



Eco-friendly Simultaneous Dyeing and Synthesis of Silver Nanoparticles on Silk Yarn Using Anthocyanin Extracts from *Melastoma malabathricum* L. Fruits

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Abstract

To address the challenge of finding sustainable and effective methods for simultaneously dyeing silk yarn and incorporating antibacterial activity without harsh chemicals or complex procedures, a successful study has demonstrated a sustainable method for dyeing silk yarn and producing silver nanoparticles (Ag NPs) simultaneously. This eco-friendly approach utilizes anthocyanin components extracted from *Melastoma malabathricum* fruits as both a natural reducing agent for Ag NPs and a colorant for the silk. The reduction of silver ions to form stabilized Ag NPs was successfully achieved at pH 10, resulting in an average particle size of 86.34 ± 17.75 nm, and leading to the coating of a silk yarn surface. After dyeing and coating with Ag NPs under basic conditions, the treated silk yarn exhibited an excellent yellow colour (positive b^* value), good colour strength (K/S) of 4.09 with the colour difference (ΔE^*) of 5.49, and good light fastness properties after exposure to simulated sunlight. Silk yarn treated at a basic pH exhibits superior antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* compared to yarn treated at acidic or neutral pHs, both before and after exposure to simulated sunlight. This improved bioactivity is demonstrated by $\log_{10}$ reductions in colony-forming units (CFUs) exceeding 4.

Keywords: *Melastoma malabathricum*; Silk dyeing; Silver nanoparticle; Anthocyanin; Light fastness.

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1. Introduction

In general, silk not only has low antibacterial properties but also has low UV protection properties, resulting in a limited application field. Various methods are used to address these and other limitations, including, for example, using nanoparticulate ZnO to improve light fastness (and wash fastness) properties of naturally dyed silk fabric^[1] and silver nanoparticles (Ag NPs) to enhance antibacterial properties.^[2] Ag NPs have excellent antibacterial activity against a broad range of microorganisms.^[3,4] Various methods have been employed to synthesize Ag NPs, covering physical, chemical, and biological processes^[5,6] as well as the synthesis of natural

fiber composites incorporating Ag NPs, as with silk fibroin.^[7,8] Several reagents have been used for the production of Ag NPs from silver ions, such as hydrazine^[9,10] or sodium borohydride plus light irradiation.^[11] However, some of the chemical reducing agents used can cause environmental problems. Therefore, the bio-reduction of silver ions to Ag NPs is of increasing interest, including in the functionalization and colouration of silk fabric using chitosan as the reductant^[12] and also with tea stem extract.^[13] Using plant extracts to access Ag NPs has gained increasing attention among researchers due to the cost-effective, green chemistry involved, as well as the simple methodology and inexpensive equipment requirements.^[14,15] Some recent examples of such bio-reductions to synthesize Ag NPs cover the use of extracts from *Colchicum luteum* root,^[16] *Mentha pulegium* and *Crocus caspius*,^[17] hulls of *Pistacia vera* and rhizomes of *Sambucus ebulus*,^[18] *Medicago sativa*,^[19] and Peruvian purple potato (Kulli papa).^[20] An aqueous extract from black rice containing anthocyanins has also been used for the simultaneous dyeing and production of Ag NPs on cellulose fabric.^[21] The Ag NPs derived from bio-reduction methods generally have a high surface area, high dispersion and smaller particle size.

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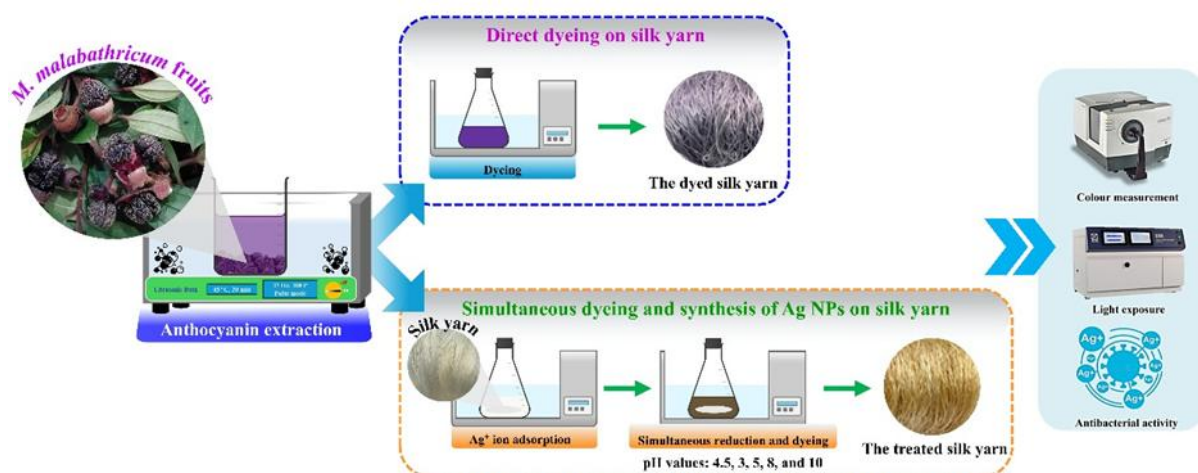


Fig. 1: Overview of our research findings.

Another plant source of interest is *Melastoma malabathricum*. This plant is a perennial shrub in the family *Melastomataceae* and is found mostly throughout South and Southeast Asia, including Thailand. Polyvinylalcohol-modified extract from leaf material of *Melastoma malabathricum* has been used to generate Ag NPs.^[22] *Melastoma malabathricum* also produces highly coloured fruits (Fig. 1) which contain two principal anthocyanin compounds, cyanidin-3-glucoside and cyanidin-3,5-diglucoside,^[23] which can be used for textile dyeing and also for separate Ag NP production utilizing the reductant properties of anthocyanins.^[20,21,24] The aqueous extract of *M. malabathricum* fruits can be used as a colourant to dye cotton fabrics by sonication dyeing in the presence of different mordants^[25] as well as ultrasound dyeing of polyester material^[26] together with silk yarn.^[27] Thus, anthocyanin compounds from the aqueous or methanolic extract of *M. malabathricum* fruits have the potential to be used as both a colourant to dye silk yarn and as bio-reducing agents for the in situ synthesis of Ag NPs on the silk at the same time. However, as far as can be ascertained, there have been no complementary studies on the simultaneous dyeing and deposition of Ag NPs on silk yarn through in situ green synthesis using *M. malabathricum* fruit extract. These new studies are now reported in this paper.

Addressing the need for sustainable textile functionalization, this research introduces a bio-inspired strategy for imparting multi-functionality to silk yarn. Specifically, this work utilizes anthocyanin extracts from *M. malabathricum* fruits to synthesize Ag NPs and colour silk yarn simultaneously. This innovative approach leverages the extract's dual potential as both a bio-reducing agent of silver ions to generate Ag NPs on the silk surface with a natural colourant. In this work, the influence of different pH values on this process was investigated. Additionally, the light fastness

properties and antibacterial activity of the treated silk yarn samples were also evaluated.

2. Materials and methods

2.1 Materials

The degummed silk yarn (Luangpirot species) was bought from Ruenmail, Muang Surin, Surin province, Thailand. Analytically pure silver nitrate (AgNO_3 , Merck, Germany), sodium hydroxide (NaOH , Merck, Germany), glacial acetic acid (CH_3COOH , Merck, Germany) and methanol (CH_3OH , Merck, Germany) were used in this study.

2.2 Instruments

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were collected using a Nicolet 6700 spectrometer (Thermo Company, USA) using a single-bounce ATR-FTIR spectroscopy accessory (Smart Orbit) with a diamond internal reflection element (IRE) at ambient temperature (25°C). The morphology of the Ag NPs was investigated using TEM, performed on a JEOL JEM-2100 microscope (JEOL Inc., MA, USA) and operated with an accelerating voltage of 120 kV. A field emission scanning electron microscope (FE-SEM Merlin Compact, Zeiss, Germany) was employed to image the morphologies and SEM-EDS spectra of the degummed silk and the treated silk yarn samples. Hydrodynamic diameters and the polydispersity index of the Ag NPs were determined using a Malvern Nano ZS light-scattering apparatus (DLS; NanoZS4700 nanoseries, Malvern Instruments Ltd., Worcestershire, UK). A thermostatted shaker bath (SWB 5050 shaking water bath; National Labnet Company, USA) was used for the simultaneous dyeing and deposition of Ag NPs on silk yarn through in situ green synthesis with the *M. malabathricum* fruits extract; solvent extraction of the fruits was assisted by sonication (Elmasonic P 30 H ultrasonic cleaning bath, Germany; 50/60 Hz, 230-240 V). Solution pH values were measured with a pH meter (Orion 420A; Thermo Scientific, USA). A colour spectrophotometer (HunterLab, Model: LabScan XE $0^\circ/45^\circ$, illuminant D65/10° observer) was used to

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measure the reflectance of dyed yarn and colour coordinate CIE $L^*a^*b^*$ values. A lyophilizer (EYELA PFP-1000, Tokyo Rikakikai Co., Ltd, Japan) was utilized to lyophilize the dye solution extracted from *M. malabathricum*.

2.3 Methods

2.3.1 Extraction of anthocyanins from *M. malabathricum* fruits

The fresh fruits of *M. malabathricum* (Walailak University grounds, Nakhon Si Thammarat, Thailand) were crushed and then mixed with acetic acid (0.5% v/v) in methanol to extract the anthocyanins from these fruits, keeping a material to liquor ratio of 1:20. The mixture was sonicated at 60 °C for 90 min using an ultrasonic bath. The liquid extract was removed by filtration, and the fruit residue was extracted a second time under the same conditions as the first extraction. The combined filtered extracts were then evaporated in a rotary evaporator at 40 °C to yield a crude extract, which was subsequently used to prepare aqueous solutions at different pH values.

The anthocyanin content of the initial crude extract obtained from the fruits was determined without further purification using the pH differential method.^[28] It was shown that the total anthocyanin content in the extract of *M. malabathricum* fruits was 574 mg/100 g of fresh fruits of *M. malabathricum*.

For the direct and simultaneous dyeing, the procedure described by Chairat *et al.* (2025)^[27] was followed. The process began by extracting fresh fruits (500 g) of *M. malabathricum* in distilled water (2.50 L) for 30 min at 40 °C, utilizing an ultrasonic bath (37 kHz sweep mode frequency). Following filtration, the resulting filtrate was freeze-dried, yielding a deep violet powder. This powder was then used for dyeing without further purification.

2.3.2 Direct dyeing of silk yarn with *M. malabathricum* fruits extract

Silk yarn was directly dyed with the aqueous dye solution (1.80×10^4 mg L⁻¹, initial pH value of dye solution was 4.5) from *M. malabathricum* fruits at 30 °C for 60 min in a thermostatted shaker bath (150 strokes min⁻¹) maintaining a material to liquor ratio of 1:25. After dyeing, the silk yarn was withdrawn from the bath and then washed with tap water for unfixed dye removal before air drying of the yarn at room temperature.

2.3.3 Simultaneous dyeing and in situ synthesis of Ag NPs on silk surface with anthocyanin dye solution from *M. malabathricum* fruits

Silk yarn was placed in an aqueous silver (I) nitrate solution (0.04 mol L⁻¹) with a material to liquor ratio of 1:25 at room temperature for 30 min in a thermostatted shaker bath operated at 150 strokes min⁻¹. The silk was then removed, squeezed and dried in a dyeing oven at 60 °C for 10 min. The crude anthocyanin dye extract from *M. malabathricum* fruits was

made up in freshly prepared aqueous solutions (1.80×10^4 mg L⁻¹) at pH values of 3, 5, 8, and 10, using sodium hydroxide solution (1.0 mol L⁻¹) and glacial acetic acid to adjust the pH values. Then, simultaneous dyeing and reduction of silver ions to generate Ag NPs on the yarn was achieved by shaking the silver nitrate-treated silk yarn with the anthocyanin dye solution (material to liquor ratio of 1:25) in a conical flask at 30 °C for 60 min in a thermostatted shaker bath operated at 150 strokes min⁻¹. After this, the treated silk yarn was removed, washed with tap water and finally air dried at room temperature. Colour strength (K/S) values for all samples, including the sample from direct dyeing above, were measured by the light reflectance technique using the Kubelka-Munk Eq. (1):^[29]

$$K/S = \frac{(1 - R)^2}{2R} \quad (1)$$

where R is the observed reflectance, K is the absorption coefficient, and S is the light scattering coefficient. A higher K/S value indicates a high dye receptivity of the substrates.

2.3.4 Treated and untreated silk yarn

Throughout this paper, treated silk yarn refers to yarn first treated with silver nitrate solution, removed and dried, and then exposed to the re-constituted *M. malabathricum* fruit extract at different pH values to induce simultaneous dyeing and Ag NP production. Untreated silk yarn refers to silk yarn that was dyed directly with *M. malabathricum* fruit extract (at pH 4.5) without any prior treatment with silver nitrate solution.

2.3.5 Characterization of Ag NPs

Freshly prepared Ag NPs were obtained by reducing silver ions to nanoparticulate silver using anthocyanin dye extract from *M. malabathricum* fruits made up in an aqueous solution. The aqueous dye solution (1.80×10^4 mg L⁻¹) was prepared at pH values of 3, 5, 8, and 10 as described above. The required amount of silver nitrate (0.17 g) was added to each of these solutions (25 mL each) at the different pH values in separate conical flasks, and then the flasks were shaken at 30 °C for 60 min in a thermostatted shaker bath operated at 150 strokes min⁻¹. The Ag NP-containing solution was then filtered through a 5-μm syringe filter before the particle size measurement of the Ag NPs by dynamic light scattering (DLS). The polydispersity index (PDI) of the Ag NPs was also determined.

2.3.6 Antibacterial activity of treated silk yarn

The antibacterial activity of treated silk yarn was carried out using the standard test method ASTM E2149 for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions. Gram-positive [*Staphylococcus aureus* (ATCC 6538); *S. aureus*] and Gram-negative [*Escherichia coli* (ATCC 25922); *E. coli*] bacteria were used as test organisms. An incubated test culture within a nutrient broth was diluted using a sterilized 0.3 mM phosphate buffer

(pH 7.4) to give a final concentration of $1.0 - 5.0 \times 10^5$ CFU/mL (CFU: colony-forming units). This solution was used as a working bacterial dilution. Before testing, all silk yarn samples were sterilized at 121°C and 15 psi for 30 min and then cut into small 5 cm lengths (0.5 – 2.0 g). Each sample was then transferred to a 250 mL Erlenmeyer flask containing 50 mL of the working bacterial dilution. All flasks were loosely capped, placed in an incubator, and shaken for 1 h at 37°C and 120 strokes min^{-1} using a wrist-action incubator shaker. After a series of dilutions using the phosphate buffer solution, 1 mL of the diluted solution was plated on the nutrient agar. The inoculated plates were incubated at 37°C for 24 h and the surviving cells were counted. The average values of the duplicates were converted to CFU/mL in the flasks by multiplying with the dilution factor. The antimicrobial activity was expressed as the % Reduction (the percentage reduction) of the organism (CFU/mL) after contact with the test specimen, compared to the number of bacterial cells surviving after contact with the control. The antibacterial activity of treated silk yarn was judged to be effective when the antimicrobial activity value (R) was ≥ 2 , as determined from the following Eq. (2):

$$R = \log_{10} (B/C) \quad (2)$$

where B = average of the number of viable cells of bacteria on the untreated silk test piece after 1 h, and C = average of the number of viable cells of bacteria on the antimicrobial test piece (treated silk) after 1 h.

2.3.7 Light exposure

The silk yarn samples were wound around a white card twice in taut layers, and a portion of each wound sample was then covered with aluminium foil. All samples were directly subjected to simulated sunlight irradiation conditions generated in a Q Sun Xenon Test Chamber (Model: Xe-3-HC), having a Xenon arc lamp and Daylight Q Filter, with an irradiance of 1.16 W m^{-2} at 420 nm. All samples were exposed to the simulated sunlight for 24 h with a relative humidity of $65 \pm 5\%$ in the chamber air at $35.0 \pm 2.0^\circ\text{C}$, together with the black panel temperature at $45.0 \pm 2.0^\circ\text{C}$.

2.3.8 Colour measurement

All treated and untreated silk yarn samples were wound around the skein holder twice in taut layers. Each measurement was evaluated from an average of three readings. The reflectance and colour coordinate CIE $L^*a^*b^*$ values were determined using a benchtop LabScan XE spectrophotometer, which is a reflectance Specular Exclude (SPEX) instrument using $0^\circ/45^\circ$ optical geometry, illuminant D65 and 10° standard observer features. The tristimulus values, X , Y , and Z , under illuminant were transformed to CIE $L^*a^*b^*$ coordinates. The colour indices obtained from the CIE $L^*a^*b^*$ measurements characterize the hue colour. The level of colour fading during simulated sunlight exposure was measured by

the ΔE^* value to compare the colour difference between the original and the faded portions in each exposure period. The ΔE^* value was calculated using the following Eq. (3):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where ΔE^* is the colour difference value, $\Delta L^* = L^*_{\text{faded portion}} - L^*_{\text{original portion}}$; $\Delta a^* = a^*_{\text{faded portion}} - a^*_{\text{original portion}}$; $\Delta b^* = b^*_{\text{faded portion}} - b^*_{\text{original portion}}$; ' L^* ' describes lightness, ' a^* ' measures redness (+positive) or greenness (–negative) and ' b^* ' measures yellowness (+positive) or blueness (–negative). The experimental data in this study were analyzed utilizing the statistical program IBM SPSS Statistics to assess the colour difference values (ΔE^*) for the treated silk yarns compared with those for the untreated yarn.

3. Results and discussion

3.1 UV-Vis study

To determine the optimal concentration of an aqueous silver (I) nitrate solution for Ag NP synthesis, concentrations of 0.01, 0.02, 0.03, and 0.04 mol L^{-1} were investigated at pH 10 using the aqueous dye solution ($1.80 \times 10^4 \text{ mg L}^{-1}$) as a reducing agent. It was observed that increasing the concentration of AgNO_3 from 0.01 to 0.04 mol L^{-1} increased the intensity of the plasmon peak of the Ag NPs as shown in Fig. 2a. Therefore, an aqueous silver (I) nitrate solution at 0.04 mol L^{-1} was identified as the most effective concentration and this concentration was then employed to investigate the effect of pH on Ag NP synthesis.

A complex interplay of processes is involved with the simultaneous anthocyanin-mediated Ag NP production on silk, together with dyeing of the yarn. However, some aspects of the processes can be teased out through analysis of the UV-Vis spectroscopic data obtained on solution-based model systems. The anthocyanin dye solution from *M. malabathricum* fruits at pH 4.67 showed a broad absorption maximum in the UV-Vis spectrum around 550 nm, as shown in Fig. 2b. Anthocyanin structures in aqueous solution, and thus the colour exhibited, depend on the solution pH. At acidic pH values ≤ 4 , the oxonium ion (flavylium ion) form of the anthocyanin predominates in the chemical equilibria, giving the solution a red colour.^[30] At higher pH values, the colour changes from pink through to blue as a result of conversion of the oxonium ion form to various intermediates, including phenolate anionic forms, particularly at pH values ≥ 7 .^[31–33]

Solution pH could also influence Ag NP production, and to gain a better understanding of this process, Ag NPs were prepared by reaction with the anthocyanin dye solution from *M. malabathricum* fruit extract ($1.8 \times 10^4 \text{ mg L}^{-1}$) as a reducing agent at pH values of 3, 5, 8, and 10. The UV-Vis spectra of the resultant Ag NP in these solutions at different pH values, compared with the spectrum of the dye alone (at pH 4.67), are illustrated in Fig. 2b. The surface plasmon vibration property of the Ag NPs could be investigated in this way. From our study, the solutions changed from a red colour

to a yellowish-brown colour at pH 8 and 10 due to the excitation of surface plasmon vibrations, thus confirming the formation of Ag NPs at these pHs. After 60 min of silver ion reduction at pH 8 and 10 at room temperature, the plasmon peaks were observed at 410 nm, indicating the formation of Ag NPs in the mixed solutions.

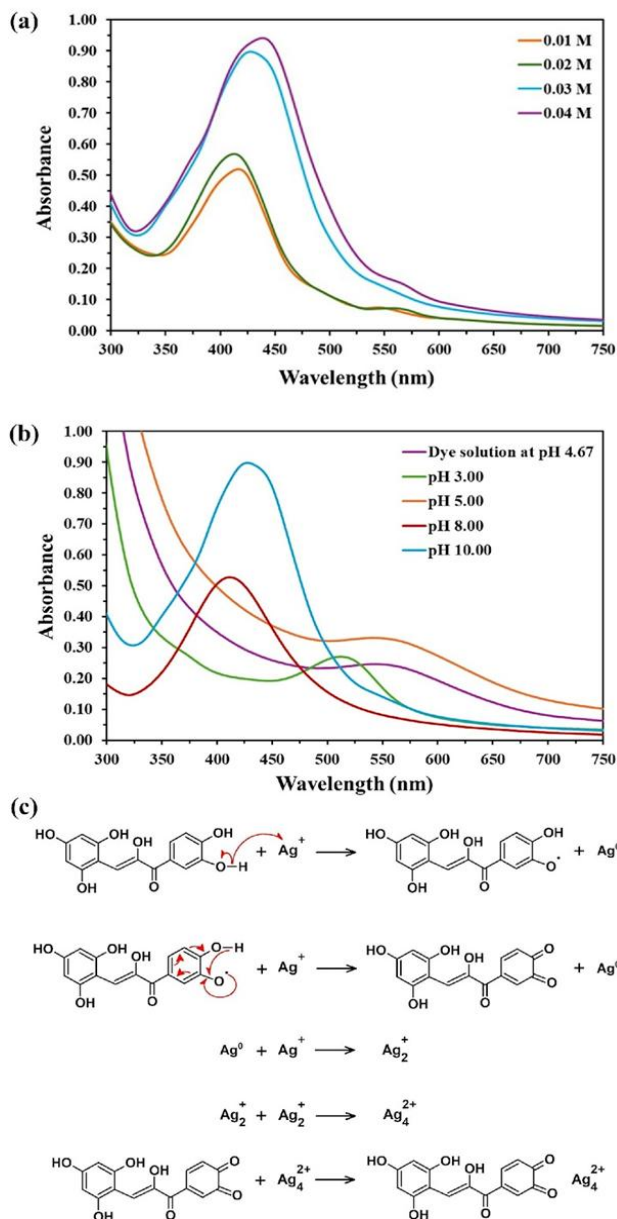


Fig. 2: (a) UV-spectra of Ag NPs in solutions at different concentrations of the aqueous silver (I) nitrate solutions at pH 10, (b) UV-Vis spectra of Ag NPs in solutions at different pHs and the anthocyanin dye solution alone at pH 4.67, and (c) the possible mechanism of Ag NPs synthesis using anthocyanin extract from *Melastoma malabathricum* fruits (chalcone forms of the aglycones shown).

At basic pH values, phenolic hydroxyl groups in the anthocyanin components (mainly cyanidin-3-*O*-glucoside and cyanidin-3,5-*O*, *O*-diglucoside) would be significantly ionised which would facilitate silver ion complexation and subsequent

electron transfer to reduce the silver (I) ion to silver (0) as shown in Fig. 2c. On the other hand, cyanidin-3-*O*-glucoside is transformed into a free radical which, in turn, reduces another silver (I) ion and is converted to ortho-quinone. Therefore, the mechanism involves the oxidation of phenolic hydroxyl groups in cyanidin-3-*O*-glucoside and cyanidin-3,5-*O*, *O*-diglucoside, leading to the formation of quinone or quinone-like structures, while the silver (I) ion is reduced to metallic silver. The cyanidin-3-*O*-glucoside acts as a reducing agent by donating electrons to silver ions to form Ag NPs. It also acts as a capping agent to prevent the nanoparticles from aggregating and helps stabilize them in solution. This behaviour is similar to that observed in the synthesis of Ag NPs through the inherent reducing property of anthocyanin compounds from black rice extract, with a solution pH of 10 being favoured.^[21]

The anthocyanins would be oxidised in this process, and they may also act as capping agents on the nanoparticles, with no other capping agents being required for stabilization of the nanoparticles.^[34] Non-ionised phenolic groups may also act as reductants, but, surprisingly, the surface plasmon resonance (SPR) band indicative of Ag NP production was not observed at 410 nm under pH 3 and 5 conditions when phenolic groups would be present. At pH 3, the UV-Vis spectrum had a maximum around 515 nm, while at pH 5, the UV-Vis spectrum was very similar to that of the dye solution (at pH 4.67) from *M. malabathricum* fruits. Presumably, the electron transfer process is slower from non-ionised phenolic groups in acidic conditions, and the reduction time of 60 minutes was insufficient to start to detect Ag NP formation. However, this result is in contrast to other work^[24] in which silver nanoparticles were synthesized from AgNO₃ using an aqueous *M. malabathricum* fruit extract at pH 4 (1 to 60 minute reaction time; mixed solution pH 4). It should be noted, though, that a different extraction procedure was used by these researchers in which diced *M. malabathricum* fruits were extracted with hot water (50 °C; 15 minutes) to give an aqueous, filtered extract (pH 6) to which the silver nitrate solution was added.^[24]

3.2 Characterization of Ag NP particle size and morphology

The particle size distribution of the Ag NPs is shown in Fig. 3. The largest size was observed at pH 3, and the size gradually decreased to about 86 nm with increasing pH from 5 to 10. This was indicative of incomplete reduction of silver ions over the 60-minute period under low pH conditions, leading to destabilized Ag NPs aggregates. This result is consistent with the UV-Vis spectroscopic observations (Fig. 2b) in which no SPR band for Ag NPs was observed at 410 nm under pH 3 and 5 conditions, but was seen at pH 8.0 and 10.0 with the complete formation of stabilized Ag NPs under the alkaline conditions; the original broad absorption band around 550 nm had also disappeared. The same result was observed by Fernando & Zhou (2019)^[35] who studied the effect of pH on

the particle size of Ag NPs. Moreover, the rate of change can be described in terms of the particle size of Ag NPs with different pHs. A higher rate of change occurred under the lower pH values of 3 and 5 at which the less stabilizing oxonium ion forms of the anthocyanins would be present, while the rate of change decreased with increasing pH due to

stabilization by the phenolate anionic forms of the anthocyanins,^[31] indicating a slower rate of aggregation compared to that in acidic conditions. This result is also consistent with the work of Al-Thabaiti & Khan (2020), who proposed a probable reduction of silver ions by cyanidin 3,5-di-*O*-glucoside into metallic Ag.^[30]

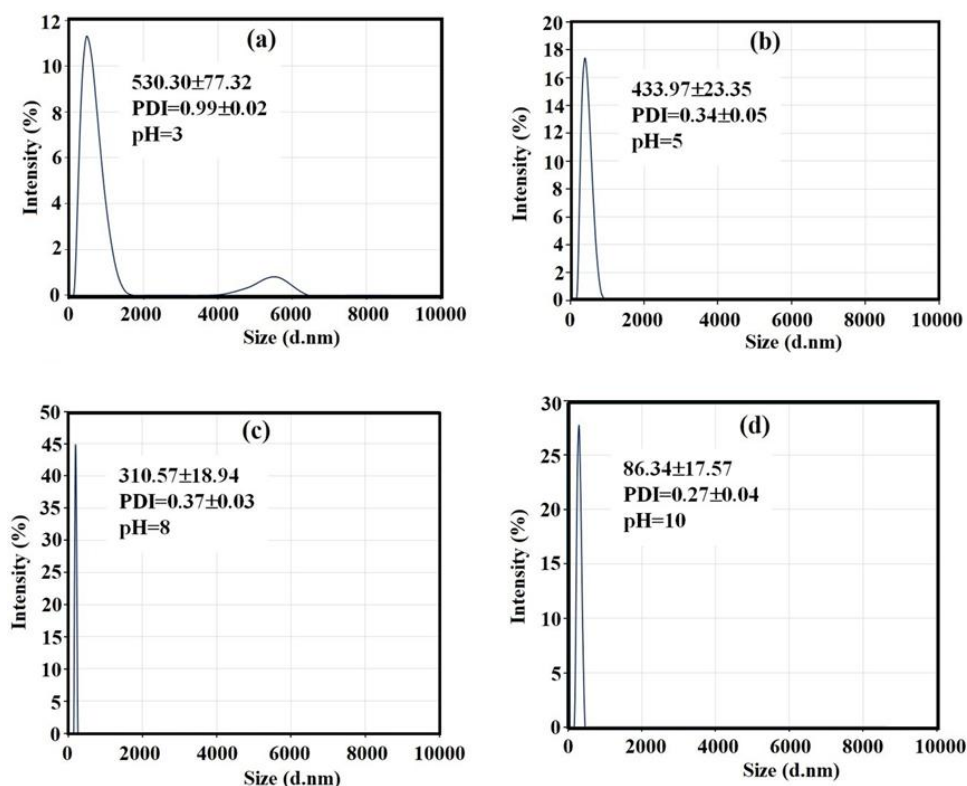


Fig. 3: The particle size distributions of Ag NPs at (a) pH 3, (b) pH 5, (c) pH 8, and (d) pH 10.

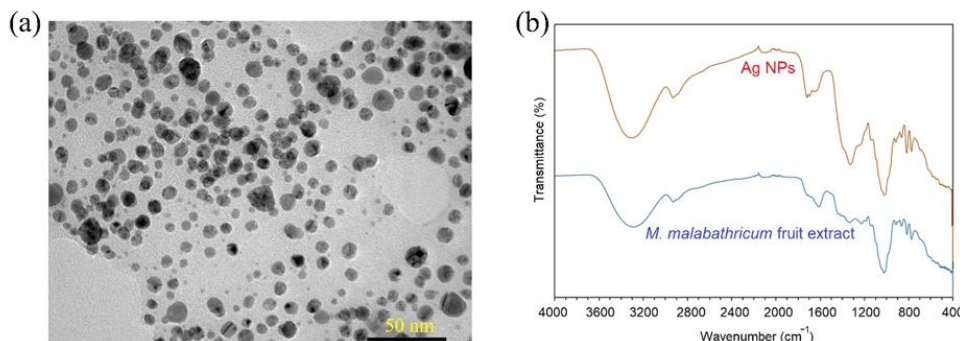


Fig. 4: TEM images of (a) Ag NPs and (b) ATR-FTIR spectra of Ag NPs (red), and *M. malabathricum* fruit extract (blue).

The size and shape of the prepared Ag NPs were analyzed using TEM, as shown in Fig. 4a. All particles are spherical. The particle size distribution shows the prepared nanoparticles having a size range from 4 to 17 nm with the average diameter of about 8.6 nm. This result suggested that the anthocyanin components (mainly cyanidin-3-*O*-glucoside and cyanidin-3,5-*O*,*O*-diglucoside) present in the *M. malabathricum* fruit extract could be used as reducing agents for silver ions as well as capping agents and stabilizers for the formed silver nanoparticles.

Fig. 4b displays the ATR-FTIR spectra of Ag NPs (red line) and natural *M. malabathricum* fruit extract (blue line). The result revealed that the ATR-FTIR spectrum of *M. malabathricum* fruit extract had no significant difference from the ATR-FTIR spectrum of the Ag NPs due to the low concentration of Ag NPs. The broad band at 3300 cm⁻¹ was assigned to O-H stretching of the sugar vibrations and phenol O-H groups. The wavenumbers at 1322 cm⁻¹ correspond to the C-O deformation of phenols, whereas bands at 1694 and 1717 cm⁻¹ are attributed to the C=C and C=O groups for aromatic rings, respectively. The absorbance at 1011 cm⁻¹ was

due to the stretching vibration of the C-O-C bond. Therefore, the spectral analysis of the concentrated *M. malabathricum* fruit extract shows the presence of O-H, C=O, C=C and C-O-C functional groups, which are characteristics of anthocyanins as reducing and stabilizing agents for silver nanoparticles.

3.3 Surface characterization of treated silk yarn

Morphologies of treated silk yarn samples at pH values of 3, 5, 8, and 10 before and after exposure to simulated sunlight were assessed and compared with the untreated silk yarn sample (only dyed with anthocyanin extract) and the results

are shown in Figs. 5 and 6, respectively. Untreated silk yarns have a smooth and clean surface. In contrast, treated silk yarn surfaces at different pHs showed some roughness due to a strong adherence of in situ-formed Ag NPs as revealed from the SEM images. Especially at pH values of 8 and 10, SEM-EDS spectra (Figs. 7b and 7c) confirmed the presence of Ag NPs on the surface of the dyed silk yarn; no silver oxide particles seemed to be formed. The high physicochemical adherence of the Ag NPs formed in situ is suggested to be due to the strong chemical affinity of Ag ions with the silk fiber. This affinity could be mediated through interactions with the

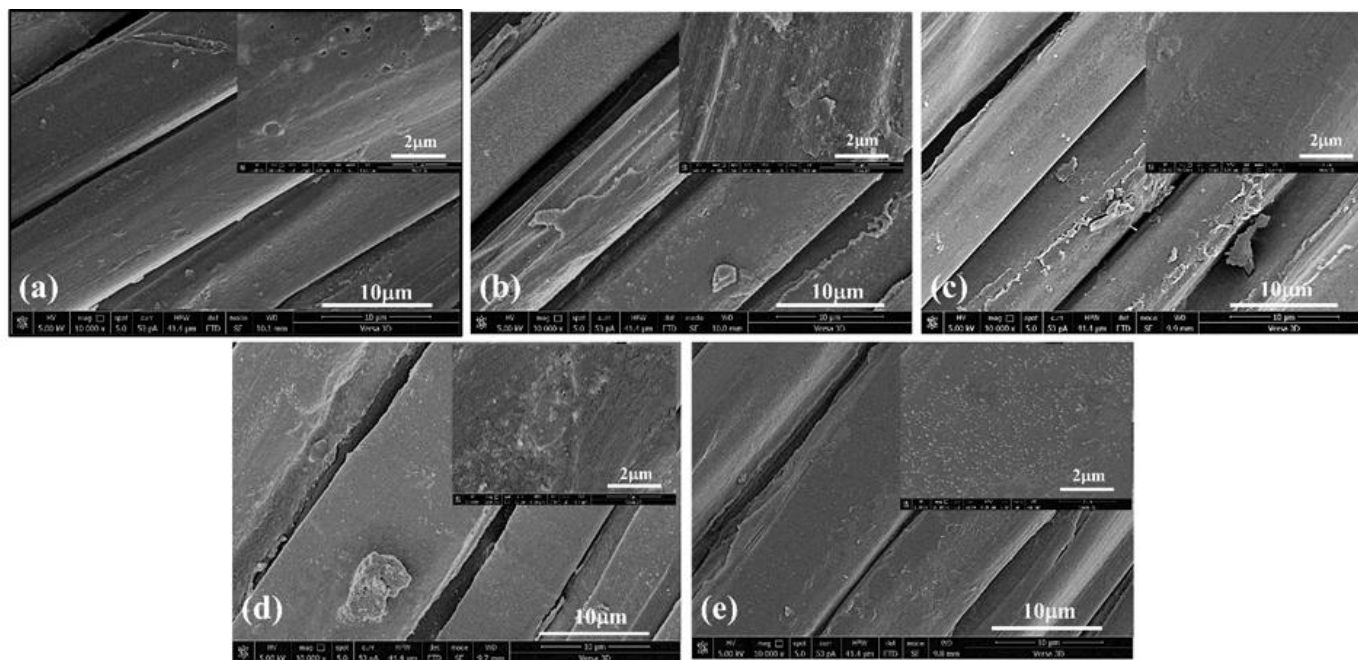


Fig. 5: SEM images of (a) untreated silk yarn and treated silk yarn before exposure to simulated sunlight using simultaneous dyeing and synthesis of Ag NPs on silk yarn at (b) pH 3, (c) pH 5, (d) pH 8, and (e) pH 10.

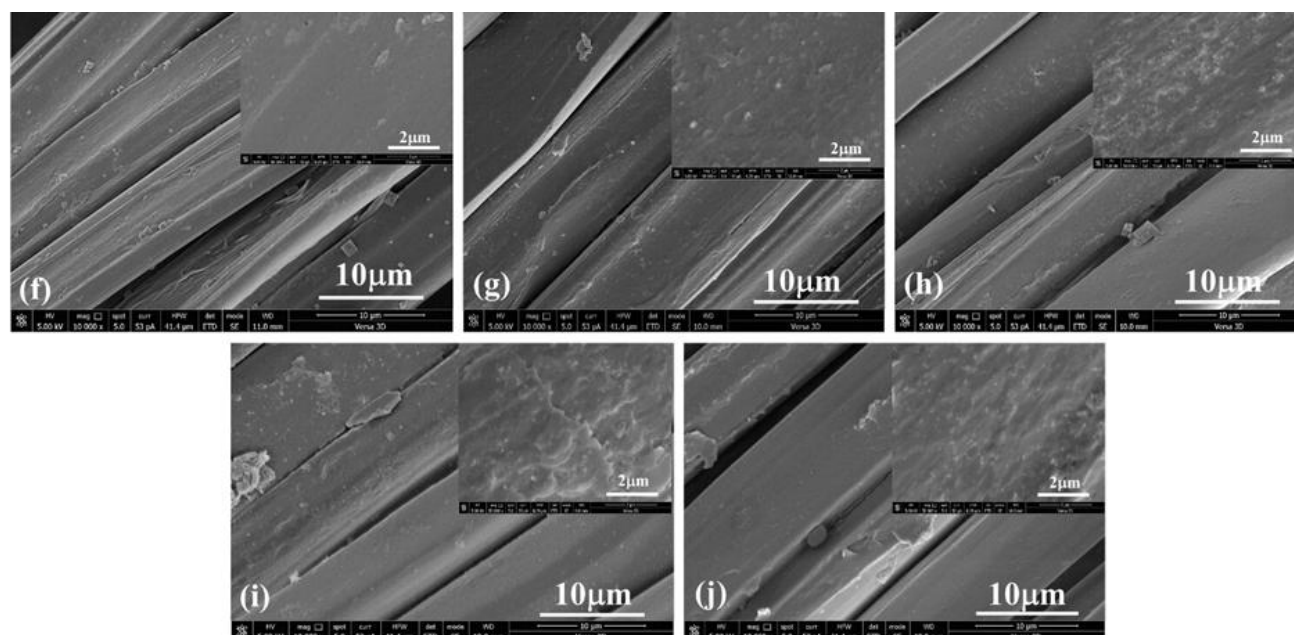


Fig. 6: SEM images of (f) untreated silk yarn with non-exposure to simulated sunlight and treated silk yarn samples at different pH values after 32 h of exposure to simulated sunlight at (g) pH 3, (h) pH 5, (i) pH 8, and (j) pH 10.

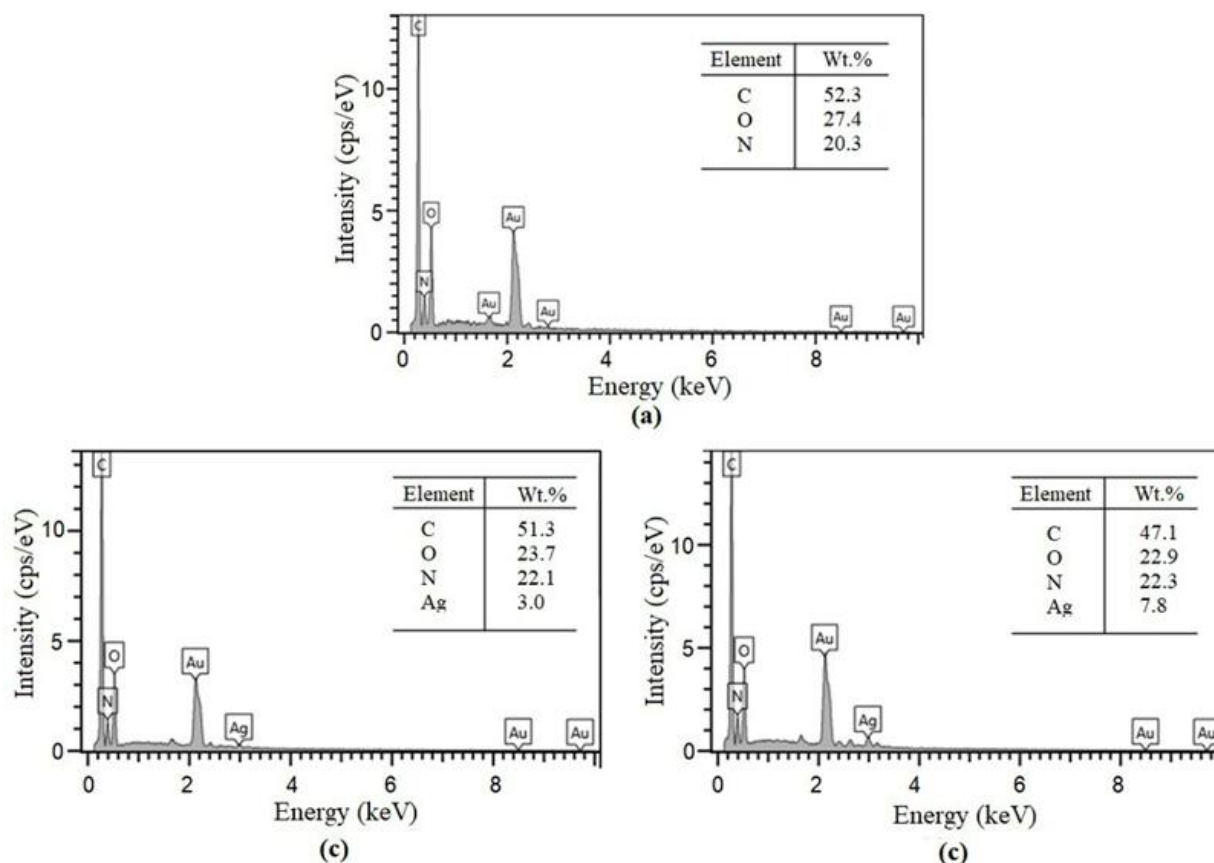


Fig. 7: SEM-EDS images of (a) untreated silk yarn and treated silk yarn before exposure to simulated sunlight using simultaneous dyeing and synthesis of Ag NPs on silk yarn at (b) pH 8, and (c) pH 10.

amide backbone ($-\text{CONH}-$), and serine or tyrosine hydroxyl ($-\text{OH}$) functional groups, or the cysteine thiol ($-\text{SH}$) group before in situ chemical reduction. Similar complexing of silver ions on the silk yarn surface to catechol or single free phenolic groups in the anthocyanin dye components could also occur before the reduction proceeds. It is most likely that the catechol group in the pendant aryl ring was oxidized twice via electron transfer from the phenolate ion moiety (at pH 10 to afford an *O*-benzoquinone substituent on the chalcone form of the anthocyanin. It is possible that there may also be a small contribution to Ag NP production through silk fibroin itself acting as a reductant via the phenolic groups in the silk tyrosine residues (3.5mole %) or possibly through the thiol group in the less abundant cysteine residues (1.0mole %)^[36] Solubilized silk fibroin plus light exposure has been used in the green synthesis of Ag NPs.^[37] In the current work, some evidence for possible light-induced Ag NPs production was also seen with silver nitrate – treated (and dried) silk yarn. Exposure of this yarn to ambient light over a 48-24 h period at room temperature resulted in a colour change from the initial light yellow to light brown, but no colour change was seen if the yarn was kept away from light.^[38]

3.4 Colour hue and changes on light exposure of the treated silk yarn

The apparent colour and colour measurements of untreated and treated silk yarn samples at different pH values of the dye

solution are shown in Tables 1 and 2, respectively. Prior to simulated sunlight exposure, the untreated silk yarn (Sample 1; only anthocyanin dyeing) and the silk yarns prepared at pH 3 (Sample 2) and 5 (Sample 3) all had a purple colour consistent with incomplete Ag NP formation during in situ reduction at these pH values in Samples 2 and 3. On the other hand, treated silk yarn at pH 8 (Sample 4) and pH 10 (Sample 5) provided a yellow colour, with the highest positive b^* value (yellowness) of all the samples being observed for the treated yarn at pH 10 (Sample 5) in line with the complete formation and deposition of Ag NPs on the surface of silk yarn at this pH. After 16 h and 32 h of exposure to simulated sunlight, all samples showed apparent colour changes, but some more than others (Table 1). These changes were also reflected in the colour measurements, which showed increasing b^* values with increasing exposure time for each sample. The increasing b^* value of the treated samples under simulated sunlight was caused mainly by the Ag NPs and oxidized dye remaining on the fiber.

After light exposure, Sample 5 again showed a deep yellow colour (highest b^* value) as well as the highest colour strength (K/S), supportive of the proposal that Ag NPs can increase the dye affinity or receptivity on the silk yarn surface. The colour changes observed after exposure to simulated sunlight may involve the photo-induced synthesis of Ag NPs by the simulated sunlight.^[39]

Table 1: Comparison of the apparent colour before and after (16 h and 32 h) simulated sunlight exposure of the untreated silk yarn (Sample 1; anthocyanin dyeing only at pH 4.5), and treated silk yarns (Samples 2-5) derived from simultaneous dyeing at different pHs and in situ synthesis of Ag NPs on the silk surface at different pH values with anthocyanin dye solution from *M. malabathricum* fruits.



Table 2: Colour measurements before and after (16 h and 32 h) simulated sunlight exposure of the untreated silk yarn (Sample 1; anthocyanin dyeing only at pH 4.5), and treated silk yarns (Samples 2-5) derived from simultaneous dyeing at different pHs and in situ synthesis of Ag NPs on the silk surface at different pH values with anthocyanin dye solution from *M. malabathricum* fruits.

Sample	pH value	K/S	CIE		
			L^*	a^*	b^*
Non-exposure to simulated sunlight					
1	4.5	1.72 (560 nm)	54.32	4.35	-5.50
2	3	2.00 (560 nm)	51.15	5.51	-0.70
3	5	2.85 (560 nm)	45.76	4.60	-2.55
4	8	2.36 (420 nm)	55.34	7.62	13.62
5	10	2.41 (420 nm)	61.31	9.12	23.02
After 16 h of exposure to simulated sunlight					
1	4.5	0.99 (560 nm)	60.66	1.63	5.14
2	3	3.72 (420 nm)	51.60	7.32	15.19
3	5	4.01 (420 nm)	46.92	7.97	11.32
4	8	3.09 (420 nm)	54.83	9.39	21.28
5	10	3.54 (420 nm)	60.17	10.71	26.54
After 32 h of exposure to simulated sunlight					
1	4.5	1.94 (420 nm)	62.40	2.20	12.83
2	3	3.66 (420 nm)	54.14	6.15	17.56
3	5	4.34 (420 nm)	49.15	7.47	16.03
4	8	3.33 (420 nm)	55.86	8.65	25.17
5	10	4.09 (420 nm)	61.17	7.92	28.38

3.5 Inhibition of photo-fading

The fading curve of anthocyanin dye from *M. malabathricum* fruits on silk yarn (Samples 1 and 5) is shown in Fig. 8. Fading of the dye on the untreated silk yarn (Sample 1) was very rapid at first and then slowed down to a constant rate. The fading rate curve of this dye corresponds to one in the type III category as described by Cox-Crews.^[39] However, the fading curve of the treated silk yarn prepared at a pH value of 10 (Sample 5) corresponds to a type I photo-fading category. Type I suggests that the rate of fading reduces steadily with time because the Ag NPs are probably dispersed well throughout the silk surface after reduction at pH 10. Further results concerning photo-fading are presented in Fig. 9 for colour difference values (calculated ΔE^*) in untreated and treated silk yarn samples prepared at different pH values after 24 h of exposure to simulated sunlight. It was found that Sample 5 at pH 10 exhibited a great improvement in the light fastness of the treated silk yarn as compared to the other samples. This is due to more Ag NPs on the silk yarn surface that can increase the absorption of visible light, as confirmed

by the SPR band at 410 nm. Therefore, the Ag NPs not only improve the colour strength but also significantly improve the colour fastness, which is a recurrent problem with natural dyes.

3.6 Antibacterial activity

The antibacterial activity results on the untreated and treated silk yarn samples are presented in Table 3. These results were obtained using the ASTM E2149 method for testing the surface. The antibacterial efficacy of untreated and treated silk yarn samples before and after exposure to simulated sunlight for 32 h against Gram-positive and Gram-negative bacteria was investigated. It was found that the Ag NP-coated and dyed silk yarn exhibited a higher percentage reduction in viable bacterial counts than untreated silk yarn, consistent with the known antibacterial properties of these nanoparticles. Further, probably limited, contributions to the antibacterial activities observed may be mediated by the anthocyanins^[40-42] or their oxidation products bound on the silk, although this was not assessed. Some surface interaction may be possible, which could synergize with the Ag NP/Ag⁺ actions.

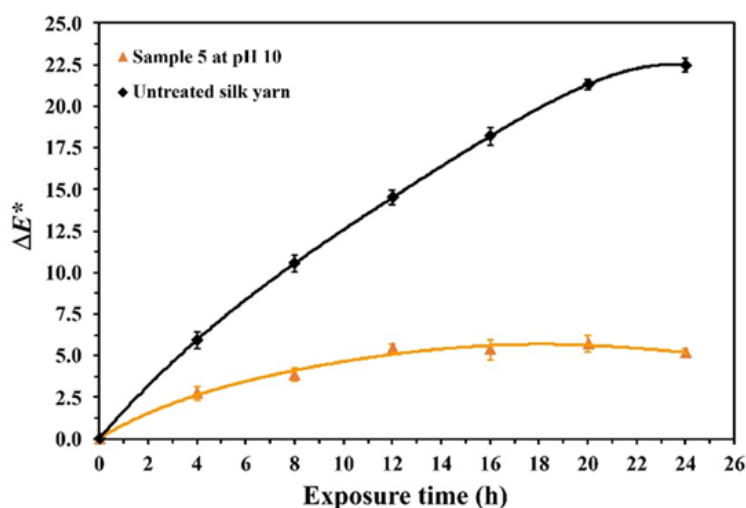


Fig. 8: Fading rate curve of anthocyanin dye from *M. malabathricum* on silk yarn (untreated with AgNO₃; Sample 1) and the treated silk yarn (Sample 5) using simultaneous dyeing and synthesis of Ag NPs on silk yarn at pH 10.

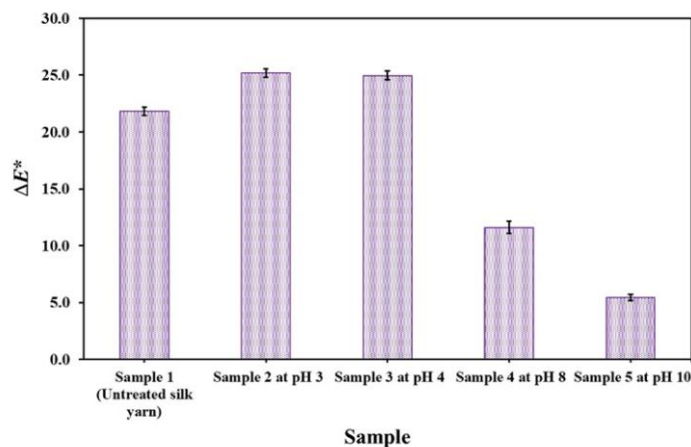


Fig. 9: Colour difference (ΔE^*) of untreated and treated silk yarn samples at different dyeing pH values after 24 h of exposure to simulated sunlight.

Significantly, the best antibacterial activity (against *S. aureus* and *E. coli*) was seen with the treated silk yarn prepared at basic pH, particularly pH 10 (Samples 5 and 5') both before and after exposure to simulated sunlight and against both bacteria; $\log_{10}$ reductions (R) in CFUs (colony forming units) greater than 4 relative to the untreated silk yarn controls were observed. This result is consistent with the complete formation of Ag NPs under alkaline conditions in 60 minutes. It can be seen clearly that the percentage reduction of *S. aureus* (Gram-positive) is lower than for *E. coli* (Gram-negative) bacteria before and after exposure to simulated sunlight, most likely because of the major difference between the Gram-positive and Gram-negative peptidoglycan cell wall components involved in the thickness of the layers surrounding the plasma

membrane. The many layers of peptidoglycan (about 30-100 nm thickness) in Gram-positive bacteria are thicker than in Gram-negative bacteria.^[43] This result agreed with those of Meikle *et al.* (2020), who confirmed that the antimicrobial efficacy of Ag NPs was higher against Gram-negative bacteria due to a thin peptidoglycan layer reducing the chances of Ag NPs penetration into the cells.^[44] Similar high activity was seen against *E. coli* for treated silk yarn both before and after simulated sunshine exposure, but lower activity against *S. aureus* for samples prepared at pH 3. Interestingly, for treated silk yarn prepared at pH 8, activity against *S. aureus* pre-exposure to sunlight was slightly better than against *E. Coli*, but after 32 h of simulated sunlight exposure, the activity against *S. aureus* had declined significantly (Table 3).

Table 3: Viable cell count and percentage reduction of *E. coli* and *S. aureus* on untreated silk yarn (Sample 1) with non-exposure to simulated sunlight, treated silk yarn samples prepared at different pH values pH 3 (Sample 2), pH 8 (Sample 4), and pH 10 (Sample 5), and untreated silk yarn (Sample 1) together with treated silk yarn samples prepared at different pH values pH 3 (Sample 2'), pH 8 (Sample 4') and pH 10 (Sample 5') after being exposed to simulated sunlight. Antibacterial activity (R) was calculated using the equation noted in the methods.

Sample	Test microorganisms	
	<i>Escherichia coli</i> (ATCC 25922)	<i>Staphylococcus aureus</i> (ATCC 6538)
Non-exposure to simulated sunlight		
Sample 1 (Untreated silk yarn)		
Number (CFU/mL)	$3.67 \times 10^5 \pm 0.23$	$4.10 \times 10^5 \pm 0.29$
Sample 2 (Treated silk yarn); pH 3		
Number (CFU/mL)	<10	$4.24 \times 10^4 \pm 0.13$
% Reduction	99.99	89.66
Antibacterial activity R ($\log_{10}$ reduction)	4.56	0.99
Sample 4 (Treated silk yarn); pH 8		
Number (CFU/mL)	<10	<10
% Reduction	99.99	99.99
Antibacterial activity R ($\log_{10}$ reduction)	4.56	4.61
Sample 5 (Treated silk yarn); pH 10		
Number (CFU/mL)	<10	<10
% Reduction	99.99	99.99
Antibacterial activity R ($\log_{10}$ reduction)	4.56	4.61
After 32 h of exposure to simulated sunlight		
Sample 1' (Untreated silk yarn)		
Number (CFU/mL)	$3.74 \times 10^5 \pm 0.05$	$3.50 \times 10^5 \pm 0.46$
Sample 2' (Treated silk yarn); pH 3		
Number (CFU/mL)	<10	$1.88 \times 10^5 \pm 0.54$
% Reduction	99.99	46.29
Antibacterial activity R ($\log_{10}$ reduction)	4.57	0.27
Sample 4' (Treated silk yarn); pH 8		
Number (CFU/mL)	<10	$7.40 \times 10^3 \pm 0.56$
% Reduction	99.99	97.89
Antibacterial activity R ($\log_{10}$ reduction)	4.57	1.67

Sample	Test microorganisms	
	<i>Escherichia coli</i> (ATCC 25922)	<i>Staphylococcus aureus</i> (ATCC 6538)
Sample 5* (Treated silk yarn); pH 10		
Number (CFU/mL)	<10	<10
%Reduction	99.99	99.99
Antibacterial activity <i>R</i> (log ₁₀ reduction)	4.57	4.57

Table 4: Comparison of bacterial reduction between results in this work and work by others using different natural plants in Ag NPs synthesis for textile coating.

Natural reducing agent source	Textile fiber	% Reduction		Reference
		<i>E. coli</i> (%)	<i>S. aureus</i> (%)	
Black rice	Cotton	97.4	99.6	[27]
<i>Scrophularia striata</i> Boiss extract	Polyamide	100	100	[49]
<i>Andrographis paniculata</i> leaves extract	Pure cotton	100	100	[50]
	Polycotton	100	100	
	Polyester	100	100	
<i>Melastoma malabathricum</i> L. fruits	silk yarn	99.99	99.99	This work

Although Ag NPs have been proven effective against both Gram-positive and Gram-negative bacteria,^[45] fungi and viruses, the exact way in which they exert their antimicrobial action is not fully understood as yet, but several mechanisms seem to be involved. Some of the mechanisms of antibacterial activity of Ag NPs that have been proposed include adhesion of Ag NPs onto the surface of the cell wall and membrane, Ag NPs (< 50 nm) penetration inside the cell and damage of intracellular structures and biomolecules, Ag NP- induced cellular toxicity and oxidative stress caused by the generation of reactive oxygen species (ROS) and free radicals, and modulation of signal transduction pathways.^[3,46,47] A further element in the mechanism of action profile is the continuous release of antibacterial silver ions from Ag NPs, which may be considered a mechanism for killing bacteria.^[34] A multi-target mechanism of action seems likely in connection with the activity of silver ions (and also nanosilver) against *S. Aureus*, with one vital target being the enzyme 6-phosphogluconate dehydrogenase.^[48] Other work by Zare-Bidaki *et al.* (2024) similarly proposed a multi-pronged mechanism for the antibacterial activity of *Colchicum luteum* root-mediated Ag NPs. This mechanism involves physical disruption of the bacterial cell envelope, chemical damage to genetic material, and functional impairment of vital cellular machinery through processes such as protein and ribosome denaturation and the generation of oxidative stress.^[16] Some of the results from this current study show that treatment pH and type of bacteria play an important role in the antibacterial activity of Ag NPs coated on the surface of the treated silk yarn before and after exposure to simulated sunlight. The sample prepared at pH 10 showed the highest antibacterial activity compared to samples prepared at other pHs.

A comparison of the percentage reduction of *E. coli* and *S. aureus* on silk yarn treated with Ag NPs (pH 10) in this study was compared to results from other reports utilizing various

natural plants for Ag NPs synthesis in textile coating as shown in Table 4. The antibacterial activity observed in this work was consistent with findings from other studies. This confirmed that the effective antibacterial activity of Ag NPs synthesized via anthocyanin extracts from *M. malabathricum* fruits and coated on silk yarn.

4. Conclusion

Silver nanoparticles (Ag NPs) were successfully synthesized on a silk yarn surface by a facile green process using anthocyanin –containing *M. malabathricum* fruit extract solution at a basic pH as a reducing agent at room temperature. The solution pH was a key factor in controlling the formation and particle size of the stabilized Ag NPs. At pH 10, the Ag NPs formed under basic conditions were smaller, averaging 86.34 ± 17.75 nm in size. In contrast, at acidic and neutral pHs, there was incomplete reduction of silver ions to Ag NPs within 1 h. Exposure of silk yarn pre-treated with silver nitrate to the anthocyanin dye solution at pH 10 resulted in simultaneous dyeing and surface Ag NP production on the yarn. This treated silk yarn exhibited very favourable properties including a high yellowness colour hue with b^* of 28.23, and the highest colour strength (K/S) of 4.09, particularly after 32 h of exposure to simulated sunlight compared with the purple coloured untreated silk yarn (dyed directly at pH 4.5 with no silver treatment). Moreover, the treated silk yarn prepared at pH 10 also exhibited significant resistance to photo-fading via a suggested fading rate type I category mechanism. The excellent improvement in light fastness compared to untreated silk yarn (and with other treated silk yarn samples prepared at lower pH values) was seen after 24 h of exposure to simulated sunlight. The antibacterial activity of Ag NPs coated on silk yarn surfaces showed a percentage reduction of *S. aureus* and *E. coli* bacteria before and after exposure to simulated sunlight at all pHs. Treated silk yarn samples prepared at a pH of 10

indicated the highest antibacterial activity against both bacteria, resulting in log₁₀ reductions (*R*) in CFUs (colony forming units) exceeding 4. The present study shows that eco-friendly simultaneous dyeing with anthocyanin dyes and Ag NPs production on silk yarn with good colour and resistance to photo-fading characteristics, plus excellent antibacterial properties, is one with considerable potential for textile coating applications. Future work will involve scaling this process for industrial use, assessing its cost-effectiveness, and exploring its applicability to various textile fibers.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

CRedit Statement

Montra Chairat: Conceptualization, Methodology, Investigation, Analysis, Writing – Original draft, Writing – Review & editing, Revision, Supervision, Project administration, Funding acquisition. **Natthaphong Naksithong:** Visualization. **Areeya Langthoh:** Visualization. **Pattarapond Gonil:** Investigation. **Choochart Warin:** Investigation, Formal analysis. **Warayuth Sajomsang:** Methodology, Investigation, Writing & editing, Revision. **John B. Bremner:** Conceptualization, Resources, Visualization, Writing – Review & editing, Revision.

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