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Research Paper

Testing potentiality of copper particles as antimicrobial agent by kirby bauer test to inhibit the activity of various microorganisms when applied to fabrics especially medical wear

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ABSTRACT

Traditional antimicrobial finishes often rely on synthetic chemicals, which is costly alongside not suitable for the environment. Commercially available antimicrobial agents, when applied to textiles, often result in the release of hazardous effluents during laundering processes. These effluents pose significant environmental threats and, in extreme cases, may have detrimental effects on human health, particularly among vulnerable populations such as infants. In light of these concerns, the current study purpose is to find out an easiest way to find out antimicrobial using copper sulphate a compound recognized for its relatively low environmental toxicity. The aim is to establish a sustainable and practical alternative to conventional chemical treatments that ensures both microbial resistance and ecological safety. When copper finished samples are tested its resulted as intermediate for Bacillus subtilis & Staphylococcus aureus.

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1. INTRODUCTION

1.1 Background of the Study

The textile industry is a significant and rapidly growing sector that offers numerous business opportunities and substantial economic benefits. With increasing global competition, there are ample opportunities for researchers and industry stakeholders to develop innovative technologies that address current challenges. A major concern for the industry is maintaining the quality and durability of fabrics. One issue frequently encountered is the deterioration of cotton fabrics caused by microbial activity, which can lead to a reduction in tensile strength [1]. Microbial attacks pose risks not only to human health but also to textiles, particularly those made from natural fibers. These fibers provide an ideal environment for microbial growth when factors like moisture, nutrients, oxygen, and suitable temperatures are present. Antimicrobial finishing is a modern development in textile treatment aimed at addressing this issue. As consumers become more conscious of hygiene and healthy living, there is a growing demand for textiles with antimicrobial properties. Such finishes inhibit the growth of harmful bacteria and have been shown to be both environmentally friendly and beneficial to health by helping to prevent infections. Additionally, they help reduce unpleasant odors, staining, and the biological degradation of garments [2]. Microbial contamination of textiles can lead to health issues for users. For example, Staphylococcus present on undergarments can cause unpleasant odors and lead to skin infections with pus formation. Escherichia coli may also produce bad odors and contribute to the development of skin ulcers. In addition, fungi like Aspergillus niger can compromise the strength of fabrics and cause discoloration [3]. Antimicrobial finishing represents a modern advancement in textile treatment. At present, a variety of antimicrobial finishes are applied for different functional purposes. One example is chitosan, a natural biopolymer recognized for its remarkable qualities such as being biodegradable, non-toxic, and possessing strong antimicrobial properties [4]. Applying chitosan coatings to conventional fibers appears to be a practical option, as chitosan has already been used in various applications, including as a thickening agent in pharmaceutical and cosmetic formulations. Additionally, this biopolymer is well known for its antimicrobial effects and wound-healing capabilities [3]. Natural dyes, herbal extracts, medicinal plants, and various antimicrobial agents are frequently used in antimicrobial textile treatments. However, these substances tend to be costly, and their extraction processes are often labor-intensive and expensive [5]. The primary goal of this research is to identify an antimicrobial finishing method that is both cost-effective and environmentally sustainable.

1.2 Current Scenario of Microbial-Related Deaths

The growth of microorganisms on textiles can lead to unpleasant odors, skin irritation, loss of fabric strength, and discoloration. This presents a significant problem, especially considering that humans are constantly in contact with textiles in daily life. Addressing this issue is essential to ensure both comfort and hygiene in everyday use. According to the GBD 2019 Antimicrobial Resistance Collaborators, an estimated 13.7 million deaths worldwide in 2019 were linked to infections. Among these, approximately 7.7 million deaths were associated with 33 different bacterial species, including both antimicrobial-resistant and susceptible strains, across 11 infectious conditions studied. These 33 bacteria accounted for about 13.6% of all global deaths and represented 56.2% of deaths related to sepsis that year as shown in Table 1 The top five bacteria responsible for the majority of these deaths were Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, and Pseudomonas aeruginosa, contributing to nearly 55% of the fatalities caused by the studied bacteria [6].

Table 1. Approximate Deaths by Different Microorganisms (Source: The Lancet)

Rank	Pathogen	Approx. Deaths	Common Infectious Syndromes
1	Staphylococcus aureus	~1,100,000	LRI, bloodstream, skin infections, cardiac, bone/joint infections
2	Escherichia coli	~950,000	UTIs, bloodstream, intra-abdominal, diarrhoea

3	Streptococcus pneumoniae	~800,000	LRI, meningitis, bloodstream, cardiac
4	Klebsiella pneumoniae	~700,000	LRI, bloodstream, UTIs
5	Pseudomonas aeruginosa	~550,000	LRI, bloodstream, UTIs
6	Streptococcus pyogenes	~500,000	LRI, skin infections, bloodstream
7	Mycobacterium tuberculosis	~500,000	LRI
8	Acinetobacter baumannii	~450,000	LRI, bloodstream
9	Enterococcus faecium	~300,000	UTIs, bloodstream, intra-abdominal
10	Non-typhoidal Salmonella	~250,000	Diarrhoea, bloodstream
11	Enterococcus faecalis	~250,000	UTIs, bloodstream
12	Group B Streptococcus	~200,000	Bloodstream, intra-abdominal
13	Proteus spp	~200,000	UTIs
14	Escherichia coli (ETEC)	~175,000	Diarrhoea
15	Haemophilus influenzae	~175,000	LRI, meningitis
16	Neisseria meningitidis	~150,000	Meningitis

1.3 Significance of the Research

The study is significant to find out a cost-effective and easiest antimicrobial finishing for 100% cotton to prevent cross infection by microorganism.

1.3.1 Purpose and Goals of the Study

1.3.2 Research Aim

1) Prevent microbial cross-contamination through the use of antimicrobial finishes.
2) Find a cost effective & environment friendly way to extract antimicrobials.

2. RELATED WORK

2.1 Microbes & How it Attacks Textile Materials

Microorganisms are tiny living organisms that cannot be seen without the help of a microscope. Typically, they are smaller than 100 micrometers, but size alone can be misleading because some microorganisms are large enough to be visible to the naked eye and can exceed 100 micrometers in size [7]. Cotton fibers are primarily made up of cellulose. Each fiber consists of 20 to 30 layers of cellulose arranged in a spiral around a central core of natural springs as shown in Figure 1 This structured organization of cellulose gives cotton fibers their strength, durability, and ability to retain moisture [8].

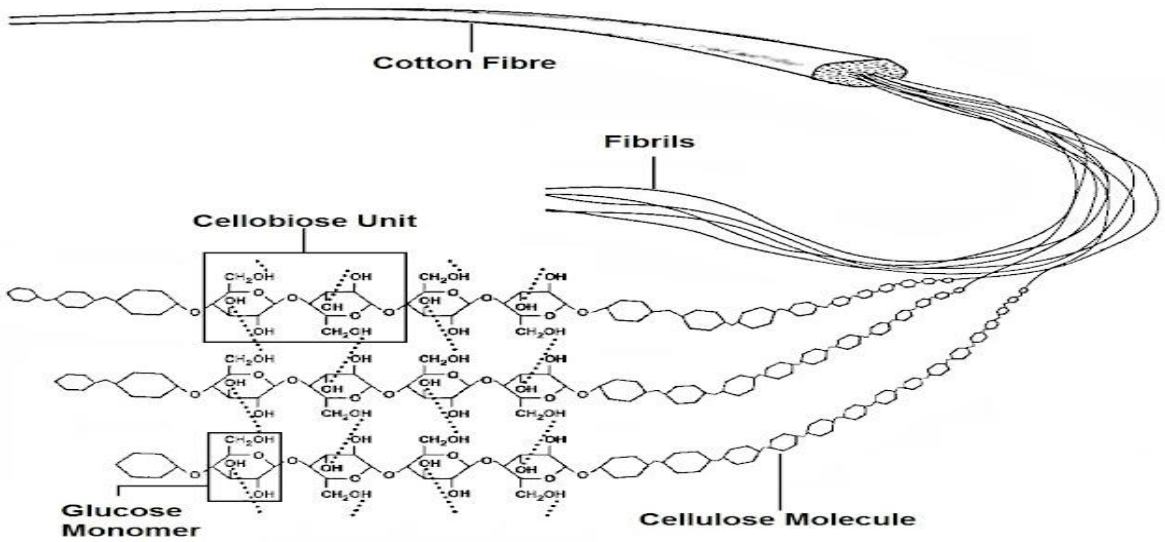

Figure 1. Chemical Structure of Cotton. (Source: Textile Academy)

The structure of cotton fibers is chemically broken down through the action of extracellular enzymes released by microorganisms as they seek nutrients. Plant fibers like cotton are particularly vulnerable to fungi that digest cellulose, known as cellulolytic fungi. These fungi produce enzymes called cellulases, which are capable of fully degrading cellulose [9]. Cellulose breakdown accelerates dramatically when exposed to specialized enzymes or biocatalysts as shown in Figure 2 these biological catalysts enable microorganisms like bacteria or fungi to metabolize cellulose efficiently. As this hydrolytic process progresses, it disrupts the structural integrity of cotton-based textiles, causing irreversible loss of durability, flexibility, and other performance characteristics essential for fabric functionality [9].

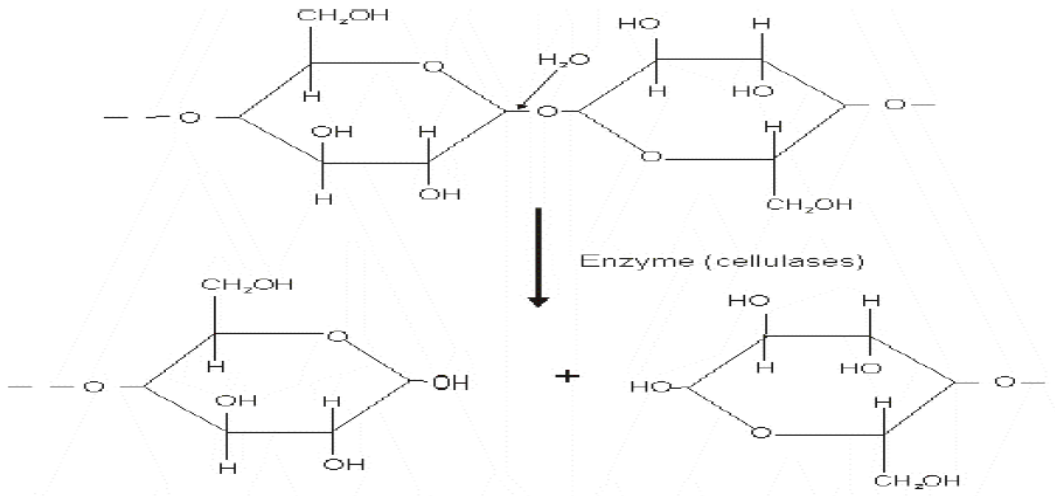

Figure 2. Breakdown of Cellulose by Enzyme

2.2 Techniques of Antimicrobial Treatment

2.2.1 Microbial Resistance Enhancement with Citric Acid and Chitosan

Citric acid and chitosan are commonly used as finishing agents to provide both durable press properties and antimicrobial functionality to cotton fabrics. These agents are typically applied using the standard pad-dry-cure technique. The solution was prepared by stirring chitosan in distilled water with citric acid at room temperature overnight, followed by the addition of a catalyst and 0.1% nonionic surfactant. Interestingly, cotton fabrics treated solely with a citric acid solution exhibit an almost complete (nearly 100%) reduction in bacterial count, even without the presence of chitosan. Furthermore, the antimicrobial effectiveness of the fabric enhances progressively with higher concentrations of chitosan when acetic acid is used as the dissolving medium [10].

2.2.2 Enhanced Antimicrobial Performance of Cotton via Novel N-Halamine Functionalization

A simple method was used to synthesize the N-halamine precursor, 1-glycidyl-s-triazine-2,4,6-trione, through the reaction of cyanuric acid with epichlorohydrin. This compound was then applied to cotton fabric using the conventional pad-dry-cure process. The resulting N-halamine-treated cotton exhibited strong antimicrobial activity, effectively inhibiting both Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) within short exposure times [11].

2.2.3 Antimicrobial Finishing with Polyhexamethylene Biguanide

Polyhexamethylene biguanide (PHMB) is a broad-spectrum antibacterial agent that has been widely used for many years as a disinfectant in both the medical and food industries. In textile applications, fabrics are soaked in a PHMB solution and then rinsed with deionized water. Studies have shown that textiles treated with PHMB exhibit rapid antibacterial action, eliminating approximately 98.2% of bacteria within 5 minutes of contact and nearly 100% within 20 minutes [12].

3. METHODOLOGY

3.1 Materials

100% Knitted Kotton fabrics collected from Pakiza Knit Composite Ltd. Copper sulfate (CuSO4), Ascorbic acid (C6H8O6) Bio-quart 200A, Poly ethylene Glycol, NaOH, Soda ash, wetting agent, sequestering agent, Detergent, Hydrogen Peroxide, Bezaktiv Red S-2B, Bezaktiv Yellow 3-R 150, Bezaktiv Blue S-RN purchased from DYSIN-CHEM LIMITED.

3.2 Synthesis Copper Particles Using Copper Sulphate

First, a glass container was filled with 100 mL of water. Copper sulfate was added and stirred thoroughly until it completely dissolved, which typically takes 5 to 10 minutes. In a separate container, 100 mL of hot water was prepared, and ascorbic acid was dissolved in it by mixing until fully dissolved. The solution was then gently heated. Next, the hot ascorbic acid solution was poured into the container with the copper sulfate solution and stirred. The mixture quickly changed color to a dark greenish-brown. Within a few seconds, metallic copper began to precipitate and settle at the bottom as salmon-colored particles. Stirring continued until all the copper had precipitated. The solution gradually lightened and took on an emerald green hue, while the layer of metallic copper at the bottom grew thicker. Once the copper particles had fully settled, the green liquid was carefully decanted. The remaining copper was then covered with a layer of distilled water to prevent oxidation from air exposure. Finally, the copper particles were transferred and stored in a clean glass container for later use [13].

3.3 Fabric Treatment

The samples were scoured using a solution containing 5 g/L of sodium hydroxide (NaOH), 3 g/L of soda ash to maintain the required pH level of 10.5, and 1 g/L each of a wetting agent, sequestering agent, and detergent. The scouring process was carried out at 90°C for one hour using an IR laboratory dyeing machine [14]. Then samples were bleached using a solution containing 5 g/L sodium silicate, 0.5 g/L sodium hydroxide (NaOH), 1.8 g/L sodium carbonate (Na_2CO_3), and 4.5 g/L of 35% hydrogen peroxide, along with 1 g/L each of a wetting agent, sequestering agent, and detergent. The bleaching process was performed at 90°C for one hour using an IR lab dyeing machine. Hydrogen peroxide, though stable in acidic conditions, becomes an effective bleaching agent in alkaline environments or at elevated temperatures. In water, it releases perhydroxyl ions (HO_2^-), which act as weak dibasic acids. These ions are highly reactive and, in the presence of oxidizable substances such as colored impurities in cotton, they decompose resulting in the bleaching effect. Sodium hydroxide enhances this reaction by neutralizing hydrogen ions, promoting oxygen release.

$H_2O_2 \sim H^+ + HO^{2-}$

$H_2O_2 \sim H^+ + HO^{2-}\ OH^- \rightarrow HO^{2-} + H_2O$

Finally, sample weighing 5 grams is selected. The dye bath is prepared using a material-to-liquor ratio of 1:15, meaning 75 mL of dye solution which contains 1 gm/L Levelling Agent, 40 g/L glauber salt, combination of Bezaktiv Red S-2B (1%), Yellow 3-R 150 (1%), and Blue S-RN (0.5%) which is used to create the desired shade. The dye bath is heated gradually to a temperature range between 60°C and 90°C. The fabric is dyed under these conditions for 30 minutes using an IR Lab Dyeing Machine. During this time, the dye is absorbed and chemically bonds with the fiber, aided by the elevated temperature and alkaline environment. Once dyeing is complete, the fabric is rinsed to remove any residual or unfixed dye. Additional washing with detergent may be carried out to enhance color fastness and ensure the fabric is clean and ready for further use or testing [13].

Eactive dyes are chosen for their ability to form strong chemical bonds with textile fibers. They contain reactive sites that interact with nucleophilic groups on the fiber—typically hydroxyl groups in cotton—to form covalent bonds. These bonds firmly attach the dye molecules to the fabric, resulting in excellent wash fastness. The reactive sites in these dyes are usually electrophilic in nature and are often part of a specific functional group. Some reactive dyes contain multiple reactive groups or a combination of different types, enhancing their bonding efficiency and color fixation [15].

3.3.1 Copper Particle Formation

At first filled one glass holder with 100 mL of water. Broken up the copper sulfate in the crate blended until every one of the solids are disintegrated this could require 5 to 10 minutes. Filled the compartment with 100 mL of heated water. Broken down the ascorbic acid in the hot water mixed until all disintegrated this may likewise require a few minutes and warmed the arrangement a while later. Then poured the hot ascorbic acid arrangement into the holder of copper sulfate solution and stirred the solution that quickly transformed into a dim greenish earthy colored tone. After a couple of seconds metallic copper started to encourage out of arrangement and settle at the base which has a salmon tone. Mixed until all of the copper precipitates out the arrangement became lighter in the end transformed into emerald green while the layer of copper became thicker.8. Allowed all the copper particles to settle to the bottom of the container, then slowly decant off the green liquid layer. Covered the copper particles with a layer of refined water to keep the copper from oxidizing in the air, particles are prepared to apply. Then Copper particle was stored in a new glass compartment.

3.3.2 Fabric Treatment with Copper Particles

The fabric samples were treated with a solution containing 15% copper nanoparticles using an oscillating dyeing machine. The finishing process was carried out at 60°C with a material-to-liquor ratio of 1:15 for 30 minutes. After treatment, the samples were dried at 80°C for 10 minutes. Samples are ready for antimicrobial test.

Antimicrobial Assessment of Cotton Fabric Samples

Samples are sent Atomic Energy Research Establishment for testing antimicrobial property of the prepared samples. The antimicrobial effectiveness was assessed using the Zone of Inhibition Test. Also known as the Kirby-Bauer Test, Disk Diffusion Test, or Agar Diffusion Test, this method provides a fast and cost-effective way to evaluate the antimicrobial activity of a substance or material against a specific microorganism. It is commonly used by researchers working with antimicrobial textiles, surfaces, and liquids to quickly measure and compare the extent of microbial inhibition [16]. The Kirby-Bauer test is a method used to assess the effectiveness of an antibiotic against a specific microorganism. If the antimicrobial agent is effective under the given conditions, bacterial growth will be suppressed in areas where the concentration of the agent in the agar exceeds the minimum inhibitory level. This area, where no bacterial growth is observed, is referred to as the zone of inhibition.

In this test, a thin, even layer of bacteria is spread onto the surface of an agar plate. The antimicrobial substance, either in solid or liquid form, is then applied—commonly by placing a disk or introducing the liquid into a small well in the agar. The plate is then incubated at a temperature suitable for the test organism, typically for 18 to 24 hours. If the antimicrobial agent diffuses into the surrounding agar and inhibits bacterial growth, a clear circular zone will form around the application site, indicating the agent's effectiveness. This test method allows for the rapid screening of multiple samples for antimicrobial activity. It can be used to evaluate various types of antimicrobial products, including liquids, coated surfaces, and solid materials treated with antimicrobial agents. Products that release antimicrobial substances—such as silver ions—into the agar medium generally produce more noticeable zones of inhibition than those where the agents remain bound to the material or are not water-soluble.

It's important to note that this test only shows whether microbial growth has been stopped—it does not confirm that the microorganisms were killed. In some cases, components in the agar medium itself can interfere with the performance of the antimicrobial agent. Additionally, this method is not suitable for testing antiviral activity, as viruses do not replicate on agar like bacteria do. Variability can also occur in the results, and the inhibition zones may not always have distinct or uniform shapes. The size of the zone of inhibition generally reflects how strong the antimicrobial agent is — a larger zone usually means the substance is more effective at stopping microbial growth. This method is quick and low-cost compared to other laboratory tests. It is especially useful for checking how well water-soluble antimicrobial agents can prevent the growth of microorganisms, even though the results are mainly qualitative. Antimicrobial activity of samples is tested against four common microorganism which are Bacillus subtilis,

Staphylococcus aureus, E. coli & Salmonella enteridis and found zone of inhibition as shown in Figure 3, Figure 4, Figure 5 and Figure 6.

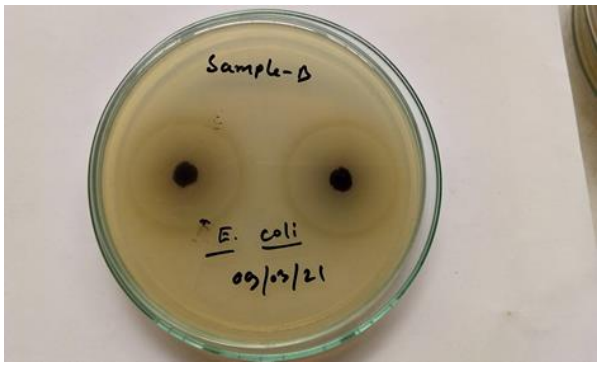

Figure 3. Assessment of E.coli

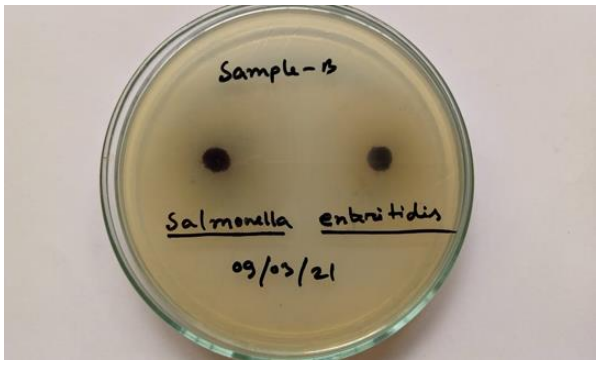

Figure 4. Assessment of Salmonella Enteridis

Figure 5. Assessment of Staphylococcus Aureus

Figure 6. Assessment of Bacillus Subtilis

4. RESULT AND DISCUSSION

Staphylococcus aureus is a gram-positive, spherical-shaped bacterium that commonly causes skin infections but can also lead to more severe conditions such as pneumonia, infections of the heart valves, and bone-related diseases. Escherichia coli (E. coli) is a type of bacteria typically found in the intestines of humans and some animals. While most strains are harmless and even beneficial for maintaining a healthy digestive system, certain strains can cause illnesses such as diarrhea, especially after consuming contaminated food or water. E. coli can be identified by counting the number of consistently yellow-brown colonies that grow on a 0.45-micron membrane filter placed on m-TEC medium, incubated at 35.0°C for 22 to 24 hours [17]. The presence of E. coli colonies can be confirmed by adding a urea substrate, which helps in their identification. Salmonella is a genus of rod-shaped, gram-negative bacteria that belongs to the Enterobacteriaceae family [18]. There are two main species within the Salmonella genus: Salmonella enterica and Salmonella bongori. S. enterica is considered the type species and is further classified into six subspecies. A comprehensive test for Salmonella enteritidis is often conducted to detect the presence of this potentially harmful and pathogenic bacterium [18]. Salmonella can be transmitted through contaminated water, air, food, or more directly via anal-oral contact. To identify and count Shigella and Salmonella, samples are cultured on selective media plates specifically designed to support their growth. he diameter of the zone of inhibition, as calculated using Equation 1, serves as a measure to evaluate the susceptibility or resistance of bacteria to a particular antimicrobial agent [19].

$$\text{Diameter} = \sqrt{\left(\frac{\pi \times Area}{4}\right)} \qquad 1$$

The final zone of inhibition measurements is compared to the standard values provided by the Clinical and Laboratory Standards Institute (CLSI) using the Kirby-Bauer antibiotic testing method. These values help determine if bacteria are susceptible, intermediate, or resistant to a specific antibiotic. Generally, a larger zone diameter indicates greater effectiveness of the antibiotic. The CLSI maintains well-established and standardized ranges for these measurements. According to CLSI M100 (2020) if the zone diameter is 15 mm or less, the bacteria are considered resistant (R), meaning the antibiotic is likely not effective. If the diameter falls between 15 mm and 29 mm, it is classified as intermediate (I). This suggests the antibiotic may work in some cases, but its effectiveness could be limited, depending on the drug concentration and infection site. If the zone diameter is 30 mm or more (or meets the susceptible breakpoint), the bacteria are susceptible (S), and the antibiotic is expected to be effective when used at standard doses. The intermediate category also acts as a buffer zone to account for minor variations in testing procedures and to reduce misinterpretations caused by small technical differences [20]. The zone of inhibition is measured in millimeters (mm) and reflects how well the product prevents bacterial growth around a sample disk.

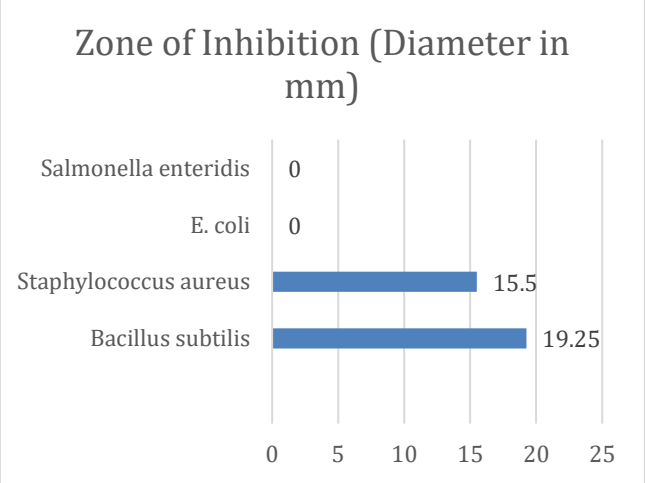

Figure 7. Zone of Inhibition Found Against the Microorganisms

Bacillus subtilis found zone of Inhibition of 19.25 mm as shown in Figure 7. This value suggests that the sample exhibits moderate antimicrobial activity against Bacillus subtilis. Based on typical CLSI standards, this might fall into the intermediate or susceptible category, indicating that the sample can inhibit the growth of this gram-positive bacterium to a notable extent. Staphylococcus aureus found zone of Inhibition of 15.5 mm as shown in Figure 7. His is a smaller inhibition zone, suggesting borderline or reduced sensitivity. Depending on the CLSI standard used, this may fall under intermediate or resistant categories, implying that Staphylococcus aureus is less affected by the sample. Escherichia coli (E. coli) found zone of Inhibition of 0 mm as shown in Figure 7. No inhibition was observed, which means the sample has no antimicrobial effect against E. coli, a gram-negative bacterium. It is considered resistant to Samples. Salmonella enteritidis found zone of Inhibition of 0 mm as shown in Figure 7. Similar to E. coli, no inhibition was detected, indicating that Salmonella enteritidis is also resistant to the sample which are showed in the graph. Bacillus subtilis (19.25 mm), showing good antimicrobial potential which can declare effective against microorganism. Staphylococcus aureus (15.5 mm), with possible limited activity which can declare as partially effective or borderline. E. coli and Salmonella enteritidis (both 0.0 mm), indicating the sample has no activity against these gram-negative bacteria which can declssare as Ineffective.

5. CONCLUSION

Researchers worldwide are actively engaged in developing antimicrobial treatments, with several studies reporting the use of antimicrobial finishes on textiles. Consumers today are increasingly conscious of hygiene and expect a wide range of textile products to have antimicrobial properties. The natural characteristics of textile fibers often support microbial growth, and the structural features or chemical processing of the fabrics can further promote this development.

Copper and its alloys—such as brass, bronze, and copper-nickel—are naturally antimicrobial. When regularly cleaned, surfaces made from uncoated copper alloys can continuously kill harmful bacteria, even those responsible for infections. Bacterial growth is generally inhibited in the presence of antibiotics. However, many strains have developed resistance to certain antibiotics, making it essential to determine the most effective treatment. To address this, a laboratory procedure known as the Kirby-Bauer disk diffusion test is used to evaluate antibiotic efficacy.

Currently, over 400 copper alloy formulations are registered with the U.S. Environmental Protection Agency (EPA) for public health claims related to their ability to eliminate six types of harmful bacteria, including resistant strains like MRSA and VRE. Among these, copper-nickel alloys are especially suitable for frequently touched surfaces due to their high strength, durability, and resistance to corrosion. In this study, copper particles were synthesized from copper sulfate and applied to a fabric sample. The findings confirmed that the treated material exhibited significant antimicrobial activity. Therefore, copper-based treatments can be effectively used in garments that are likely to come into contact with microorganisms.

Recommendations

From this study we can recommend to use copper finished application is for those garments which has possibility to come in contact with microbes' special preference would be medical wears. Sample demonstrates antibacterial activity mainly against gram-positive bacteria, especially Bacillus subtilis. However, it appears ineffective against gram-negative strains, such as E. coli and Salmonella enteritidis, highlighting its limited spectrum of activity. This could inform its potential use in targeting specific bacterial infections or guiding further formulation improvements.

Acknowledgments

The objective of this study was to explore the effectiveness of functional fabrics in inhibiting microbial activity. The experimental work was conducted at the Textile Coloration Laboratory of the National Institute of Textile Engineering and Research (NITER), in collaboration with the Fiber and Polymer Institute under the Bangladesh Council of Scientific and Industrial Research. Antimicrobial testing,

specifically the widely used Kirby-Bauer disk diffusion method, was carried out at the Microbiology Laboratory of the Institute of Food and Radiation Biology (IFRB), part of the Atomic Energy Research Establishment.

In this research, copper particles were synthesized from copper sulfate and applied to fabric samples to evaluate their antimicrobial efficacy. Based on the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines, an antimicrobial agent is considered effective if the diameter of the inhibition zone meets or exceeds the susceptible breakpoint. If the zone is smaller than the resistant breakpoint, the agent is considered ineffective. Zones falling between these two thresholds indicate intermediate activity. Four common microorganisms were selected for testing, and the results were evaluated in accordance with these standards

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All financial support was received for this study was contributed by the three authors of this paper equally. The authors declare no competing interests

Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Mohammad Tanvirul Hasnat	✓	✓	✓	✓		✓		✓	✓	✓	✓			
Md. Taslim	✓	✓				✓	✓	✓			✓	✓	✓	
Md. Mahabub Hasan	✓			✓	✓	✓				✓				

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Conflict of Interest Statement

The authors confirm that there are no financial conflicts of interest associated with this research. However, it should be noted that both the corresponding author and co-author are employed by the institution from which the sample data was obtained.

Informed Consent

Respecting privacy is a fundamental legal right and must not be violated without the individual's informed consent. When the disclosure of personal information is essential for scientific purposes, researchers are required to secure comprehensive informed consent, including written authorization from the participant, before including them in the study. In alignment with this, we confirm that informed consent was obtained from all individuals who participated in this research.

Ethical Approval

This study did not involve the use of human participants or animals. All procedures were conducted in accordance with relevant national regulations and institutional guidelines, and the research received approval from the affiliated institutions of the authors.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Details of the materials used in the experiments, including reagents and software tools, are provided in the supplementary files accompanying this publication.

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