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Phytochemical-Assisted Green Synthesis of Antioxidant and Antimicrobial Silver Nanoparticles Using *Cichorium Intybus* Extract for Enhanced Medical Cotton Fabrics

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Abstract
This study evaluates antimicrobial cotton fabrics functionalized with silver nanoparticles (AgNPs) biosynthesized using Cichorium intybus L. (chicory) leaf extract. The eco-friendly synthesis leveraged phytoconstituents as dual reducing and stabilizing agents. Characterization via UV-vis spectroscopy (λmax=400 nm) and TEM confirmed spherical AgNPs (4.5–10.9 nm), while SEM-EDX verified uniform coating on cotton. The AgNP-cotton demonstrated enhanced antimicrobial efficacy against Gram-positive Staphylococcus aureus (25 mm inhibition zone), Gram-negative Escherichia coli (23 mm), fungal Candida albicans (25 mm), and Aspergillus niger (21 mm), attributed to AgNP-induced membrane disruption and ROS generation. Phytochemical analysis identified flavonoids, phenolics, and alkaloids with high concentrations (128.17 mg RE/g flavonoids; 459.94 mg GAE/g phenolics). Antioxidant assays revealed strong radical scavenging (IC50: 9.21 µg/mL ABTS; 17.14 µg/mL DPPH). This sustainable integration of phytochemicals and nanotechnology presents eco-friendly antimicrobial textiles with potential for medical applications, such as wound dressings and hospital linens, offering alternatives to conventional treatments.
Keywords: Cichorium intybus; UPLC-QTOF MS; Silver nanoparticles; Green synthesis; Antimicrobial textiles; Antioxidant activity.

1. Introduction

The integration of plant-based antimicrobials with advanced technologies, such as nanoparticle synthesis, has significantly enhanced their potency and stability. This synergistic approach not only amplifies antimicrobial efficacy but also mitigates longstanding challenges, including rapid degradation and low bioavailability. Ongoing investigations into medicinal plants and their bioactive compounds are poised to play a pivotal role in tackling global health issues while simultaneously driving the development of sustainable, eco-friendly innovations across diverse industries [1].

Chicory (*Cichorium intybus*, Family: Asteraceae) is a versatile perennial plant with a centuries-old legacy in traditional medicine and culinary applications. Its roots, leaves, and vibrant blue flowers are abundant in bioactive compounds such as polyphenols, flavonoids, sesquiterpene lactones, carotenoids, tannins, vitamins, and minerals. These phytochemicals endow chicory with diverse therapeutic properties, including antioxidant, anti-inflammatory, and antimicrobial activities, as well as benefits for digestive health, liver function, and blood sugar regulation [2].

Chicory's therapeutic potential is underscored by its rich array of bioactive compounds, including inulin (a prebiotic fiber that enhances gut health), chicoric acid (renowned for its antioxidant and anti-inflammatory effects), and flavonoids such as quercetin and kaempferol, which exhibit potent antimicrobial and cardioprotective properties. These constituents collectively position chicory as a promising candidate for modern medicine, functional foods, and nutraceutical applications [3-4]. Beyond its traditional uses, emerging research highlights chicory's integration with advanced extraction and formulation technologies, broadening its capacity to address

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complex health challenges. These include metabolic disorders [5], chronic inflammation [6], oxidative stress-related conditions, and potential roles in mitigating cardiovascular and neurodegenerative diseases [7]. Such findings reinforce Chicory's significance in developing preventative healthcare strategies.

The integration of nanotechnology has revolutionized the utilization of chicory's bioactive compounds by enhancing their efficacy and bioavailability. Nanoparticle-based delivery systems offer significant advantages, including improved stability, absorption, and targeted delivery of Chicory-derived compounds, thereby maximizing their therapeutic potential [8]. This approach holds promise for medical and pharmaceutical applications, where chicory could serve as a cornerstone for advanced drug delivery systems or multifunctional therapeutic formulations. Moreover, chicory's eco-friendly cultivation and sustainable production further bolster its appeal in the development of health-promoting products, aligning with the increasing demand for natural and safe alternatives. As ongoing research continues to refine its applications, chicory is well-positioned to play a pivotal role in advancing health and wellness across various industries [9].

In recent years, nanotechnology has emerged as a transformative approach for improving the efficacy and stability of antimicrobial agents, alongside plant extract research. Among these, silver nanoparticles (AgNPs) are particularly notable due to their exceptional antimicrobial efficacy, attributed to their high surface area-to-volume ratio and ability to interact with microbial cell membranes. AgNPs can penetrate microbial cell walls, trigger reactive oxygen species (ROS) production, and disrupt DNA replication, leading to microbial cell death. They exhibit broad-spectrum activity against diverse pathogens, including bacteria, fungi, and viruses. Combining AgNPs with plant-derived extracts has demonstrated synergistic potential, and enhanced antimicrobial effects while improving the release kinetics and stability of bioactive constituents [10-11].

The use of silver nanoparticles in textiles has garnered significant attention, particularly in healthcare environments where antimicrobial fabrics play a critical role in infection control and hygiene maintenance. Cotton fabrics infused with silver nanoparticles have demonstrated promise in applications such as wound dressings, surgical gowns, and other medical textiles, owing to their effectiveness in inhibiting microbial colonization [12].

The incorporation of AgNPs derived from *C. intybus* into cotton fabrics is an environmentally responsible advance in antimicrobial textile development. This approach combines the bioactive features of plant-derived compounds with the accuracy of nanoparticle technology, allowing the development of multifunctional materials with improved antibacterial activity. This technique, which harnesses *C. intybus*' inherent antibacterial properties as well as the targeted activity of nanoparticles, provides a sustainable alternative to standard synthetic therapies, meeting worldwide desires for green technology solutions.

This study investigates the antimicrobial potential of cotton fabrics functionalized with silver nanoparticles (AgNPs) synthesized using *C. intybus* extract. A comparative analysis was conducted to evaluate the antimicrobial activity of the plant extract alone versus that of the AgNP-treated fabric, highlighting the pivotal role of nanoparticle synthesis in enhancing bioactive properties. Although several studies have examined the individual antimicrobial effects of plant extracts and silver nanoparticles, there is a notable gap in research concerning the integration of *C. intybus*-derived AgNPs into textile materials. Furthermore, limited attention has been given to directly comparing the efficacy of the crude extract and its nanoparticle form in functional applications. This work addresses these gaps by demonstrating how green-synthesized AgNPs can significantly enhance antimicrobial performance when applied to cotton fabrics, offering a sustainable, eco-friendly strategy for developing advanced medical and hygienic textile.

2. Experimental

2.1. Extraction and fractionation of C. intybus

The aerial parts of *C. intybus* were harvested from fields in Beheira governorate, Egypt during March 2020. The plant specimen was taxonomically authenticated at the El-Orman Botanical Garden, Department of Flora and Taxonomy, Giza, Egypt, by a plant taxonomy specialist. A reference specimen (C.in3-2020) was preserved in the Laboratory of Medicinal Chemistry at the Theodor Bilharz Research Institute, Egypt.

The aerial parts of *C. intybus* air-dried, and finely ground to prepare for extraction. The powdered biomass was subjected to maceration in 90% methanol at a 1:10 (w/v) solid-to-solvent ratio, with continuous stirring for 24 hours at room temperature. The resultant mixture was filtered, and the methanolic extract was concentrated under a vacuum using a rotary evaporator, then stored at 4°C for subsequent analysis. To isolate bioactive constituents, the crude extract was sequentially partitioned using solvents of increasing polarity - petroleum ether (60–80°C), methylene chloride, ethyl acetate, n-butanol, and water-enabling the separation of compounds according to their polarity-based solubility profiles [13].

2.2. Preliminary qualitative Phytochemical analysis

The *C. intybus* extract and its derived fractions underwent phytochemical screening following standardized protocols to detect key bioactive constituents. These included flavonoids, tannins, phenolics, terpenes, alkaloids, saponins, steroids, and glycosides, employing standard qualitative methods as described by Harborne and Mondal [14-15].

2.3. Estimation of total phenolics content (TPC)

The overall amount of phenolic compounds in the The total phenolic content (TPC) of the C. intybus extract was quantified using the Folin-Ciocalteu assay according to Hadjira., 2024[16]. Briefly, 100 µL of the extract (100 µg/mL) was mixed with 500 µL of Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate solution. The mixture was vortex-mixed for 15 seconds, then diluted with distilled water to a final volume of 10 mL. After incubation at 25°C for 120 minutes to enable chromogenic development, absorbance was measured at 765 nm using a UV-Vis spectrophotometer. TPC was calculated as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) based on a gallic acid standard calibration curve.

2.4. Estimation of total flavonoids content (TFC)

The total flavonoid content (TFC) of the *Cichorium intybus* extract was analyzed according to the technique reported by Indiarto, 2024[17]. Briefly, 0.5 mg of the extract was dissolved in 3 mL of methanol. Subsequently, 0.2 mL of potassium acetate solution and 0.2 mL of aluminum chloride solution were added sequentially. The mixture was then brought to a final volume of 10 mL with distilled water and incubated in the dark for 30 minutes to complete the reaction. Absorbance readings were taken at 415 nm using a UV spectrophotometer. The TFC was calculated and expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract).

2.5. Antioxidant Activity Assessment

2.5.1. ABTS Assay (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid))

The ABTS assay quantifies antioxidant activity by measuring the ability of antioxidants to quench the ABTS radical cation (ABTS•+), a blue-green chromophore with a maximum absorbance at 734 nm. To prepare the stable ABTS•+ solution, 7 mM ABTS aqueous solution is reacted with 2.45 mM potassium persulfate ($K_2S_2O_8$), followed by incubation in the dark for several hours until the characteristic blue-green color develops. For the assay, a diluted *C. intybus* extract sample is mixed with the ABTS•+ working solution. After incubating the reaction mixture at room temperature for 5-10 minutes, the reduction in absorbance at 734 nm is measured using a UV-Vis spectrophotometer. The decrease in absorbance is proportional to the sample's antioxidant activity, as antioxidants neutralize the ABTS•+ radical. Results are expressed as Trolox equivalents, derived by comparing the sample's absorbance reduction to that of a standard Trolox solution [16].

2.5.2. FRAP Assay (Ferric Reducing Antioxidant Power)

The FRAP (Ferric Reducing Antioxidant Power) assay evaluates the reducing capacity of antioxidants by quantifying their ability to convert the Fe^{3+}-TPTZ (ferric tripyridyltriazine) complex into its blue-colored Fe2+ form under acidic conditions. The FRAP reagent, prepared fresh before use, consists of a mixture of 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride ($FeCl_3•6H_2O$), and acetate buffer (pH 3.6) in a 10:1:1 volume ratio. Antioxidants in the sample reduce Fe^{3+} to Fe^{2+}, which binds to TPTZ to form a blue chromophore with maximum absorbance at 593 nm. For the assay, *C. intybus* extract is mixed with the FRAP reagent and incubated at 37°C for 4–10 minutes. Absorbance at 593 nm is measured spectrophotometrically, with the intensity of the blue coloration directly proportional to the sample's reducing power. Results are expressed as millimolar ferrous sulfate ($FeSO_4$) equivalents per milligram of extract (mM $FeSO_4$ eq/mg extract), calibrated against a standard curve [18].

2.5.3. DPPH Assay (2, 2-Diphenyl-1-picryl hydrazyl)

The DPPH assay employs the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which exhibits a characteristic violet color and maximum absorption at 517 nm. Antioxidants interact with this radical by donating a hydrogen atom, resulting in a spectrophotometrically measurable color transition from violet to yellow [19]. In this essay, a *C. intybus* extract is mixed with a 0.1 mM methanolic DPPH solution and incubated in darkness at room temperature for 30 minutes. The subsequent decrease in absorbance at 517 nm is recorded, with the magnitude of the reduction in color intensity correlating directly with the sample's antioxidant capacity. Antioxidant activity is typically expressed as a percentage of DPPH radical scavenging relative to control. Ascorbic acid is

commonly employed as a reference standard, and results are expressed as IC50 values - the concentration of antioxidants required to scavenge 50% of the initial DPPH radicals [20].

The ABTS, DPPH, and FRAP assays collectively provide a comprehensive assessment of the antioxidant potential of *C. intybus*. These methods evaluate antioxidant activity through distinct mechanisms: ABTS and DPPH measure radical scavenging capacity, while FRAP quantifies reducing power. The combination of these complementary analytical tools offers a robust approach for detecting and quantifying antioxidant compounds in *C. intybus* extract, ensuring a multifaceted evaluation of its bioactivity [21].

2.5.4. UPLC-QTOF-MS-Based Characterization of Bioactive Compounds in C. intybus extract

Ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS, AB SCIEX, Concord, Canada) was employed to identify bioactive components in the *C. intybus* extract. The extract was injected into the UPLC system, where chromatographic separation was performed using a C18 column. The system operated in negative ion mode, enabling detection and identification of compounds based on their mass-to-charge ratio (m/z) and fragmentation patterns. Data generated by the QTOF-MS system were analyzed to yield detailed mass spectra, facilitating the identification of bioactive constituents in the extract [22]. This technique provides high sensitivity and resolution, making it particularly effective for resolving complex mixtures of natural compounds, thereby enhancing the understanding of the chemical profile of *C. intybus* and its potential therapeutic applications.

2.6. Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were effectively synthesized by combining *C. intybus* extract with an aqueous silver nitrate solution using a 1:2 volume ratio of silver nitrate to *C. intybus* extract, which served as both the reducing and stabilizing agent. A 4 mM aqueous solution of $AgNO_3$ was freshly prepared in a 250 mL volumetric flask and utilized as the precursor. For the reduction of Ag^+ ions, 200 mL of *C. intybus* extract was combined with 100 mL of the $AgNO_3$ solution at the optimized concentration. The reaction mixture was incubated at 40 °C for 90 minutes in a sealed incubator to facilitate nanoparticle formation [23]. The formation of nanoparticles was initially indicated by a visible color change, a phenomenological sign of nanoparticle synthesis, and further confirmed by UV-Vis spectroscopy. The spectroscopy analysis revealed a distinct absorption peak at around 420 nm, characteristic of the surface plasmon resonance effect in silver nanoparticles. After synthesis, the nanoparticles were purified by centrifugation, washed with distilled water to eliminate unreacted substances, and stored in a dark environment to prevent degradation until further use [24].

2.7. Treatment of Cotton Fabrics with Silver Nanoparticles

Cotton fabric samples were immersed in the silver nanoparticle solution and gently stirred for 2 hours to ensure uniform nanoparticle deposition across the textile matrix. Post-treatment, the fabrics were air-dried at ambient temperature and subsequently heated at 80°C for 1 hour. This thermal treatment strengthened nanoparticle adhesion to the fiber surfaces, thereby improving the structural stability and durability of antimicrobial functionality [25].

2.8. Characterization of Synthesized Silver Nanoparticles

2.8.1. UV- Visible Spectroscopy

The eco-friendly reduction of silver nitrate to silver nanoparticles (AgNPs) was monitored over a 24-hours using UV–vis spectroscopy (Shimadzu 2401PC, Shimadzu Corporation, Kyoto, Japan). The sample was diluted with deionized water, if necessary, before analysis. UV–vis spectra of the nanoparticles were recorded in a quartz cuvette, with measurements taken in the range of 200–800 nm, and the scanning speed was adjusted to capture the relevant absorbance peaks associated with nanoparticle formation [26].

2.8.2. Transmittance Electron Microscopic (TEM) Analysis

The suspension containing AgNPs biosynthesized from the *C. intybus* extract was analyzed using TEM with a JEOL model 1200 EX electron microscope (JEOL Ltd. , Japan).. To prepare the TEM samples, a drop of the AgNPs suspension was placed onto carbon-coated copper grids and allowed to evaporate. The samples were left to dry for 4 minutes. The shape and size of the AgNPs were determined by examining the TEM micrographs [18].

2.8.3. Scanning Electron Microscopy / Energy Dispersive X-ray Spectroscopy (SEM/EDX) Biosynthesis of gold nanoparticles (AuNPs)

SEM/EDX analysis was performed using a JEOL JSM- SEM/EDX analysis was performed using a JEOL JSM-5800LV (JEOL Ltd., Japan) to determine the elemental composition of the AgNPs deposited on the surface. The SEM/EDX spectra were obtained directly from the surface of the metal, provided the deposit was sufficiently thick for analysis. This technique allowed for a detailed examination of the morphology and elemental distribution of the AgNPs.

2.8.4. Antimicrobial Activity Testing

The antimicrobial efficacy of both the *C. intybus* extract and the biosynthesized AgNPs-treated fabrics was evaluated against several microbial strains: *Staphylococcus aureus* ATCC 6538 (Gram-positive bacteria), *Escherichia coli* ATCC 25922 (Gram-negative bacteria), *Candida albicans* ATCC 10231 (yeast), and *Aspergillus niger* NRRL A 326 (fungus). The agar cup plate diffusion method was employed to assess the antimicrobial activity [27]. For bacterial and yeast strains, nutrient agar plates were prepared with the following composition (g/L): peptone (5), meat extract (3), and agar (15-20), adjusted to pH 7.0. Each plate was uniformly inoculated with 0.1 ml of a 107-108 cells/ml suspension of the test microbes. For fungal strains, Czapek-Dox agar plates were used with the following composition (g/L): sucrose (30), sodium nitrate (2), dipotassium phosphate (1), magnesium sulfate (0.5), potassium chloride (0.5), ferrous sulfate (0.01), and agar (15-20), with pH 7.1. Each plate was seeded with 0.1 ml of the fungal inoculum (106-107 cells/ml). A 1 cm hole was created in the center of each plate using a gel cutter (Cork borer) under sterile conditions. A drop of melted agar was added to the hole and allowed to solidify, forming a base layer. Afterward, 0.1 ml of AgNPs solution was added to the hole. The plates were placed in a refrigerator at 4°C for 2-4 hours to facilitate maximum diffusion of the test solution. Subsequently, the plates were incubated at 37°C for 24 hours for bacteria and yeast and at 30°C for 48 hours for fungi to ensure optimal growth conditions. The antimicrobial activity was determined by measuring the diameter of the inhibition zone, expressed in millimeters (mm). The experiment was repeated multiple times, and the mean of the readings was recorded [28]. Additionally, the antimicrobial effects of the AgNP-treated cotton fabrics were assessed using the disc agar diffusion method [29].

2.8.5. Analytical statistics

All data examined statistically via the SPSS program, edition "13". The findings were displayed as the mean ± standard deviation.

3. Results and Discussion

3.1. The Qualitative Phytochemical Analysis of C. intybus Extracts

Phytochemical screening of *C. intybus* extracts was performed, and the results were aligned with findings documented in prior studies [30-31]. The extracts were subjected to qualitative analysis to identify the presence of various phytochemicals, as summarized in Table 1. The analysis revealed the presence of multiple bioactive compounds, including phenols, flavonoids, alkaloids, tannins, glycosides, and steroids. We acknowledge that flavonoids are typically more soluble in polar solvents; however, certain flavonoid aglycones, due to their low polarity and lipophilic nature, can exhibit partial solubility in non-polar solvents such as petroleum ether. Additionally, trace amounts of polar compounds may be co-extracted due to the complexity of plant matrices or during the initial extraction phase [32].

The secondary metabolites are widely recognized for their broad-spectrum biological activities, such as antimicrobial, antioxidant, and anti-inflammatory effects. The identified phytochemicals underscore the therapeutic potential of *C. intybus* and substantiate its applicability in pharmacological and medicinal contexts.

Table 1: The Phytochemical composition identified in leaf extracts of *C. intybus.*

Phytoconstituents	Methanol extract	Petroleum ether extract	Methylene chloride extract	Ethyl acetate extract	Butanol extract
Flavanoids	+	+	+	+	+
Phenolics	+	-	+	-	+
Tanins	+	+	-	+	+
Alkaloids	+	+	-	+	-
Terpenes	+	+	+	+	+
Saponins	-	-	-	-	-
Steroids	+	+	-	+	-
Glycosides	+	-	-	-	+
+ presence of constituents, - absence of constituents					

3.2. Quantitative Analysis of Phenolic and Flavonoid Content in C. intybus Extracts

Table 2 summarizes the total phenolic and flavonoid content of *C. intybus* solvent-derived extracts, revealing significant variations among extraction solvents. The methanolic extract demonstrated a significantly higher total phenolic content (459.94 ± 1.00 mg GAE/g extract) compared to the butanol extract (416.41 ± 0.00 mg GAE/g extract). This marked disparity suggests that methanol is a more efficacious solvent for the extraction of phenolic secondary metabolites from *C. intybus*, which are widely recognized for their potent antioxidant and antimicrobial activities. The methanolic extract exhibited a significantly elevated flavonoid content, quantified as 128.17 ± 1.05 mg RE/g extract, in marked contrast to the butanol extract (105.91 ± 0.97 mg RE/g extract). Flavonoids, secondary metabolites renowned for their antioxidant, anti-inflammatory, and antimicrobial bioactivities, likely exhibit potentially augmented efficacy in the methanol-derived extract due to the enhanced concentration of these phytoconstituents. These findings align with prior research [33-34], which documented analogous phytochemical profiles in *C. intybus* extracts, confirming methanol as an efficient solvent for the extraction of bioactive Phytoconstituents. The higher concentrations of phenolic and flavonoid in the methanolic extract may underpin its superior biological efficacy, particularly its antioxidant and antimicrobial bioactivities, positioning it as a promising candidate for pharmacological and nutraceuticals applications. The results further underscore the potential of *C. intybus* extracts, particularly the methanolic fraction, as natural reservoirs of therapeutic secondary metabolites.

Table 2: Total phenolic content (TPC) and total flavonoid content (TFC) of *C. intybus* extracts.

Extract	TFC (mg rutin/g extract)	TPC (mg gallic acid equivalent/g extract)
Methanol	128.17 ±1.05	459.94±1.00
Petroleum ether	4.81 ± 1.53	34.22 ± 0.15
Methylene chloride	7.62± 0.87	288.30 ± 0.87
Ethyl acetate	101.27 ±0.78	26.62 ± 1.56
n-Butanol	105.91 ± 0.97	416.41 ± 0.94

3.3. Antioxidant Activity of C. intybus Extract

The antioxidant capacity of *C. intybus* extract was assessed using three distinct assays: ABTS, FRAP, and DPPH. These methodologies offered complementary insights into the extract's free radical scavenging capacity and electron-donating potential.

ABTS assay: The extract exhibited significant radical scavenging activity, as evidenced by a dose-dependent attenuation of absorbance at 734 nm. The IC50 values for the methanol extract and its fractions ranged from 9.21 ± 1.24 µg/ml to 46.19 ± 0.87 µg/ml, markedly elevated compared to the reference compound Trolox (1.99 ± 1.07 µg/ml). The robust antioxidant efficacy suggests the extract's capacity to effectively neutralize the ABTS radical cation, highlighting its potential utility in mitigating oxidative stress mediated by reactive oxygen species (ROS) and other free radicals.

FRAP Assay: The ferric ion-reducing capacity of the extract was quantitatively assessed via the FRAP assay, revealing a significant dose-dependent antioxidant activity characterized by an absorbance of 17.24 ± 0.57 mM FeSO4 equivalents per mg extract. These findings indicate robust electron-donating properties attributable to the high phenolic and flavonoid content, which mediate electron transfer processes via redox-active functional groups, thereby reducing ferric ions (Fe^{3+}) to their ferrous (Fe^{2+}) state. This mechanistic action corroborates the extracts potential efficacy in mitigating oxidative stress through disruption of radical chain propagation, highlighting its therapeutic relevance in attenuating oxidative damage in biological systems

DPPH Assay: The DPPH radical scavenging assay demonstrated the dose-dependent scavenging capacity of the extract, as evidenced by a visible color change indicating the reduction of DPPH radicals. The IC50 of the methanolic extract was 17.14 ± 1.25 µg/ml, indicating high antioxidant efficiency. The DPPH assay, in conjunction with the ABTS and FRAP results, suggests that the extract is rich in antioxidant compounds, such as flavonoids, tannins, and phenolic acids, which are known to neutralize radicals through hydrogen donation. Since lower IC50 values indicate stronger antioxidant activity, the data confirm that the methanol extract, rich in phenolic and flavonoid compounds, had the most potent radical scavenging ability.

Combinatorial antioxidant profiling ($ABTS^{+}$ decolorization, ferric-reducing antioxidant power [FRAP], and DPPH radical scavenging) quantitatively validated the *C. intybus* methanolic extract's potent free radical-neutralizing capacity, validating its translational viability for multidisciplinary applications including ROS-scavenging therapeutic adjuvants, lipid peroxidation-inhibiting food additives, and oxidative stress-mitigating polymer matrices.

Table 3: Antioxidant activity of *C. intybus* extracts

Extract	ABTS (IC_{50} µg/ml)	DPPH (IC_{50} µg/ml)	FRAP (mM $FeSO_4$equivalent/mg extract)
Methanol	9.21±1.24	17.14±1.25	17.24±0.57
Petroleum ether	46.19±0.87	70.14±0.94	3.33±1.21
Methylene chloride	31.56±0.93	57.21±0.83	9.17±0.74
Ethyl acetate	13.47±2.42	25.64±0.79	14.15±1.48
n-butanol	18.17±1.08	28.41±1.37	15.41±2.11
Quercetin	-	-	22.14±1.05
Ascorbic acid	-	3.21±0.55	-
Trolox	1.99±1.07	-	

Results are the mean of triplicate (n=3) and expressed as the mean ± standard deviation.

3.4. Phytochemical Composition Analysis of C. intybus Extract

Phytochemical characterization of *C. intybus* methanolic extract via UPLC-QTOF-MS resolved structurally diverse secondary metabolites, encompassing flavonoids, alkaloids, terpenoids, and phenolic acids [35]. These phytoconstituents (Table 4, Fig. 1) exhibit pharmacologically relevant bioactivities, notably antipathogenic and redox-modulatory capacities [36]. Among them, flavonoids and phenolic acids were found in high concentrations, which correspond with the potent antioxidant properties observed in various assays such as ABTS, FRAP, and DPPH. Mechanistically, these redox-active species mediate radical neutralization via hydrogen atom transfer and single electron transfer pathways, thereby attenuating reactive oxygen species propagation and oxidative stress indices in vitro. Beyond their redox-modulating properties, alkaloids and terpenoids fractions isolated from *C. intybus* extract exhibit synergistic antimicrobial enhancement through distinct microbicidal mechanisms. Alkaloids are widely known for their broad-spectrum antimicrobial properties, including the inhibition of microbial growth through mechanisms such as enzyme inhibition or disruption of cell processes. Terpenoids, on the other hand, can interact with microbial cell membranes, altering their integrity and permeability. This membrane disruption enhances the overall antimicrobial efficacy of the extract, making it effective against a variety of pathogenic microorganisms, including bacteria, fungi, and yeast.

The phytochemical diversity of *C. intybus* extract correlates with its polyvalent bioactivity profile, validating its utility as a viable biosource for both redox-active phytoconstituents and broad-spectrum antimicrobial metabolites. This renders the phytocomplex suitable for translational applications in phytotherapeutic development, nutraceutical preservative systems, and green antimicrobial formulations. The robust dual functionality-antioxidant capacity coupled with microbicidal efficacy-reinforces its viability as a multisectoral bioprospecting target, spanning pharmaceutical innovation, agricultural biocontrol, and sustainable material science.

3.5. Characterization and Antimicrobial Efficacy of C. intybus-Mediated Biosynthesized AgNPs

AgNPs synthesized via C. *intybus* leaf extract were demonstrated successful characterization using UV-visible spectroscopy and TEM.

UV-Vis Spectrophotometric Analysis: The UV-vis spectrum (Fig. 2) exhibited a distinct plasmon resonance band (original: "characteristic peak") at λmax ≈ 400 nm, confirming the reduction of Ag^+ ions to Ag^0 nanoparticles. This absorbance corresponds to the coherent oscillation of conduction electrons in monodispersed AgNPs, indicative of narrow size distribution and colloidal stability. The persistent absorbance intensity over time confirmed negligible aggregation, a critical prerequisite for biomedical applications.

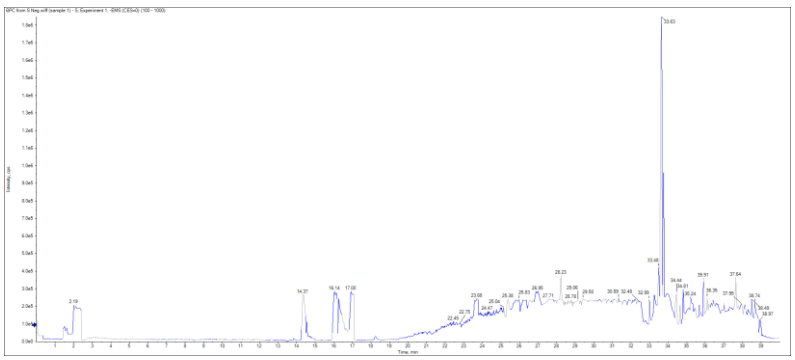


Fig. 1. UPLC-QTOF-MS chromatogram delineating phytochemical constituents detected in the methanolic extract of *C. intybus* under negative ionization mode.

Table 4. Phytochemical profiling and structural characterization of secondary metabolites in *C. intybus* methanolic extract via UPLC-QTOF-MS in negative ionization mode

Identified Compound	Retention time (min)	Exact mass	Mass fragmentation	Elemental formula	Class of compound	Ref.
Caftaric acid	32.805	312.05	311, 277,179,163, 135	$C_{13}H_{12}O_9$	Esterified phenolic acid	[37]
Chlorogenic acid	2.457	354.31	191, 179, 135	$C_{16}H_{18}O_9$	Esterified phenolic acid	[18]
Coumaric acid	16.08942	164.16	119	$C_9H_8O_3$	phenolic acids	[38]
Caffeic acid	14.43498	180.16	161,135,107,91	$C_9H_8O_4$	Phenolic acids	[18]
Chicoric acid	22.88323	474.371		$C_{22}H_{18}O_{12}$	Esterified phenolic acid	[39]
Cichoriin	25.37923	340.28	178,150,134	$C_{15}H_{16}O_9$	Coumarins	[37]
β-sitosterol	28.04075	414.718	414,396, 381, 273, 255	$C_{29}H_{50}O$	Sterol	[40]
Luteolin	14.38252	285.037	241,177,133,109,65	$C_{15}H_{10}O_6$	Flavone	[41]
4,5-Dicaffeoylquinic acids	22.85752	516.4	515, 353, 191, 179	$C_{25}H_{24}O_{12}$	Quinic acids derivatives	[37]
Malic acid	14.3825	134.1	133, 115, 89,71	$C_4H_6O_5$	Dicarboxylic acid	[42]
Quinic acid	27.0007	192.17	191, 93, 85	$C_7H_{12}O_6$	Carboxylic acid	[43]
Ellagic acid	14.65058	303	302,261,150	$C_{14}H_6O_8$	Hydrolyzable tannins	[44]
5-*p*-Coumaroylquinic acid	33.6029	338.85	337,219, 173	$C_{16}H_{18}O_8$	Quinic acids derivatives	[18]
Protocatechuic acid	14.3825	154.020	153,109,91	$C_7H_6O_4$	Phenolic acids	[43]
Isovanillic acid	16.3287	168	167,153, 124	$C_8H_8O_4$	Phenolic acids	[45]
Oleanolic acid	33.35725	456.70		$C_{30}H_{48}O_3$	Triterpenoid	[46]
Ascorbic acid	2.21555	176.12	121, 103, 91	$C_6H_8O_6$	Lactones	[47]
Kaempferol-3-*O*-glucoside	35.112	447.094	331, 284,255, 227	$C_{21}H_{20}O_{11}$	Flavonoids	[48]
Kaempferol-3-*O*-alpha-L-arabinoside	35.55362	419.55	288, 287, 258, 213, 165, 153	$C_{20}H_{18}O_{10}$	Flavonoid-3-*O*-glycosides	[48]
Isovitexin	22.89363	433.05	415, 313	$C_{21}H_{20}O_{10}$	Flavonoid *C*-glycosides	
Genistein	27.0007	269.046	117	$C_{15}H_{10}O_5$	Isoflavone	[49]
Luteolin	14.301	285.037	241,177,133,109,65	$C_{15}H_{10}O_6$	Flavone	[18]
Rutin	10.4309	611.5	593, 575, 489, 465, 449,345	$C_{27}H_{30}O_{16}$	Flavonoid-3-*O*-glycosides	[50]
Gluconic acid	1.9413	195.0524	177,151,129,121	$C_6H_{12}O_7$	Carboxylic acid	[38]
Acacetin	21.0676	285	270,257,243	$C_{16}H_{12}O_5$	Flavonoids	[18]
Myricetin	1.626767	319.1	317, 151	$C_{15}H_{10}O_8$	Flavonols	[51]
Naringenin 7-*O*-glucoside	12.6514	433	432, 271, 253, 151, 119	$C_{21}H_{22}O_{11}$	Flavonoids	[52]
Esculin	33.70305	339.1977	183	$C_{15}H_{16}O_9$	Coumarins derivative	[53]

TEM Analysis: High-resolution TEM micrographs (Fig. 3) revealed quasi-spherical AgNPs with diameters ranging from 4.5 to 10.9 nm. The small size and spherical shape confer a high specific surface area, potentiating microbial membrane penetration and oxidative damage, thereby amplifying bactericidal efficacy. The facile biosynthesis and colloidal stability of AgNPs underscore the dual role of *C. intybus* phytoconstituents as reductants and capping agents. This green protocol harnesses the natural phytochemical constituents of the plant extract, which act as biocompatible reducing agents to convert silver ions into nanoparticles and stabilize them during formation. The method avoids the use of hazardous chemicals often employed in conventional synthesis, thus making it environmentally friendly and sustainable [54]. Furthermore, surface functionalization of AgNPs with *C. intybus*-derived phytochemicals, including phenolic acids, flavonoids, and other secondary metabolites [55], confers synergistic bioactivity through the integration of redox-active phenolic and flavonoid moieties. These metabolites enhance antimicrobial efficacy via mechanisms such as reactive oxygen species (ROS) generation and membrane permeabilization, while concurrently exhibiting antioxidant and anti-inflammatory properties [56].

This eco-compatible synthesis strategy combining reduced ecotoxicological impact with multifunctional bioactive properties positions green synthesis as a scalable and sustainable platform to conventional chemical reduction or lithographic methods. The colloidal stability of AgNPs confirms the capping efficacy of *C. intybus* phytochemicals in preserving nanoparticle monodispersity, a prerequisite for biomedical payload delivery and catalytic reproducibility. By utilizing renewable plant biomass, this method eliminates stoichiometric toxic reagents, adhering to green chemistry principles of atom economy and waste minimization, thereby advancing benign-by-design nanotechnologies.

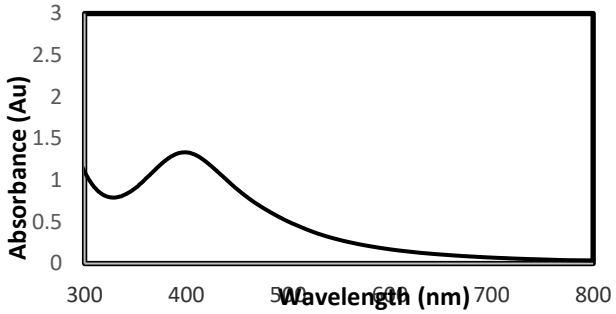


Fig. 2: UV/Vis absorption spectrum of phytosynthesized AgNPs derived from *C. intybus* extract.

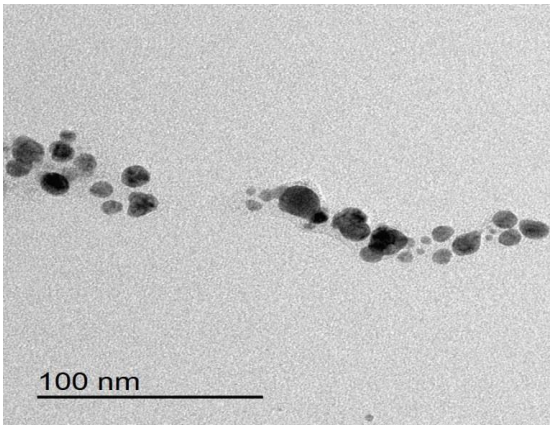


Fig. 3: TEM micrograph of phytosynthesized AgNPs generated via *C. intybus* methanolic extract-mediated reduction.

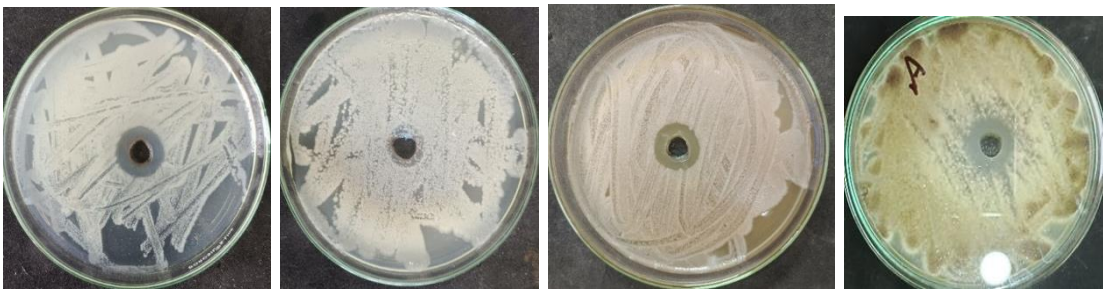
3.5.1. Antimicrobial Performance of C. intybus Extract and AgNP-Functionalized Substrates.

The antimicrobial efficacy of *C. intybus* extract and AgNP-functionalized cotton substrates was assessed against a panel of microbial pathogens, including fungal (*A. niger* and *C. albicans*) and bacterial (*E. coli* and *S. aureus*) strains, using the standardized Agar Cup Plate Diffusion Method. The clear zone diameters (in mm) indicate the effectiveness of each treatment, with larger zones signifying higher antimicrobial activity (Table 5, Figure 4, 5). The *C. intybus* extract exhibited moderate inhibitory efficacy against all tested microbial strains, suggesting the presence of bioactive phytochemical constituents indicates that the extract contains bioactive compounds with antimicrobial properties. Pronounced efficacy was observed against *A. niger* and *S. aureus*, demonstrating targeted antifungal and antibacterial mechanisms (enhanced specificity). In contrast, a reduced inhibitory effect against *E. coli* indicates potential intrinsic resistance mechanisms in Gram-negative bacteria. Notably, cotton substrates functionalized with biosynthesized AgNPs exhibited a statistically significant enhancement in antimicrobial activity compared to the phytochemical extract alone, suggesting synergistic interactions between AgNPs and plant-derived compounds.

The superior antimicrobial efficacy of AgNPs is attributable to their inherent physicochemical properties, which compromise microbial membrane integrity, induce Reactive Oxygen Species (ROS) generation, and inhibit DNA replication processes via ligand-mediated interactions (Idris *et al*., 2024; Rodrigues *et al*., 2024). AgNP-functionalized fabrics demonstrated potent inhibitory activity against *C. albicans* and *S. aureus*, clinically relevant pathogens associated with nosocomial infections and biofilm formation on biomedical textiles. Notably, the broad-spectrum antimicrobial potential observed against *E. coli* validates its applicability in mitigating multidrug-resistant Gram-negative infections (enhanced clinical context).

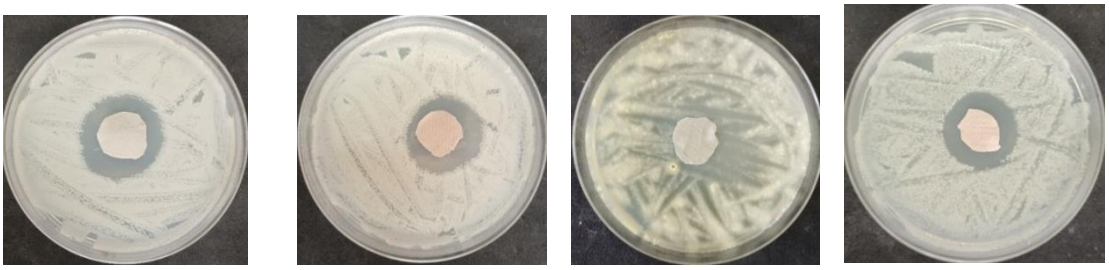
AgNPs demonstrate broad-spectrum antimicrobial efficacy against clinically relevant pathogens, including *E. coli* and *S. aureus* [25]. Functionalization of cotton matrices with AgNPs suppressed bacterial colonization,

making them suitable for healthcare and hygiene applications [58-59]. Annealing at 80°C facilitated the covalent immobilization of AgNPs to cellulose hydroxyl groups within cotton matrices through hydroxyl-mediated coordination, ensuring long-lasting antimicrobial effects [25]. In conclusion, the data presented in **Table 5** and **Figures 4–5** demonstrate that AgNP-treated cotton fabrics exhibit superior antimicrobial efficacy compared to *C. intybus* extract alone across all tested microbial strains , demonstrating a synergistic interaction between phytochemical constituents and nanoparticle-mediated mechanisms. These findings underscore the potential of sustainable textile engineering for high-performance antimicrobial applications, particularly in clinical settings requiring infection-resistant materials such as bioactive wound dressings. Functionalization of cotton with *C. intybus*-synthesized AgNPs yields a multifunctional coating that integrates potent biocidal activity with reduced environmental toxicity, outperforming conventional synthetic antimicrobial agents in both durability and ecological safety.



(a) **(b)** **(c)** **(d)**

Fig. 4: The antimicrobial activity of *C. intybus* methanolic extract against (a) *S. aureus*, (b) *E. coli*, (c) *C. albicans,* and (d) *A. niger*.



(a) **(b)** **(c)** **(d)**

Fig. 5: The antimicrobial activity of AgNPs-treated cotton fabric against (a) *S. aureus*, (b) *E. coli*, (c) *C. albicans,* and (d) *A. niger*.

Table 5. *In vitro* antimicrobial efficacy of *C. intybus* methanolic extract and phytosynthesized AgNPs-functionalized cotton fabric against different test microbes

Sample	Clear zone (mm)			
	S. aureus	***E. coli***	***C. albicans***	***A. niger***
***C. intybus* extract**	18	15	17	18
AgNPs-treated cotton fabric	25	23	25	21

3.5.2. Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDX) Analysis of Cotton Fabrics Functionalized with C. intybus-Biosynthesized AgNPs

The SEM/EDX analysis revealed nano scale surface modifications of cotton fabrics. As shown in Figure 6a–c, SEM micrographs acquired at 2,000× and5,000×magnification confirmed the homogeneous deposition of AgNPs (20–50 nm diameter) on cellulose fibrils. EDX spectra (Figure 6c) exhibited characteristic peaks of Ag in their pattern, confirming the immobilization and loading of AgNPs onto the bio-treated cotton sample. In contrast, untreated cotton fabrics (Figure 7a–c) displayed smooth topographic profiles devoid of AgNPs aggregates, with EDX spectra confirming no detectable Ag signatures.

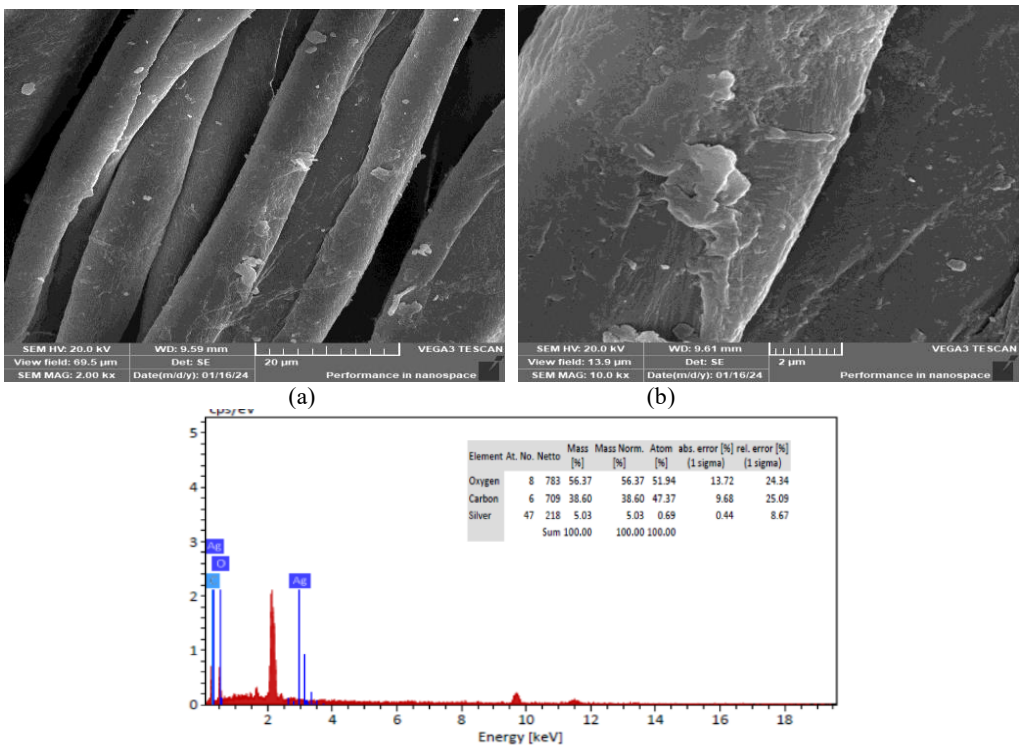


Figure 6. (a) SEM micrograph (2,000× magnification), (b) SEM micrograph (5,000× magnification), and (c) EDX spectra of cotton fabrics functionalized with *C. intybus* extract-biosynthesized AgNPs.

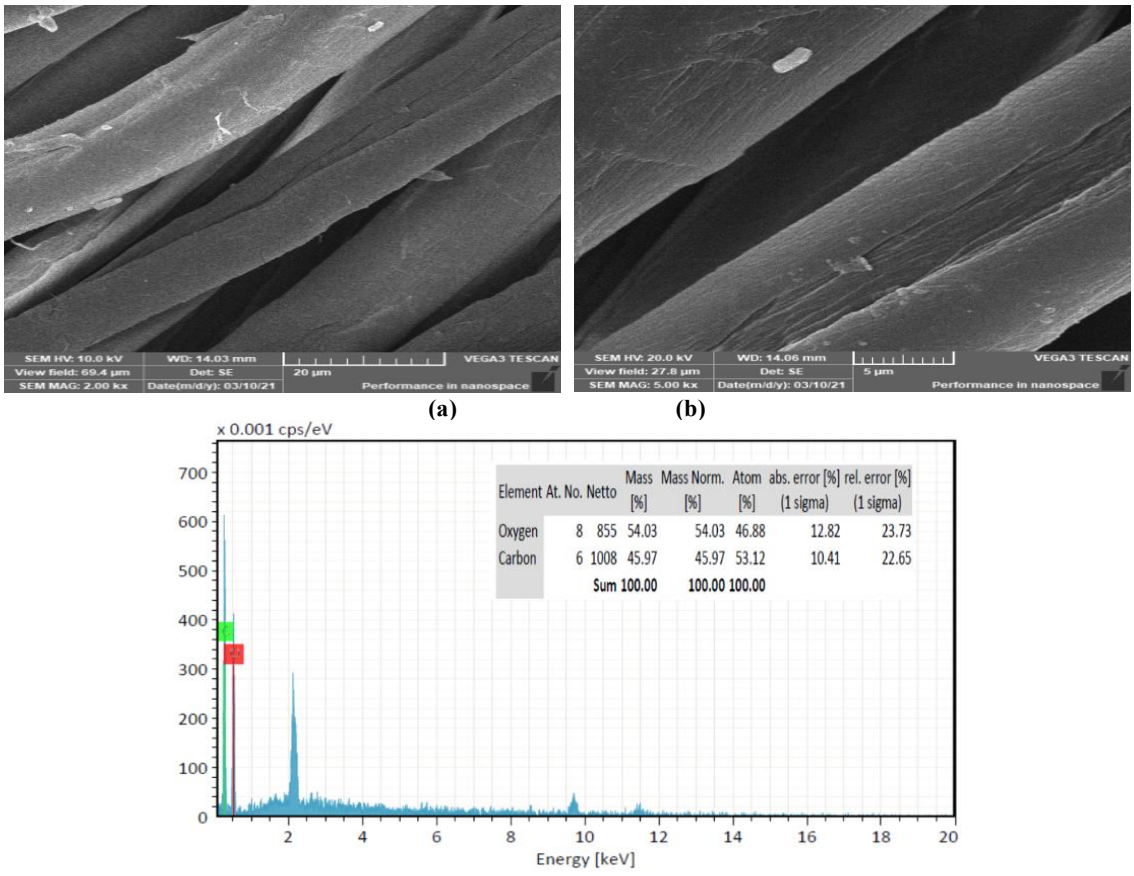


Figure 7. (a) SEM micrograph (2,000× magnification), (b) SEM micrograph (5,000× magnification), and (c) EDX spectra of untreated blank cotton fabrics.

4. Conclusion

The dual antioxidant and antimicrobial functionality exhibited by *C. intybus* –derived AgNPs functionalized onto cotton substrates underscores the potential utility of phytochemical-mediated nanotechnology in biomedical and hygienic textile engineering. This study establishes that *C. intybus* extract serves dual roles as a natural antioxidant reservoir and a capping/stabilizing agent during the phyto-synthesis of AgNPs, thereby enhancing the ROS scavenging capacity and bacteriostatic activity of the composite material. Further research could explore the long-term stability and durability of these treated fabrics, as well as their safety in clinical environments.

5. Conflicts of interest

No conflicts of interest have been reported.

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