
Sustainable application of natural dye from Clitoral plant for dyeing and surface finishing of textile fibers and its microbial effect to overcome environmental pollution

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Ali, N. F. and Abd-Elsalam, I. S. (2024). Sustainable application of natural dye from Clitoral plant for dyeing and surface finishing of textile fibres and its microbial effect to overcome environmental pollution. *International Journal of Agricultural Technology* 20(2):493-500.

Abstract Result showed that the natural dye from *Clitoria ternatea* actively antimicrobial activity against *E. coli*, *S. aureus*, *C. albicans* at pH 3. Moreover, the natural dye from *C. ternatea* greatly inhibited *E. coli*, *B. subtilus*, *S. aureus*, *C. albicans* at concentration of 4%. It is suggested that the natural dye from *C. ternatea* may feasibly develop to be surface finishing of textile fibres and antimicrobial products. The ability of extract to change colour of *C. ternatea* is proved to be distinguishing feature. There are found to be four different coloured anthocyanin forms that can alternately change colour based solely on pH by adding a weak acid like lemon or lime juice. The application of natural dye produced from clitoria plant affected antimicrobial activity. The results exhibited an excellent colour strength (K/S) and fastness properties.

Keywords: Clitoria, Natural dye, Neem, Antibacterial, Dyeing

Introduction

The identification and use of natural colourants, among which blues are uncommon and frequently susceptible to processing and storage conditions, have gained attention as a result of these worries (Chu *et al.*, 2016). Butterfly pea (*Clitoria ternatea*) flower extracts have a longer shelf life than similar plant-based colourants, are generally easier to use, and can be used as a natural blue colourant (Siti Azima *et al.*, 2017). A rising number of people are interested in finding and using natural colourants as a result of these worries; blues are particularly uncommon and can be sensitive to processing and storage conditions (Chu *et al.*, 2016). According to Siti Azima *et al.* (2017) stated that extracts from the blossoms of the butterfly pea are long shelf life, and can be used as a natural blue colourant.

Artificial dyes are often used by food manufacturers to replicate or enhance desired colours in their products. Nonetheless, research has shown that these dyes may be harmful to human health (Chu *et al.*, 2016) and increased children's hyperactivity (McCann *et al.*, 2007).

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These concerns attention to the identification and usage of natural colourants, among which blues are unique and often susceptible to processing and storage conditions (Chu *et al.*, 2016). Extracts from the flowers of the butterfly pea (*Clitoria ternatea*) are a natural blue colourant that can be used for a longer period of time and with less effort than comparable plant-based colourants. The extract's capacity to change color of four distinct colored forms of anthocyanin, and they can alternate depending only on pH. Deep blue to purple hues in the blooms are nearly equal proportion of red flavylum and blue quinoidal forms. The BPFE appears as pink or light purple when the pH is decreased, which is typically accomplished by adding a little acid like lemon or lime juice. This causes more of the flavylum (red) form. Green results from chalcone (yellow) and quinoidal (blue) forms when the pH is elevated. This is typically accomplished by adding the spice saffron. The objective was to investigate the natural dye from Clitoral plant for dyeing and surface finishing of textile fibres and its microbial activity effects.

Materials and methods

Wool fibres Mill of 100% wool fibres was supplied from Misr Co. which El Mehalla El-Kobra, Egypt for spinning and weaving. The fibres were washed in a bath containing 2g/l non-ionic detergent (Nonidet) at 40°C to remove any impurities and then thoroughly washed with water and then dried by air at room temperature. Wool fibres 10/2 fibres supplied by El Mahalla company, Egypt. Neem oil extract was purchased from EL Gmhoria company, Egypt. Chitosan with high molecular weight of 210,000, and Poly (D-glucosamine), were purchased from ROTH, Germany. Dyestuff was from Algal Pigments Scientific classification as Kingdom, Protista, Chlorophyta, Charophyta, Zygnematophyceae, Zygnematales and Spirogyra species.



Figure 1. Flower of *Clitoria ternatea* L.

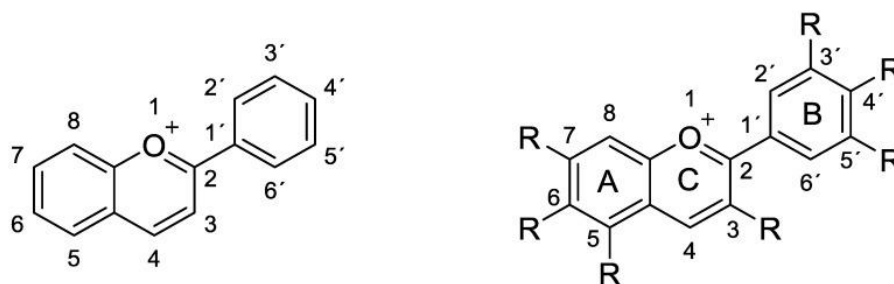


Figure 2. Chemical structure of anthocyanin pigment (R_ H,OH)

The microwave equipment was Samsung M 245 with an output of 1,550 watts operating at 2450 MHz. The extraction of dyes was done. To eliminate all moisture from the flowers, *Clitoria ternatea* L. flowers were cleaned and dried at a low temperature in an oven. To maximize the surface area, the flower was then pulverized into tiny bits, roughly 1 mm in size. Next, 1.2 g of the flower to 10 ml of water were mixed with 0.12 g/ml and with 0.12 g/ml to create the blue natural dye (Kanchana *et al.*, 2013). Centrifuge was used to separate the dye for 15 minutes at 4 °C and 10,000 rpm. To get the natural dye, the mixture was filtered by whatman filter paper HCl and NaOH were used to alter the pH.

The process of dyeing, wool fibres were dyed in a microwave. The wool fibres were coloured using 2 g/l of blue dye that was isolated from the *C. ternatea*, with a liquor ratio of 1: 50. The dye was microwaved for 5 minutes at pH 5. The warm water was used to wash the dyed fibres, and then cold water. After 30 minutes of washing at 50°C with 5g/l non-ionic detergent, the clothes were cleaned and allowed to air dry at room temperature (Ali *et al.*, 2014 ; Ali and abd-Elsalam2020)). Colour strength was measured by a spectrophotometer called the Ultra Scan PRO to measure colour strength. After determining the samples' reflectance, the K/S was measured spectrophotometrically at λ_{max} 385 nm. K/S analysis was performed on coloured wool fibres. Wool fibres were dyed and undyed that measured using the CIELAB coordinates (L^* a^* b^*) with using an Ultra Scan PRO spectrophotometer (Hunter Lab) equipped with 108 standard observers and a D65 illuminant.

A fastness property of fastness was based on ISO standard procedures. The colour fastness to rubbing, washing, perspiration, and light were tested using ISO 105X12 (1987), ISO 106-C06 (1989), and ISO 105-E04 (1989) standards. The samples' colour changes were evaluated in comparison to a precise grayscale.

Antimicrobial activity was evaluated. Peptone (5.0 g), beef extract (1.5 g), yeast extract (1.5 g), NaCl (5.0 g), and agar (20 g) at pH 7.5 be made as the nutrient agar medium (g/L). The agar was made and autoclaved at 121 °C for 20 minutes. Tested microorganisms were *Escherichia coli*, *Bacillus*

subtilis, *Staphylococcus aureus* and *Candida albicans* which were cultured for the entire night at 37 °C and 120 rpm. The wool samples were layered on the seeded medium. The zones of inhibition were evaluated after overnight incubation period at 37 °C. Untreated wool samples were evaluated in the second set of trials (Ali and Abd-Elsalam, 2022).

The antimicrobial activity was tested *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans*. The tested pathogens were made inocula of about 0.5 McFarland standard (1.5×10^8 CFU /ml) (McFarland, 1907). 25.0 μ l inoculum was then added to each plate of 20 ml of sterile nutrient agar medium.

Results

Effect of dye concentration

The impact of various dye concentrations of 2,4,6,8, and 10 g/l were resulted to show that a concentration of 6 g/l produced the highest dyeing, followed by a decrease in colour strength as seen in Figure 3. Conversely, at low concentrations, the colour strength was noticeably diminished.

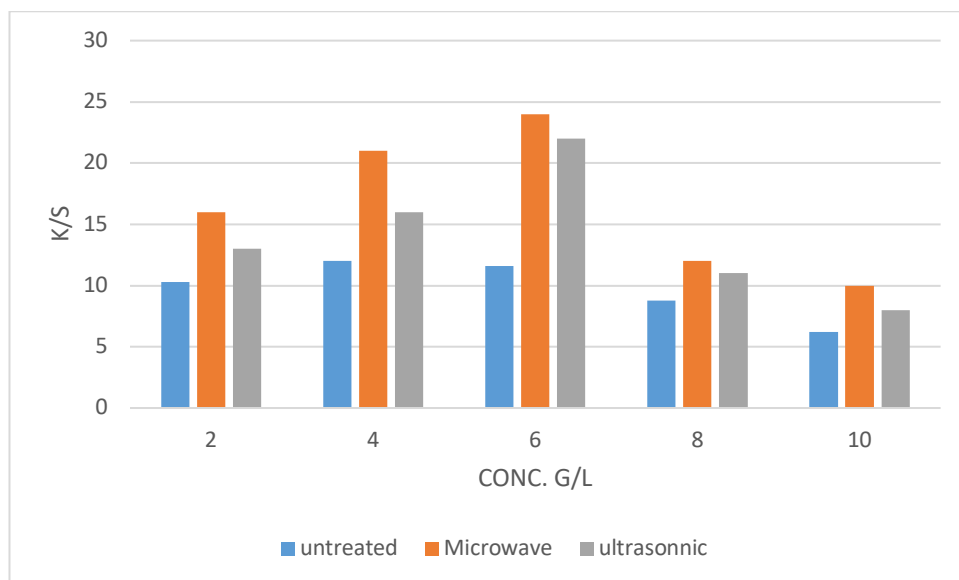


Figure 3. Effect of dye concentration

Effect of time on the colour strength

The findings showed that the length of time (1–5 min) had an impact on the colour strength of the microwave-dyed wool fibers (Figure 4). The dye under study was a strong affinity for the fibers. The findings demonstrated that as time went on, dyeing efficiency improved and peaked at five minutes.

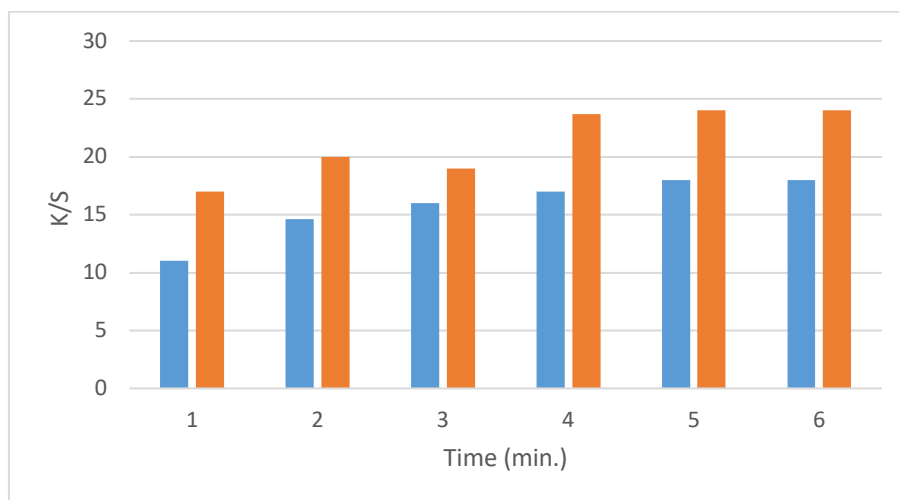


Figure 4. Effect of time on colour strength

Effect of pH on the colour strength for dyed wool fibres pre-treated with neem oil

The pH of wool fibres coloured with natural dye under examination showed an impact on the colour strength. In an acidic media, the dye was a strong affinity for the fibres. Anionic dye molecules were absorbed by cationised amino groups by electrostatic attraction in an acidic solution. The colour strength of wool fibres treated with the dye under examination produced the highest colour strength (K/S) value at pH 5 (Figure 5).

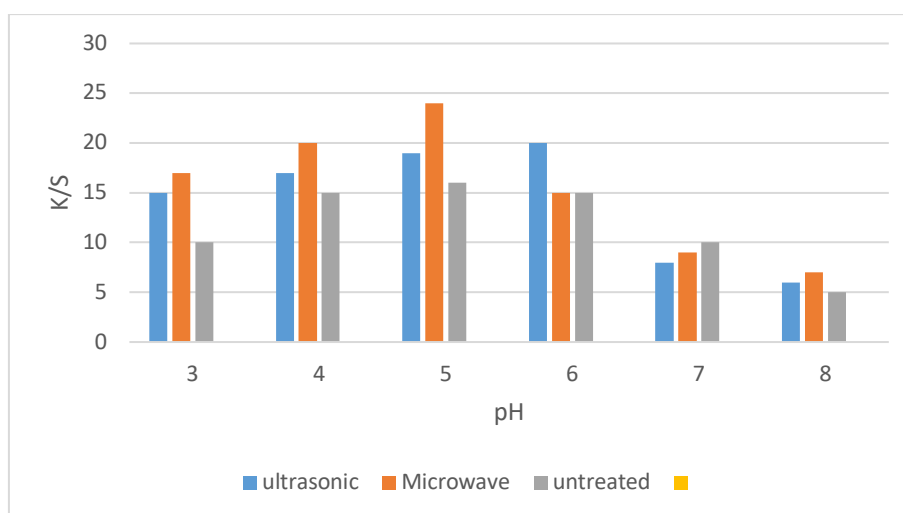


Figure 5. Effect of pH on the color strength

Antimicrobial activity

The results showed that the tested sample revealed no activity against *E. coli*. On the other hand, it showed remarkable significant inhibition *B. subtilis*, *C. albicans* and *S. aureus*.

Table 1. Effect of time of dyeing on the inhibition zone

Time (min)	Inhibition Zone(mm)			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>S. aureus</i>
Control	-	-	-	-
1	-	10.25	10.10	-
2	-	10.88	10.32	10.20
3	-	20.31	10.00	10.31
4	-	30.21	-	10.30

The results showed that the tested sample revealed great activity against *E. coli*. However, it showed low significant inhibition against *S. aureus*, *C. albicans* as shown in Table 2. The maximum inhibition was obtained by using pH 3 at which a noticeable inhibition.

Table 2. Effect of pH stability on the inhibition zone

pH	Inhibition zone(mm)			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>S. aureus</i>
Control	-	-	-	-
1	10.32	-	-	-
2	10.32	10.33	-	10.32
3	10.45	20.23	10.10	10.10
4	10.42	10.69	-	-

The results revealed great activity against *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans* as shown in Table 3. The maximum inhibition was obtained by using concentration 4% at which a noticeable inhibition were recorded against the tested pathogens. At the low conc. 1 %, only inhibition activity was noticed against gram +ve bacteria. By increasing the concentration abroad spectrum inhibition was noticed against *B. subtilis*. (Figure 6 and 7).

Table 3. Effect of dye concentration on the inhibition zone

Conc.%	Inhibition zone(mm)			
	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i>	<i>S. aureus</i>
Control	-	-	-	-
1	-	10.45	-	-
2	6.07	10.65	8.09	-
3	9.09	20.35	10.21	10.25
4	10.01	40.21	10.45	10.10
5	10.45	20.10	10.21	10.34

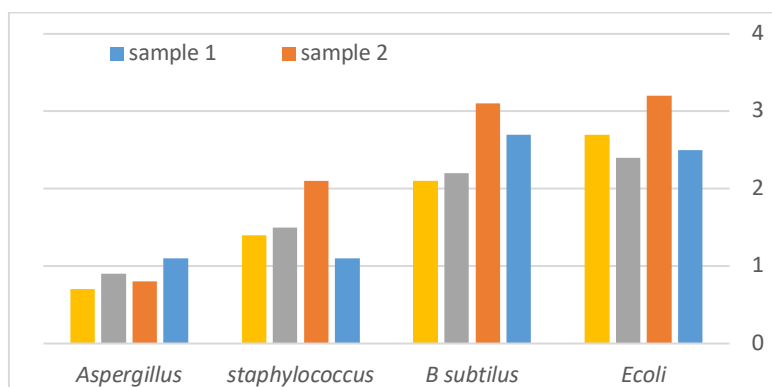


Figure 6. Antimicrobial activity of tested samples

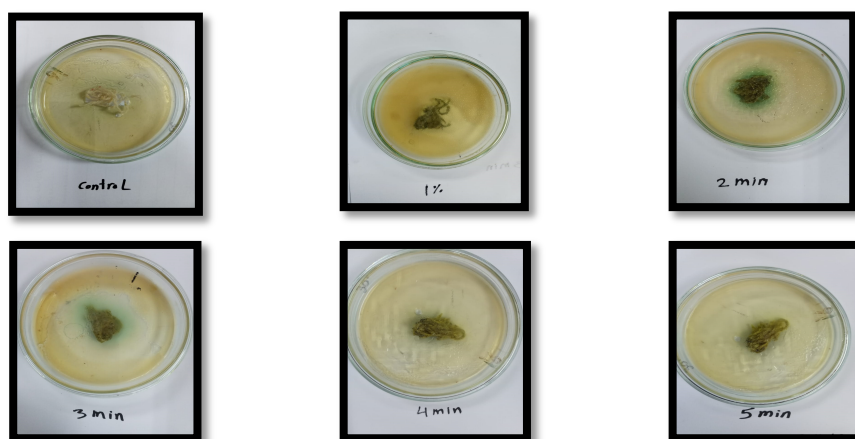


Figure 7. Antimicrobial activity inhibition zone at different time intervals

Discussion

Through the extraction method from the flowers of *Clitoria ternatea*, it showed blue aqueous solution which is the mixture of anthocyanins. The outcome demonstrated that the blooms are shown to be dark blue colour variation that can be used as a natural fabric dye (Chu *et al.*, 2016; Siti Azima *et al.*, 2017;). All tests were yielded aqueous solution hues and bluish hues. Lemon extract was added as a mordant, which caused the colours to show for the samples of fabric differed from one another. Variables were found including the methods used for dyeing and mordanting, samples of extracts and mordant. Visual inspections were yielded clearly to demonstrate the colour changes. The wool sample under experimentation revealed an excellent colour depth and shading. The outcome demonstrated that the blooms to be a dark blue colour variation that can be used as a natural fabric dye (Nguyen *et al.*, 2011). The pH of the surrounding solution was significantly affected on BPFE and the anthocyanins it is connected with, especially when it comes to the extract's colour. The deep blue to purple hue is produced by a nearly equal mixing of the quinoidal (blue) and flavylum

(red) forms of anthocyanin at its normal pH range of 6.0–8.0; deviations from this range may result in undesirable colour changes in the final product. (Terahara *et al.*, 1990; Terahara *et al.*, 1996; Siti Azima *et al.*, 2017). Concentrations influenced the dyed wool fibres' colour strength. The dye that was being studied had a strong affinity for the fibres. The colour strength of wool fibres dyed with the dye under examination produced the maximum colour strength (K/S) value at conc 6g/L, according to the results. The pH of the dye bath had an impact on the colour strength of wool fibres dyed with the natural dye under investigation. In an acidic media, the dye exhibited a strong affinity for the fibres. The anionic dye molecules can be adsorbed by the cationized amino groups in an acidic solution by the process of electrostatic attraction. The microwave dyeing process affects the colour strength of the coloured wool fibres over time. The dye being studied has a strong affinity for the wool's fibres and colour strength.

It concluded that the natural dye from *Clitoria ternatea* revealed great activity against *E. coli*, *S. aureus*, *C. albicans* at pH 3. It was greatly inhibited *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans* at concentration of 4%. It is suggested that the natural dye from *C. ternatea* may possibly develop to be surface finishing of textile fibres and antimicrobial products.

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(Received: 12 January 2024, Revised: 22 February 2024, Accepted: 10 March 2024)