/stripper Speed	210–215 m min^{-1}	Reduction of neps and web uniformity
	Top and Bottom Random Roller Speed	1000–1160 m min^{-1}	Makes the fiber orientation Randomizes to reduce directional strength variation
	Top and Bottom Doffer speed	22–25 m min^{-1}	Transfers the formed web from the cylinder to the next stage
	Top and Bottom Scrambler speed	13.50–15 m min^{-1}	To improve isotropy and web cohesion by disruption of fiber alignment
	Top and Bottom Take off Apron Speed	20-24 m min^{-1}	Maintaining uniform web thickness and tension, also controls web delivery rate
	Pressure of Jet Head HP1	20 Bar	Initialization of light fiber entanglement for web stabilization
Aquajet (Hydroentanglement)	Pressure of jet head HP2	30-40 Bar	Increases bonding and web integrity
	Pressure of jet head HP3	27-55 Bar	Development of internal fiber entanglement and strength
	Pressure of jet head HP4	27-55 Bar	Enhances uniformity and consolidation
	Pressure of jet head HP5	25 Bar	Provides high strength bonding
	Pressure of jet head HP6	30-40 Bar	Finalizes surface quality and fabric strength
	Drum - 1 & 2	98-101 %	Enables water drainage during entangling and Support web transport
	Diverting Belt	118-119 %	Transfers web while maintaining alignment
	Intake Dryer belt Ratio	100-105 %	Controls residence time and shrinkage in drying
	Drum Dryer- 1 & 2	100%	Regulates drying

Process Stage	Parameters	Operating Range / Specification	Primary Functional Role
	Ratio		efficiency and fabric relaxation
	Rotation Speed Suction fan #01,02 & 03	90,70 & 75 %	Removes moisture-laden air to aid drying
	Dryer Temperature (°C)	90-120	Supplies heat for moisture evaporation
	Moisture (%)	6.0	Monitors residual moisture for final fabric quality
	Line speed	23 m min^{-1}	Productivity–bonding balance
Water Management	Water quality	Reverse osmosis (RO) treated	Nozzle protection, consistency
	Hygiene control	H_2O_2 (50 %), controlled dosing	Reduction of process bioburden
Drying	Drying temperature	~120 °C	Structural stabilization
Winding (Bastian)	Web tension	2–8 N	Defect free roll formation
	Roll diameter	600–1200 mm	Storage and converting suitability
	Traverse control	Servo-driven	Uniform roll build

2.2 Hydrogen Peroxide (H_2O_2) Traces Test

To confirm that H_2O_2 used as a process hygiene aid did not remain in the final fabric, residual H_2O_2 levels were assessed on representative samples of the hydroentangled nonwoven [18]. Extraction of fabric specimens (~5 g) was conducted in deionized water, and the concentration of H_2O_2 in the extract was measured by a colorimetric assay based on titanium oxysulfate, which is capable of detecting peroxide down to 0.1 ppm [19]. Duplicate measurements were taken for each batch and the results were all below the detection limit. This indicated that no trace of H_2O_2 was left in the dried and wound fabric. This allowed the subsequent antimicrobial activity that was observed to be related to material properties or controlled experimental treatments (such as benzalkonium chloride) as opposed to residual process oxidant [20].

2.3 Agar Diffusion Test for Nonwoven Fabric against Known Bacterial and Fungal Strains

The antibacterial activity of the bleached Spunlace nonwoven cotton fabric was investigated using the standard microbiological technique, that is, the Kirby-Bauer disc diffusion method and was modified according to the experimental requirements. All experimental steps, including inoculum preparation, sample incubation, and antimicrobial testing, followed standard protocol. The nonwoven fabrics were tested for their antimicrobial activity against selected bacterial and fungal pathogens, such as *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 14028), and *Aspergillus niger* (ATCC 16404), using an agar diffusion (zone of inhibition) assay [21]. The selected microorganisms are representative of clinically important bacterial and fungal species that are frequently found in healthcare environments and hygiene products. *Staphylococcus aureus* was selected as a typical gram-positive bacterium linked to skin and wound infections. *Pseudomonas aeruginosa* was chosen because of its opportunistic character and inherent antibiotic resistance. Aspergillus niger was selected as a representative environmental fungal contaminant. Whereas, *Salmonella typhimurium* was selected as a representative enteric pathogen relevant to public health and hygiene research. These microbes collectively provide a comprehensive evaluation of the bleached Spunlace nonwoven cotton fabric's antibacterial efficacy. Fabric samples (1 cm x 1 cm) were inoculated into nutrient agar plates (Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi) pre-inoculated with a standardized microbial suspension (~10^6 CFU mL^{-1} for bacteria and 10^5 spores mL^{-1} for *Aspergillus niger*). To determine the zones of inhibition around the fabric, plates were incubated at 37 °C for 24 h (bacteria) or 28 °C for 48–72 h (fungi), and the diameters were measured in millimeters [15]. All tests were performed in duplicate. H_2O_2-treated fabrics served as a negative control and BKC-treated fabrics served as a positive antimicrobial control [16]. This approach enabled the direct assessment of microbial growth inhibition due to the controlled antimicrobial treatment or due to intrinsic properties of the fabric. For the H_2O_2-treated samples, the antimicrobial activity was evaluated on the bases of visible microbial growth after incubation.

3.RESULTS AND DISCUSSION

3.1 Traces of H_2O_2

The H_2O_2 traces for sample A were found to be 3976 ppm. While in case of sample B, the traces were found to be 15904 ppm. The elevated level of traces confirms the higher dosage of H_2O_2 in case of sample B. Samples treated with 3% of H_2O_2 inhibited the visible growth of *P.aeruginosa* and

S.aureus.

3.2 Antimicrobial Properties of Developed Fabric

As mentioned in the M&M section, the anti-microbial properties of the fabric were tested against ATCC test pathogens. There are two aspects of the results. Results vary when there is a concentration increase in H_2O_2 traces [18] during the processing. Moreover, different activity was seen when BKC was applied [16]. No Zones of inhibitions (ZOIs) were observed when fabric treated with 1% of H_2O_2 was placed on bacterial lawns plates of *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. However, there was a considerable improvement in antibacterial properties of the fabric when treated with 3% of H_2O_2 (sample B) [23]. Although *Salmonella* growth seemed to be not affected by the increase in concentration of H_2O_2 [24], there was absolutely no growth in case of *P.aeruginosa* and *S.aureus* [15]. The absolutely-no-growth can be explained as the excellent antibacterial disseminating properties of the fabric into the medium. These results show how the antimicrobial characteristics of H_2O_2 have an impact through direct contact with the fabric as opposed to dispersing into the agar medium. Similar results were observed for oxidizing disinfectants, whose antimicrobial efficacy primarily depends on contact with the surface rather than diffusion via agar [24]. The other aspect of the results was a completely different agent used in the processing (0.3% Benzalkonium chloride a (QUATS) compound with known anti-microbial properties [16], developed solely for this experiment) (Table 2). In case of *Salmonella,* a zone of inhibition measuring 18.8mm was observed (Fig 2A-L). The ZOI in case of *S.aureus* was found to be 30.05 mm. In case of *P.aeruginosa*, no ZOI was found. This shows the better potential of BKC compared to the H_2O_2 against *Salmonella* [25] when applied to the fabric in processing (Table 3). Kampf classified BKC as an efficient quaternary ammonium compound with activity against a wide spectrum of microbes. This makes it one of the most extensively used disinfectants in healthcare and industrial applications [15]. The structural features of Spunlace nonwoven cotton textiles may also have an impact on the observed antibacterial activity. Spunlace fabrics, in contrast to traditional woven textiles, are produced by hydroentanglement without the use of chemical binders. This results in a structure that is highly absorbent, soft, porous, and has significant fiber entanglement. These properties improve the contact area between microorganisms and the fabric surface, potentially improving antimicrobial agent interactions with microbial cells [11,12]. Because of these structural characteristics, Spunlace nonwoven materials are widely used in medical wipes, wound dressings, and disposable medical products. The current findings are consistent with prior research revealing that cotton-based nonwoven materials may successfully minimize microbial contamination and have significant potential for healthcare and hygiene applications [8,9,25]. Because of its softness, absorbency, and suitability for healthcare applications, Spunlace nonwoven cotton is a viable material for infection-control measures. There are some limitations to the current investigation. The use of duplicate experimental observations may restrict the reproducibility and robustness of the results. Additionally, a small number of clinically relevant bacteria were used to evaluate the antibiotic activity. Future research should include more experimental replicates, a broader range of bacterial and fungal pathogens to further confirm the findings of this study.

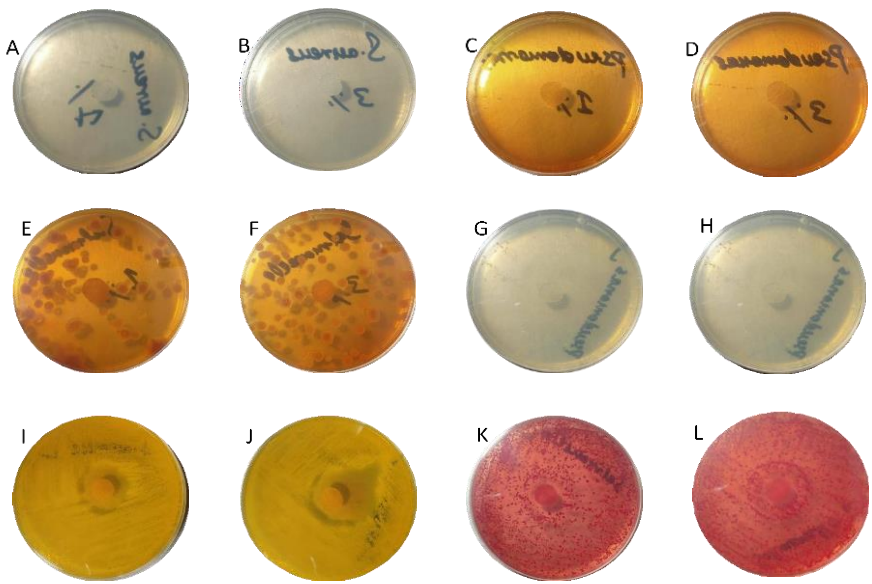

Figure 2. Antibacterial Activities Exhibited by the Fabric's Samples. (A) *Staphylococcus aureus* Treated with 1% H_2O_2 (Replicate 1) (B) *Staphylococcus aureus* Treated with 3% H_2O_2 (Replicate 2) (C) *Pseudomonas aeruginosa* Treated with 1% H_2O_2 (Replicate 1) (D) *Pseudomonas aeruginosa* Treated with 3% H_2O_2 (replicate 2) (E)

Salmonella typhimurium Treated with 1% H_2O_2 (Replicate 1) (F) *Salmonella typhimurium* Treated with 3% H_2O_2 (Replicate 2) (G) *Pseudomonas aeruginosa* Treated with 0.3% BKC (Replicate 1) (H) *Pseudomonas aeruginosa* Treated with 0.3% BKC (Replicate 2) (I) *Staphylococcus aureus* Treated with 0.3% BKC (Replicate 1) (J) *Staphylococcus aureus* Treated with 0.3% BKC (Replicate 2) (K) *Salmonella typhimurium* Treated with 0.3% BKC (Replicate 1) (L) *Salmonella typhimurium* Treated with 0.3% BKC (Replicate 2).

Table 2. Fabric Sample Treated with 1% and 3% H_2O_2

S.No	Bacterial Strains	Sample A (Treated with 1% H_2O_2)	Sample B (Treated with 3% H_2O_2)
1	*Staphylococcus aureus*	-	-
2	*Pseudomonas aeruginosa*	-	-
3	*Salmonella typhimurium*	+	+
4	*Aspergillus niger*	+	+

Note: As the antimicrobial activity of H_2O_2-treated samples was evaluated based on visible microbial growth, no ZoIs were observed. That is why, quantitative ZoIs values are not reported. ***Key***: += growth, -= No growth

Table 3. Zone of Inhibition of the Sample C (Fabric Sample Treated with 0.3% Benzalkonium chloride (BKC))

S. No	Bacterial Strains	Plate 1	Plate 2	Mean of Plate 1 and 2
1	*Staphylococcus aureus*	34.5 mm	25.6 mm	30.05
2	*Pseudomonas aeruginosa*	-	-	-
3	*Salmonella typhimurium*	17.66 mm	18 mm	17.83
4	*Aspergillus niger*	-	-	-

4. CONCLUSION

This study evaluated the antimicrobial efficacy of chemically-treated nonwoven fabrics using two widely recognized biocidal agents, that is, H_2O_2 (1% and 3%) and BKC (0.3%) as finishing agents. Three clinically important bacterial strains, that is, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*, along with one fungal strain, *Aspergillus niger*, were selected as test microorganisms. The experimental results showed that H_2O_2-treated fabrics at both concentrations (1% and 3%) exhibited 100% growth inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This is a result of the high sensitivity of these two microorganisms to the oxidative effect of H_2O_2. However, when grown under the same conditions, *Salmonella typhi* and *Aspergillus niger* showed visible growth. Thus, it was noticed that these organisms showed a remarkable resistance to H_2O_2 at the tested concentration. The antimicrobial properties of the fabrics treated with (0.3% Benzalkonium Chloride) showed greater antimicrobial activity than H_2O_2. However, strong inhibitory effect was observed with well-defined zones of inhibition of 30.05 mm against *Staphylococcus aureus* and 17.83 mm against *Salmonella typhi,* respectively. This shows comparatively greater susceptibility of *Staphylococcus aureus*. The *Pseudomonas aeruginosa* and *Aspergillus niger,* however, did not show any zone of inhibition, This indicates that they are non-susceptible to 0.3% concentration of BKL under the tested conditions. This would clearly indicate that there are no single antimicrobial compounds that would be effective against all types of microorganisms, as demonstrated by the selective antimicrobial activity of both chemical agents. These limitations may be overcome. Moreover, a wider spectrum of activity may be found by future studies with higher concentrations and multiple applications of antimicrobial agents and the use of newer compounds with biocidal activity. The results of this study are scientifically very valuable. These results may help in the development of efficient antimicrobial nonwoven products to be used in hygiene products, surgical gowns, hospital beds' linen products, as well as PPE and for opening new innovation centers in the medical textile industry.

Author Contributions

Syed Ahmad: methodology, writing-original draft, writing-review & editing. **Shahid Nawaz:** supervision, writing-original draft, writing-review & editing. **Muhammad Nadeem Raza:** project administration, resources.

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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Generative AI Disclosure Statement

The authors did not use any type of generative artificial intelligence software for this research except for the graphical abstract.

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