DEVELOPMENT OF ANTIFUNGAL AND UV PROTECTIVE COTTON FABRIC USING GREEN-SYNTHESIZED CUO NANOPARTICLES AND A. NILOTICA ALGAE

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Development of Antifungal and UV Protective Cotton Fabric Using Green-Synthesized CuO Nanoparticles And *A. Nilotica* Algae Methanol Extracts

BY

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ABSTRACT

An innovative method for functional finishing of cotton fabric combining methanol extract of *A. nilotica* algae with copper oxide nanoparticles. Given the continuous need to create functional cotton fabric that is both risk-free and highly effective in comparison to alternative techniques, green synthesis nanoparticles have garnered a great deal of interest in the modern world. In the present work, cotton fabric was functionalized successfully with biosynthesized copper oxide nanoparticles and Azolla nilotica algae methanol extract. Cu_2SO4 was used as a precursor and methanol extract both as a reducing agent and combined with CuO NPs, which were applied through the pad-dry-cure method. Characterization was done using SEM, XRD, TGA, FTIR spectral analysis, and UV-vis spectrophotometer. The average particle size obtained was 21.63 nm. Optimization was done using Box Behenken Design while applying the two active agents. Then, the fabric was evaluated for its antifungal and ultraviolet radiation protection. The optimum value of 20.542mm zone of inhibition of antifungal activities (C. albicans), and UPF with a value of 90.34 was obtained. Therefore, CuO NP and algae extract-coated cotton fabric can be used for medical and other antimicrobial and ultraviolet protection needed applications.

Keywords: Antifungal; Copper oxide nanoparticles; UV protection; green-synthesis; methanol extract

1. INTRODUCTION

Nowadays modern society and end users are demanding more and more surface-modified textile fabrics, which led to the creation of sophisticated textile materials with new and particular features including increased resistance and durability (Sfameni et al., 2023). Cotton is among the dominant textile fibers in the world in the apparel sector due to its numerous advantages, including biocompatibility, non-melting, biodegradability, hydrophobicity, softness, and comfort (Shikder et al., 2023). Thus, such properties of cotton make it widely used in the clothing industry (Mahbubul Bashar and Khan, 2013). Currently, researchers tend to create functional textile materials that can proactively interact with the environment and the wearer's physical state and provide practical protection (Ma et al., 2024). For example, different functional finishes were introduced for cotton fabric previously including, flame retardant, waterproof (Tian et al., 2020), antibacterial and self-cleaning properties, wrinkle resistance, and UV protection properties(Mollick et al., 2023). Given the widespread usage of cotton in homes, businesses, the medical field, etc., antifungal and UV protection qualities are regarded as essential functional properties for cotton fabric. Cotton is a naturally occurring fiber that degrades easily and has a lower UV protection rating. Numerous researches have been done in the past with different chemicals and plant extracts to impart functional properties on cotton fabrics with respect to antifungal and ultraviolet protection(Kamala Nalini and Vijayaraghavan, 2020). The earliest known usage of functional textile treatments dates back to the Egyptians, who covered their mummy wrappings with herbs and spices. Among the artificial and natural materials that have been created are triclosan, quaternary ammonium, polybiguanides, N- halamines, platinum, and chitosan, which are synthetic and natural substances used for antimicrobial properties(Kamel and Hassabo, 2021). Fruits and vegetables contain phenolic compounds, a well-known class of secondary metabolites used for the cotton finishing process. In addition, cotton was treated with a chemical comprising silicon and nitrogen to functionalize cotton fabric(Nabil et al., 2018). Moreover, nonmaterial's composed of metals, metal oxides, ceramics, polymers, and carbon are being employed as finishing agents to impart the necessary functional qualities(Vigneshwaran and Arputharaj, 2020). However, because synthetic fragrances have certain detrimental effects on the environment, attention has turned to natural resources although, plant extracts have limited efficiency and endurance. Nanotechnology becomes an important in multifunctional finishing of cotton fabric through its application in the textile sector, producing fibers with a range of uses and functions, including. UV protection, antibacterial, and anti-odor properties(Patra and Gouda, 2013). Metal Nanoparticles are used alone and combined to make functional cotton fabric. Nanopaticles(Radetić and Marković, 2019) such as copper and copper oxide nanoparticles have been used to impart finish on cellulose-based textiles previously. With the use of peanut waste shell extract as an environmentally friendly reducing agent, a highly effective multifunctional cotton fabric was created using a greener method that involved one-step in-situ production of AgNPs onto chitosan-coated cotton fabric. Although metal nanoparticles impart better functional properties, the cost of meal nanoparticles such as silver is high. Using metal nanoparticles in a synergetic way increases the cost of finishing cotton fabric (Butola and Kumar, 2020). Therefore, materials with low cost and plant extract with better bioactive components in environmentally friendly ways are gaining attention for the fictionalization of cotton fabric. The present study addresses the previously mentioned concerns by combining the advantages of methanol-extracted *A. nilotica* algae and copper oxide nanoparticles to create a cotton fabric with antifungal and UV protection capabilities. The Box-Behnken Design approach has been used to create an ideal formulation for optimization using Design-Expert software. Methanol-extract and CuO Nano particles treated cotton fabric were examined and combined to impart functional cotton fabric.

2. LITERATURE REVIEW

This section reviews previous research on developing functional materials, specifically evaluating the antifungal and UV protection properties of cotton fabrics. The literature review aims to provide background on the key issues addressed in this study, highlighting the significance of the current research. It also explores the various factors influencing the antifungal and UV protection capabilities of cotton, setting the stage for further investigation into enhancing these properties through innovative treatments and technologies.

2.1. CHEMICAL FINISHING OF TEXTILES

Chemical finishing refers to applying chemicals to fabrics to achieve specific properties. This process alters the fabric's elemental composition, as shown by differing analyses before and after treatment. In lightweight construction and membrane manufacturing, textile finishing involves processing outer layers for activation, functionalization, and modification. Multifunctional finishing, in chemical finishing, denotes that the treated textiles can imparted with more than two desired functions using a single process (Wagaw et al., 2024). Multifunctional finishing enhances materials by adding diverse properties like durability, stain resistance, and aesthetic appeal (Anik et al., 2025).

2.2. FUNCTIONALIZING MATERIALS

TRADITIONAL CHEMICALS

Traditionally, organic compounds have been used to impart functional properties such as triclosan for antibacterial activity, benzophenones for ultraviolet protection, dimethylol dihydroxy ethylene urea for wrinkle resistance, fluorocarbons for hydrophobicity, long-chain hydrocarbons and poly dimethylsiloxanes for softness, etc.

PLANT EXTRACTS: Plant extracts, derived from natural materials, are gaining attention in textile dyeing for their ability to produce sophisticated, elegant shades. Beyond aesthetic appeal, these plant-based dyes are being explored as multifunctional agents for enhancing fabric performance. The functional properties of these extracts include antifungal and UV protection properties (Melo Miranda et al., 2025).

NANOMATERIALS: Chemical textile finishing imparts diverse functionalities but poses environmental risks, damaging ecosystems, flora, fauna, and human health through lifecycle impacts. Nanotechnology offers immense commercial potential in textiles, providing long-lasting properties that traditional methods often fail to achieve, even after laundering (Shabbir and Mohammad, 2017).

SYNTHESIS OF CuO NPs: It has interesting physicochemical features and are present in various oxidation states, viz., Cu^0, Cu^I, Cu^{II}, and Cu^{III}. They are chemically inert and thermal stability and synthesis with different methods (Grigore et al., 2016). Biological methods have been used for this research.

SYNERGY AND ITS MECHANISM: Synergy refers to the collaboration of agents, substances, or forces that produce a combined effect greater than their individual contributions. This mechanism enables innovative solutions and improvements, (Pezzani et al., 2019).

2. MATERIALS AND METHODS

2.1. MATERIALS

The Hawassa University Technology Institute Textile and Garment Department provides 100% plain cotton woven fabric with the following specifications in

Table 1. Algae have been collected from Fikir Haik Hawassa Ethiopia. Identification of the species was done at the agriculture laboratory room. It was identified as *A. nilotica* algae. The chemicals used were, copper sulfate (99.0%, pure), Ethanol, and sodium hydroxide (99.8% pure) were purchased from the market. All chemicals used were analytical-grade chemicals.

Table 1. Specification of plain cotton fabric

Fabric structure	Fabric type	Count (Ne)	EPI (Ends per inch)	PPI (Picks per inch)	Weight (gm/m^2)	Thickness (cm)
Plain weave	Cotton	30s	60	60	145	0.1

2.2. PREPARATION OF PLANT EXTRACT

To prepare fine-sized algae powder the collected algae was washed with tap water to remove specks of dirt. Then, dry with sun shad, and powdered using a mixer grinder. The resulting powder was passed through a 250μm sieve mesh. The extraction was done Soxhlet extraction method by adding methanol in a round bottom flask and heating it at $60^{o}C$ till its boiling point. The apparatus allows the rising methanol vapor to pass through the condenser, where it cools and condenses back into liquid form. After that, this liquid falls onto the 15g of powder inside the extraction chamber. Then, extraction was continued for 4 hours. Finally, methanol was removed through a rotary evaporator then extract become ready for the next processes.

Figure 1. Experimental setup of soxhlet extraction processes

2.3. CuO NPs SYNTHESIS PROCESSES

To produce CuO NPs, 0.3MCuSO4 was dissolved in 200ml of distilled water within a 1000ml baker at room temperature. Then algae extract which was prepared with the soxhlet extraction method was combined with CuSO4 solution at a 2:1(v/v ratio i.e. extract to copper sulfate), simultaneously, sodium hydroxide solution was added to the mixture dropwise to maintain the pH of the solution 8 though buffering the solution. Then the mixture was heated on a magnetic stirrer at 60°C for 3hrs and the solution was allowed to cool at room temperature. Finally, the dark brown color was separated from the cooled mixture using a centrifuge at 10,000 rpm for 10 minutes. Then it was washed with distilled water to remove impurities and make the pH neutral. The dark brown precipitate was kept in an oven at 60°C for 24 hours. To ensure that all of the moisture had evaporated, the dried CuO nanoparticles were placed in a furnace set at 400 °C for 2 hours. Finally, copper oxide nanoparticles are packed with desiccators for later usage as shown in **Figure 2.**

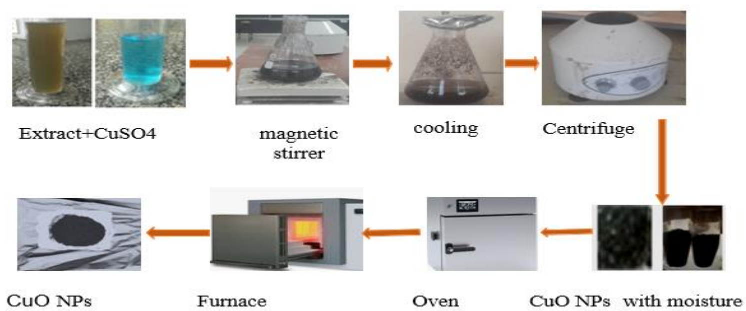


Figure 2. Experimental setup of copper oxide nanoparticle synthesis process

Percentage yield of biosynthesized copper oxide nanoparticles: The percentage yield of biosynthesized copper oxide nanoparticles refers to the efficiency of the nanoparticle production process. It is calculated by comparing the actual amount of copper oxide nanoparticles obtained to the theoretical maximum that could be produced. Higher yields indicate more efficient synthesis and better utilization of resources. The extraction yield was calculated using the following formula.

$$Yiled = \frac{Final\ weight\ of\ NPs}{Initial\ weight\ of\ precursor} x100 \qquad (1)$$

CHARACTERIZATION: Copper oxide nanoparticle was characterized by using UV-Vis spectrophotometers typically covering a wavelength range from 190 nm to 1100 nm at a best resolution ranging from 1nm to 5 nm with an operating range of 20-25°C. Fourier Transform Infrared Spectroscopy (FTIR) Perkin Elmer version over the frequency range from 4000-500 with a resolution of 0.5cm^{-1} with an operating range of 5-45°C used to identify functional groups present. X-ray diffraction (XRD) was used to identify the crystal structure, crystallographic orientation, average particle size, and phase

composition of nanomaterial. The other characterization for copper oxide nanoparticles is a scanning electron microscope (SEM) which is used to determine the surface morphology of the copper oxide Nan particle to investigate its morphological structure.

2.4. FACTOR SELECTION AND OPTIMIZATION PROCESSES

The Box-Behnken design was used to develop an experimental plan with 14 runs, utilizing Design Expert software. It focused on three variables: methanol extract of *A. nilotica* algae, CuO nanoparticles (NPs), and temperature, with ranges of 10-30%, 0.1-0.5M, and 30-50°C, respectively. The pad-dry-cure method applied the active agents to cotton fabric, using a 1:30 material-to-liquor ratio and a pH of 8. The fabric was dried at 80°C for 30 minutes and cured at 140°C for 3 minutes.

ANTIFUNGAL TEST: The zone of inhibition method was utilized to assess the treated fabric's antifungal activity (C. albicans) using a qualitative agar diffusion plate test following "European Committee on Antimicrobial Susceptibility Testing (EUCAST)" methods(Kahlmeter et al., 2006). The bacteriostatic agar medium was made by mixing dried agar powder with distilled water, heating the mixture to a boil, and autoclaving it for 15 minutes at 121°C. After autoclaving, the agar was cooled to 45–50°C and then put into sterile Petri dishes. Using a sterile inoculating spreader, the agar was then allowed to solidify at room temperature until the depth reached 2 mm. To inoculate the agar plate, a 24- hour broth culture of the test fungus was equally dispersed across its surface. To conduct this test, a 15 × 15 mm sample of the treated fabric was placed on an agar plate that had been inoculated with a specific strain of fungus. The agar plates were then incubated at 37°C for 18 to 24 hours. A larger zone of inhibition suggested increased antifungal efficacy.

UV-PROTECTION TEST: This test method determines the fabric's effectiveness in protecting from ultraviolet radiation. The sample was exposed to UV radiation, and the amount of radiation transmitted and blocked was measured. The result is expressed in terms of the ultraviolet protection factor which was categorized into UPF protection categories such as, excellent (40-50+), very good (25-39), and good (15-24) categories (Bashari et al., 2019). Each sample was calculated using the following formula as per the AATCC 183-2000 method.

$$UPF = \frac{\int_{280\,\text{nm}}^{400\,\text{nm}} E_\lambda \times S_\lambda \times T_\lambda \times \Delta\lambda}{\int_{280\,\text{nm}}^{400\,\text{nm}} E_\lambda \times S_\lambda \times \Delta\lambda} \quad (2)$$

Where, $E\lambda$ = relative erythemal spectral effectiveness, $S\lambda$ =solar spectral irradiance, $T\lambda$ = average spectral transmittance of the sample, and $\Delta\lambda$ = measured wavelength interval (nm)

AIR PERMEABILITY TEST: It is challenging to accurately determine from other metrics since it depends on the porosity of the fabric, the amount of air voids in the fabric, yarn specifications, thickness, and other factors. Since it has to do with breathability, it is the primary method for testing cloth comfort properties. The FX 3300 air permeability tester ASTMD737-96 was used for the testing.

TENSILE STRENGTH TEST: This test assesses the fabric's strength and flexibility after it has been treated with nanoparticles. The test was carried out following ISO 13934/2 guidelines, and the findings provide significant information about how the Nano treatment affects the fabric's mechanical qualities, including its tensile strength and elongation characteristics.

TEAR STRENGTH TEST: Using a recorded constant-rate-of-extension type tensile testing machine, the ASTM D5587 test technique measures the tearing strength of textile materials using the trapezoid process.

FABRIC STIFFNESS TESTING: Variations in fabric stiffness and bending angle length have an impact on the drapes and handle properties of fabrics. The stiffness of the fabric was assessed to know the effect of finishing chemicals on stiffness in both the warp and weft directions. ISO13934 stiffness test method was used, to measure the bending lengths of the treated and control samples, through measuring a 25 by 25 cm sample fabric, and the test was done by averaging three samples.

$$Bending\ length = falling\ length\ x\ (\theta) \qquad (3)$$

3. RESULT AND DISCUSSION

3.1. CHARACTERIZATION OF PLANT EXTRACT

The FTIR spectra determined the plant extract's potential functional biomolecules. The spectra of the methanolic extract of Azolla nilotica algae used for the reduction and stabilization of CuO NPs in the biosynthesis process are shown in **Figure 3.** The peak at 3250 cm^{-1} is the highest wave number observed in the methanol extract of *A. nilotica*, indicating strong O-H bonding associated with hydroxyl groups. In contrast, the peak at 750 cm^{-1} reflects the bending vibrations of O-H groups. The stretching of -CH bonds in aliphatic groups is represented by a peak at 2152 cm^{-1} additionally, the absorption at 1654 cm^{-1} corresponds to the bonds of COO^- anions and C=C aromatic structures. The conjugation of C=O groups from aromatic rings is indicated by a peak at 1250 cm^{-1}, while the bending vibrations of the C-OH alcoholic group and C-O single bond vibrations from ether linkages are observed at 1062 cm^{-1}.These functional groups C-O, O-H, C=O, and COOH- exhibit a strong affinity for binding metals, facilitating the formation of highly stable nanoparticles(Neouze and Schubert, 2008).

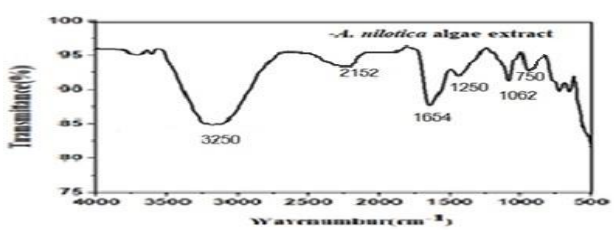


Figure 3. FTIR Spectra of hot water extract of *A. nilotica* algae extract

3.2. CHARACTERIZATION OF CUO NPS

The methanolic extract of Azolla nilotica was used to synthesize CuO NPs. The extraction yield obtained was 67.8% among 9.58g of copper sulfate with an actual yield of 6.5g of CuO NPs characterization was made with the following parameters.

FTIR ANALYSIS: The extract bimolecular contributes to the formation of CuO NPs, improving their ability to reduce, stabilize, and cap the nanoparticles. A broad absorption peak was observed in the FTIR spectrum of CuO NPs which is caused by the adsorbed water molecules and functional group of the reducing agents. The adsorption of water is due to the high surface area to volume ratio of the nanoparticles. Figure 4 (A) shows the FTIR spectra recorded in the solid phase at a range of 4000 - 500 cm^{-1}. The peak at 3125 cm^{-1} corresponds to the strong O-H stretching vibration of hydroxyl groups. In contrast, the peak at 775 cm^{-1} is attributed to the bending vibrations of O-H groups. The peak at 2150 cm^{-1} is related to the -CH stretching of aliphatic groups, while the 1590 cm^{-1} peak represents the vibrations of COO^{-} anions and C=C aromatic conjugates. The peak at 1400 cm^{-1} signifies the conjugation of C=O groups in aromatic rings, and the 1022 cm^{-1} peak is associated with the bending vibrations of the C-OH alcoholic group as well as C-O single bond vibrations in ether linkages. Additionally, the presence of the -C=O bond indicates the aromatic nature of the synthesized nanoparticles.

UV-VIS SPECTROSCOPIC ANALYSIS: UV-visible spectroscopy is a particularly useful technique for studying metal nanoparticles since peak positions, and shapes are sensitive to particle size. The signature of the creation of nanoparticles is the surface Plasmon resonance peak according to this study. When the photon and the free electron energy in the nanoparticles are identical, the electrons oscillate and produce a surface Plasmon(Szunerits et al., 2014). The characteristics of surface Plasmon resonance (SPR) with absorption of 422 nm were discovered in this research. This shows the presence of copper oxide nanoparticles in the nanoparticle's UV-visible absorption spectrum.

SEM ANALYSIS: Figure 4 (C), illustrates how the surface morphology of copper oxide nanoparticles was observed using SEM at magnifications of 6000x and 3000x. This analysis was important because the antifungal activity of the nanoparticle depends on the morphology of the nanoparticles. The SEM picture of the samples displays white backgrounds with low and high aggregation as well as some scattered dark spots that may represent phytochemical components dispersed on copper oxide nanoparticles. As can be seen from Figure 4 (C), the nanoparticles appeared small and some agglomerated, with different morphologies from spherical shapes of various sizes to amorphous shapes. According to SEM pictures of copper oxide nanoparticles captured at 6000x magnification, the particles are distributed well and have a uniform, well-defined crystal structure with few aggregations. The micrograph-derived CuO nanoparticles recorded at 3000x magnification by SEM exhibited as voluminous and a little agglomerated. This is usually explained as a common way to minimize their surface-free energy due to polarity and the strong electrostatic attractive inter-particle force of CuO NPs(Foroozesh and Kumar, 2020).

XRD ANALYSIS: The synthesized CuO NPs from copper sulfate and *A. nilotica* algae extract were confirmed 2θ degrees using X-ray diffraction measurements. The monoclinic crystal system CuO (C2/c space group, JCPDS card no. 45-0937) can be used to index all CuO NPs' peaks. The absence of distinct impurity peaks indicates that premium CuONPs were produced (Ahamed et al., 2014). Furthermore, the well-defined and very sharp peaks of CuO NP reflection observed from the XRD patterns graph showed that the obtained CuO NPs contained a high crystalline nature(Phang et al., 2021). The mean average crystal size of the synthesized CuO NP was calculated using Scherrer's formula. The average crystal size of CuO NPs corresponding to the highest intense peaks is 21.63 nm.

$$D = \frac{K\lambda}{\beta \, Cos\,(\theta)} \qquad (4)$$

Where, D is the nanoparticle crystalline size, K represents the Scherrer constant (0.98), λ denotes the wavelength (1.54), and β denotes the full width at half maximum (FWHM).

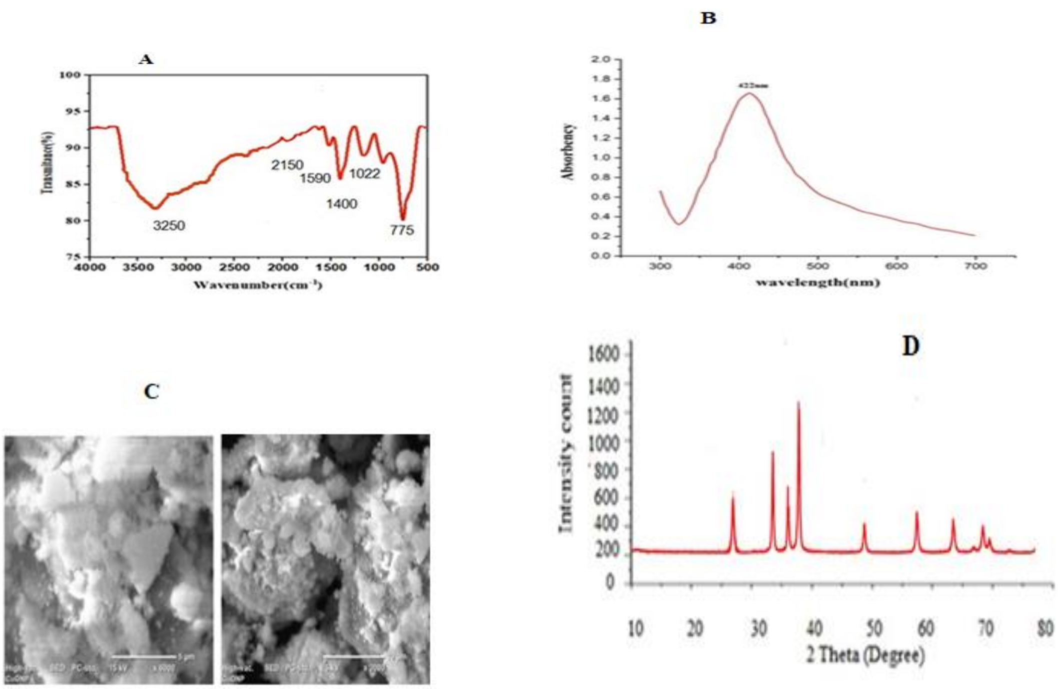


Figure 4. FTIR spectra of Copper oxide nanoparticles A, UV-visible analyses of copper oxide nanoparticles B, SEM images of synthesized copper oxide nanoparticles C, and XRD patterns of CuO NPs synthesized using *A. nilotica* algae extract D.

3.3. DATA PRESENTATION

Formulation techniques in coatings development were made by employing the two active agent's the 14 experimental runs are shown in **Figure 5** (A), which provide a better understanding of how various parameters affect coating qualities. The antifungal test for the experimental runs was done, and the results

were presented by the zone of inhibition method.

However, the samples without inhibition zones concede that they do not have an antifungal effect. Therefore, the control sample, which was not treated with antifungal active agents, did not show a clear inhibition zone. This means the antifungal activity of the untreated cotton fabric was too much less compared to the treated one. The test mechanism was done by putting a 15x15mm fabric sample on a nutrient agar plate, inoculating it with a fungal culture, and then incubating it. Following incubation, the circumference of the inhibition zone surrounding the fabric sample was measured carefully. The measured test results were expressed in millimetres of diameter, as shown in **Figure 5** (B). Each sample was levelled with a number and letter, for example, **F1** indicates the sample for run number one and likewise forothers.

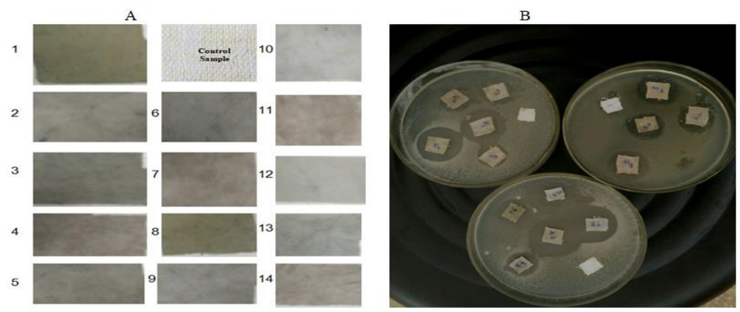


Figure 5. Coated sapless for fourteen experimental runs (A) and antifungal tests for total run samples (B)

Table 3 experimental run for the optimization process

	Factor1	**Factor 2**	**Factor 3**	**Response 1**	**Response 2**
Run numbers	**Methanol extracts%**	**B: CuO NPs**	**Temperature**	**ZOI antifungal activities**	**UPF**
	%	Molarities	^{o}C	mm	ratings
1	30	0.3	50	15	78.08
2	40	0.1	50	15	82.38
3	20	0.1	40	10	73.05
4	30	0.1	40	13	76.4
5	30	0.5	30	21	77.27
6	30	0.5	40	25	100
7	30	0.1	50	12	74.8
8	40	0.3	30	18	88.7
9	20	0.3	50	14	83.51
10	20	0.5	40	18	89.3
11	30	0.3	50	16	84.89
12	40	0.3	40	17	95.93
13	30	0.5	30	20	90.43
14	20	0.3	30	15	84.8

3.4. DATA ANALYSIS ON ANTIFUNGAL ACTIVITIES (RESPONSE 1)

Data was analyzed for the response of zone of inhibition for susceptibility of antifungal activities *(C.*

albicans) concerning the active agents treated on cotton fabric with the help of design expert software analysis tools. The effect of factors of methanol extract, copper oxide nanoparticles, and temperature on the response inhibition of *C. albicans* fungal activity was analyzed using the following parameters.

ANALYSIS OF VARIANCE

Table 4 ANOVA on the zone of inhibition of *C. albicans* fungi

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	151.96	9	16.88	38.59	0.0016	Significant
A- methanol extracts	28.12	1	28.12	64.29	0.0083	
B-CuO NPs	120.12	1	120.12	274.57	< 0.0001	
C-Temperature	2.00	1	2.00	4.57	0.0993	
AB	36.32	1	36.32	76.76	0.0013	
AC	3.78	1	3.78	5.87	0.0789	
BC	3287	1	32.87	58.71	0.0091	
A^2	0.0500	1	0.0500	0.1143	0.7523	
B^2	1.25	1	1.25	2.86	0.1662	
C^2	0.4500	1	0.4500	1.03	0.3679	
Residual	1.75	4	0.4375			
Lack of Fit	1.25	3	0.4167	0.8333	0.6466	Not significant
Pure Error	0.5000	1	0.5000			

It is evident from the preceding table that (A, B, C, AB, AC, and BC) are important model terms. Whereas (A^2, B^2, and C^2) are not significant terms for the provided model since their P-value is more than 0.05 of the 95% confidence intervals. The majority of terms (A, B, C, AB, AC, and BC) had a significant impact on the response of the zone of inhibition antifungal activities, according to the ANOVA table. The lack of fit F-value of 0.8333 implies the lack of fit is not significant relative to the pure error. The P-value at a 95% confidence interval shows 0.6466 which is greater than 0.05(0.6466>0.05) lack of fit is not significant. Non-significant lack of fit is good for the required model to fit. This indicates that the response zone inhibition of *C. albicans* fungal activity with the factor methanol extract, copper oxide nanoparticles, and temperature is properly observed by a quadratic model.

FIT STATISTICS: The Predicted R^2 of 0.9703 is in reasonable agreement with the Adjusted R^2 of 0.9819; i.e. the diffusion-to-noise than 0.2. Adequate precision measures the signal-to-noise ratio. Ratio greater than 4 is desirable. The ratio of 45.145 indicates an adequate signal. This model has been used to navigate the design space.

REGRESSION EQUATION: The regression equation includes only significant factor coefficients. The quadratic model, involving three factors, is expressed by a formula that captures their individual effects.

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+...+\beta_nXn \quad (5)$$

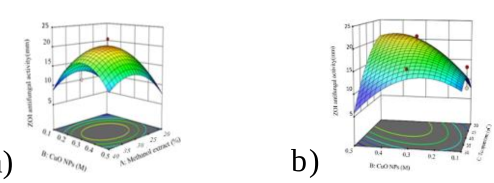
Where, β_0 is the intercept, representing the expected value of Y when all X values are zero. β_1, β_2, β_n are the coefficients for each independent variable.

Y=18.5+3.21A+4.87B+3.56AB+2.99BC implies the equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The high levels of the factors have a higher impact in this equation with a coefficient of 4.87 which implies it is the main responsible factor for inhibiting *C. albicans* since the coded equation is useful for identifying the relative impact of the factors by comparing them with the factor coefficients.

FFECT OF MAIN FACTOR ON RESPONSE: The effect of the main factor on response is a simple method which was done for evaluating the influence of a single factor on the response in design expert software. It used to calculate the effect of primary factors, through analyzing the average change in the response variable by raising the level of a single factor. Factor A (methanol extracts) present in *A. nilotica* algae extracts, such as flavonoid and tannin have been demonstrated antifungal activity against *C. albicans* species(Hsu et al., 2021). The graph of the methanol extract against *C. albican* shows an increasing effect. Therefore, further increasing the concentration of methanol extract of *A. algae* extracts would likely lead to even greater antifungal potency and inhibition of *C. albicans(Rahman et al., 2023)*. In the case of factor B has a sharp increasing effect as shown in **Figure 6**. As the concentration increased from 0.1 to 0.5M, there is an increase in susceptibility for *C. albicans* was observed. This implies the antioxidant activity of copper oxide nanoparticles increases as the concentrations increase and a clear inhibition zone has been observed(Bezza et al., 2020).

Figure 6. Effect of main factors on the zone of inhibition of antifungal activities

3D SURFACE: The combined effect of important independent variables is displayed on the 3D surface. Which come from copper oxide nanoparticles, methanol extract, and temperature on the three-dimensional graph that corresponds to the response on the zone of inhibition for antifungal activities *(C. albicans)*. The combined effects of the components on the 3D graph are shown in **Figure 7.**



a) b)

Figure 7. Methanol extract with CuO NPs (a) and CuO NPs with temperature (b) on 3D surface of the zone of inhibition of antifungal activities

The three-dimensional surface (a) shows the combined effect of factors with methanol extract and copper oxide nanoparticles on the response ZOI of antifungal activities. The maximum zone of inhibition of fungal activity for *C.albicans* was 18mm obtained with the factor CuO NPs 0.3 molarity and methanol extracts of 20% respectively as shown in **Figure 7** (a). This is because the nanoparticles' release of copper ions causes the negatively charged fungal cell wall to burst, denaturing proteins and ultimately resulting in cell death. Concurrently, the fungal cell wall and the hydroxyl radicals from the Azolla nylotica algae extract react, causing the fungal cell wall to burst(Naika et al., 2015). The combined effect of factors with Copper oxide nanoparticles and temperature on the response zone of inhibition of antifungal activities in **Figure 7** (b). The maximum zone of inhibition of fungal activity for candida albicans was 18mm and was obtained with the factor CuO NPs 0.3 molarity and temperature of 45 degree centigrade respectively. As the temperature increases the antifungal activity also increases since the release of copper ions toward the negatively charged fungi (*C.albicans*) activities of CuO NPs increase(Wahab et al., 2023).

3.5. DATA ANALYSIS ON UPF (RESPONSE2)

ANOVA: Analysis of variance for the effect of previously mentioned factors on the response of ultraviolet radiation protection was analyzed by ANOVA.

Table 6. ANOVA on percent blocking of ultraviolet radiation protection

Source	Sum of Squares	df	Mean Square	F-value	P-value
Model	437.22	9	48.58	57.55	0.0007
A- methanol extracts	45.60	1	45.60	54.03	0.0018
B-CuO NPs	11.90	1	11.90	14.10	0.0199
C-Temperature	1.54	1	1.54	1.82	0.2481
AB	361.94	1	361.94	428.80	< 0.0001
AC	0.0025	1	0.0025	0.0030	0.9592
BC	48.05	1	48.05	58.33	0.0158
A²	3.19	1	3.19	3.78	0.1237
B²	9.51	1	9.51	11.26	0.0284
C²	0.0022	1	0.0022	0.0026	0.9617
Residual	3.38	4	0.8441		
Lack of Fit	2.88	3	0.9588	1.92	0.6466 Not significant
Pure Error	0.5000	1	0.5000		

Table 6 shows that significant model terms include (A, B, AB, BC, and B²), while other factors are not significant, as their P-values exceed 0.05 within the 95% confidence interval. The lack of fit F-value of 1.92 indicates the lack of fit is not significant relative to the pure error.

The P-value of 0.4776, greater than 0.05, further confirms this. This non-significant lack of fit suggests the quadratic model effectively captures the UPF with the factors of phenolic compounds, copper oxide nanoparticles, and temperature.

FIT STATISTICS: The Predicted R^2 of 0.8910 is in reasonable agreement with the Adjusted R^2 of 0.9751; i.e. the difference is less than 0.2. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 23.475 indicates an adequate signal. Therefore, the model was used to navigate the design space.

REGRESSION EQUATION: In the regression equation, only coefficients of significant factors are included. The quadratic model of three factors is given by the previous equation (5) for determining the dependent variables or the response. From the coded equation the regression formula will be: $Y= 87.5+4.50A+3.01B+7.73AB+3.21BC-1.72B^2$. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing them with the factor coefficients. The factors with higher coefficients have a higher impact on the response. In this equation factor AB with a coefficient of 7.77 has a significant impact on the response which implies, that it is the main responsible factor for the response UPF. This is due to the nature f CuO NPs which helps to create a barrier that absorbs and scatters UV rays on its structure, and morphology, preventing them from penetrating the fabric. *A. nilotica* algae extracts contain anti-oxidants capable of reacting with UV radiations and creating a barrier as copper oxide nanoparticles do (Čuk et al., 2021). When these are combined, cotton fabric with better UPF has been obtained. The negative coefficients show an inverse relation against the response.

EFFECT OF MAIN FACTOR ON RESPONSE: The effect of a single factor on the response is a simple approach to assess how one variable influences the outcome in Design Expert software. The average change in the response variable, caused by increasing the amount of a single factor, is used to quantify the impact of primary factors, as shown in Figure 8. As the value of factor A increases, the UPF (Ultraviolet Protection Factor) increases from left to right. This is due to the increased presence of hydroxyl free radicals in the methanol-extracted *A. nilotica* algae extract, which is derived from flavonoids, alkaloids, tannins, and other compounds (El-Serafy et al., 2024). The graph demonstrates a positive correlation between methanol extract concentration and UPF, indicating that further increasing the concentration enhances UV protection.

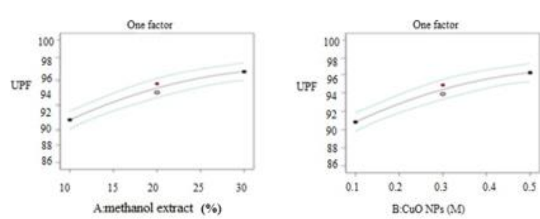


Figure 8. Effect of main factors on UPF

Factor A (methanol extract) of *A. nylotica* algae has an increasing effect on the response ultraviolet protection factor as we go from 10-30 percent. This is due to the presence of ultraviolet barriers in the extract such as flavonoids, tannins, polyphenols, etc. have the capability of trapping radiations. Copper oxide nanoparticles or factor B have a sharp increasing effect as shown in the graph. As the concentration increased 0.1 to 0.5 molarity, there was an increase in the UPF, which means the positive holes that absorb ultraviolet radiation in copper oxide nanoparticles increased as the concentration increased. The electrons and holes react with oxygen and water molecules to generate O_2^-, H_2O_2, and hydroxyl radicals based on the previous literature (Liu et al., 2021).3D SURFACE: The combined effect of copper oxide nanoparticles (CuO NPs), methanol extract, and temperature on ultraviolet protection factor (UPF) is illustrated in the 3D surface plots shown in Figure 14. The 3D surface (c) demonstrates the interaction between CuO NPs and methanol extract, revealing how these factors affect UPF. The highest ultraviolet protection was achieved with a CuO NPs concentration of 0.3 M and a methanol extract concentration of 20%. Additionally, the 3D surface (d) illustrates the effect of CuO NPs and temperature on UPF, showing that the maximum ultraviolet protection was obtained with CuO NPs at 0.3 M and a temperature of 45°C. These results highlight the optimal conditions for UV protection.

c)

d)

Figure 9. Methanol extract of *A. nylotica* algae with CuO NPs (c) and CuO NPs with temperature on 3D surface of ultraviolet radiation protection factor

3.6. OPTIMIZATION PROCESSES

The optimization of the application of a methanol extract of *A. nilotica* algae extract and copper oxide nanoparticles using the pad dry cure method. The maximum optimized result for the response zone of inhibition of the antifungal activity (*C. albicans*) and ultraviolet protection factor was obtained as follow

Table 8. Solution found for the optimization process

Methanol extract	CuO NPs	Temperature	C. albicansUPF	Desirability	
28.756	0.500	50.0	20.54290.339	0.894	Selected

The optimization process yielded a single solution, with the optimum results showing a zone of inhibition for antifungal activity of 20.54 mm and a UPF rating of 90.339. These values were achieved by applying 28.756% methanol extract of *A. nilotica* algae and 0.5 M copper oxide nanoparticles onto plain cotton fabric. Cotton fabric treated with both copper oxide nanoparticles and methanol extract exhibited enhanced UPF and antifungal activity. This improvement is attributed to the copper ions released from the nanoparticles, which exist in different oxidation states (Cu^{+} and Cu^{2+}) and interact with the negatively charged fungal cells. These ions disrupt fungal metabolism by attaching to the cells, inhibiting growth and suppressing respiration (Kamel et al., 2022). The methanol extract of *A. nilotica* algae also affects fungi, similar to the copper oxide nanoparticles, due to its antioxidant properties that interact with fungal cells and contribute to better UV protection (Hamed et al., 2020). Together, the free radicals from copper oxide nanoparticles and the extract trap ultraviolet radiation. The combined effect of these active agents, as shown in Table 8, demonstrates improved antifungal and UV protection properties for the cotton fabric.

3.7. TESTING AND EVALUATION

A combination of methanol extract and copper oxide nanoparticles was applied to the fabric, and elected parameters were evaluated. Every test is necessary to determine the properties of the cotton fabric treated with active agents after coating.

TENSILE STRENGTH TEST: Tensile strength was done by averaging three samples and the result was expressed in terms of percent elongation at break. The effect of copper oxide nanoparticles and methanol extract of *A. nilotica* algae on plain cotton fabric have been shown in **Table 9.**

Table 9. Tensile strength test

S/No.	Sample	Force (N/mm^2)		Elongation at break(%)	
		Warp	Weft	Warp	Weft
1	Control sample	298	274	20.3	14.59
2	Treated sample	298	274	21.5	15.5

Elongation at the break of the treated sample was increased due to the deposition of antioxidants from the extract and copper oxide nanoparticles on the cotton fabric. Both of these components act as reinforcement agents, enhancing interactions between the fibers through the formation of hydrogen bonds, creating a complex with the cotton fabric. As shown in Table 9, the results indicate that the fabric's warp-wise tensile strength is greater than its weft-wise tensile strength. This difference occurs because weft yarn lacks size material, while warp yarn has size material applied during the weaving preparation process. Additionally, there are more ends per inch of fabric than picks per inch.

FABRIC STIFFNESS TESTING: This test was done to analyze the flexibility and handling properties of the treated fabric. It helps to know the impact of the finishing agents on stiffness. Three tests have been done and their average result is shown in **Table 10**.

Table 10. Stiffness test

Sample type	Bending length (cm)	
	Warp	Weft
Control sample	1.6	1.5
Treated sample	1.8	1.6

The result shows that the bending length of the treated plain cotton fabric was greater than that of the control sample. It suggests that the finishing agents are responsible for increasing the stiffness. The formation of grain boundary structures, appropriate nanoparticle loading, and increased interfacial bonding are the main causes of the increase in stiffness of the fabric treated with nanoparticles(De Cicco et al., 2017).

TEAR STRENGTH TEST: Using a recorded constant-rate-of-extension type tensile testing machine, the ASTM D5587 test method measures the tearing strength of textile materials using the trapezoid process. The tear strength of the treated fabric decreases warp-wise but there is an increasing weft-wise direction as shown in **Table 11**. This is due to the reduction in slipping paste of the warp threads in the fabric. However, there is an increase in tear weft-wise direction since the slipping past for weft threads is better than the warp thread in the sample (Shahidi et al., 2018).

Table 11. Tear strength test

Sample type	Tear strength (gram-force)	
	Warp	Weft
Control sample	559.4	528.31
Treated sample	551.8	549.7

AIR PERMEABILITY: The ease of airflow through a fabric is a crucial attribute of textile materials that determines its level of comfort and breathability (Shahid et al., 2021). The air permeability of the control sample is about 18.8 Cm³/cm²/s whereas; the air permeability for methanol extract and copper oxide nanoparticles treated fabrics is 13.5 Cm³/cm²/s. There is a decrease in the air permeability of the fabric due to changes in the surface characteristics. This is because the pore blockage, relaxation shrinkage, and roughness produced by the treated active substances decrease air permeability by raising airflow resistance.

4. CONCLUSION

The goal of this research was to develop an environmentally friendly finishing technique that would yield cotton fabric with better anti-fungal and ultra violet properties. Cotton fabric was functionalized using methanol extract of *A. nilotica* algae and copper oxide nanoparticles. Qualitative analysis was used to identify phytochemicals such as tannin and flavonoids. An algae methanol extract and a magnetic stirrer were used in environmentally friendly synthesis of copper oxide nanoparticles. Using SEM, UV-vis spectrophotometer, FTIR, and XRD, the resultant copper oxide nanoparticles were studied. An average particle size of 21.63 nm was obtained. The optimum results obtained were, antifungal activities (*C. albicans*) with a zone of inhibition of 20.542mm, and UPF of 90.339. These values were obtained with factors or independent variables of copper oxide nanoparticles with a value of 0.5 molarity, a methanol extract of 28.756%, and temperature values of 50 degrees centigrade. The fungal activities were due to methanol extract containing hydroxyl (^{-}OH) radicals that could interact with the cell walls of fungi and hinder the growth of microorganisms. Likewise, copper oxide nanoparticles also release Cu^{2+} and Cu^{+} which can hinder the growth of bacteria through reacting with negatively charged bacterial cells. The hydroxyl radicals brighten the surface of the fabric and the band gap created due to copper oxide nanoparticles enhances the ultraviolet protection ability of a cotton fabric. The final optimized sample was used to evaluate the coated fabric's key characteristics, including tensile strength and water and air permeability. Its comfort was unaffected yet an outstanding result was obtained. An impressive outcome was achieved without affecting its comfort.

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