JSAT
Journal of Science and Agricultural Technology
Research Article
https://doi.org/10.14456/jsat.2022.7
e-ISSN 2730-1532, ISSN 2730-1524

Utilization of *Carissa carandas* Linn. aqueous extracts as reducing agent for traditional cotton fabrics dyeing with indigo from *Strobilanthes cusia* Nees

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Received: July 29, 2022. Revised: September 21, 2022; September 28, 2022. Accepted: October 3, 2022.

ABSTRACT

This current study investigated the aqueous extracts of *Amaranthus viridis* and *Carissa carandas*as reducing agents in indigo dyeing fabric with *Strobilanthes cusia* compared to the traditional plant *Tamarindus indica*. Freshly prepared extracts were subjected to preliminary phytochemical screening. It was found that *C. carandas* extract revealed the presence of pH 2.6 and the content of phenolic, flavonoid, and tannin of 0.93 GAE/mg DW, 0.92 QE/mg DW, and 7.55 ECGC/mg DW, respectively. This might be responsible for the reduced properties. The reducing power measured by DPPH assay and ferric ion reducing antioxidant power (FRAP) confirmed that 10% (w/v) of *C. carandas* extract possessed the excellent property closely to *T. indica* extract, whereas 15%-20% (w/v) *C. carandas* extract with 10% (w/v) bio-mordant was found to contain the closely colorimetric characteristics (CIE L*a*b*) to *T. indica* extract. It can be concluded that *C. carandas* extract has high potential as a reducing agent in fabric dyeing with *S. cusia* and might be a promising low-cost reducing agent for developing green dyeing for clothing in the near future.

Keywords: *Carissa carandas*, cotton fabrics, dyeing, reducing agent, indigo

INTRODUCTION

Cotton fabric dyeing with natural dyes is an important culture of the northern people in Thailand, including Chiang Mai, Phrae, Nan, and Lampang provinces. The fabulous dyes obtained from plants such as *Strobilanthes cusia* Nees (indigo plant) have been used for many decades to meet the community way, customer requirements, and serve the conservative trend of natural products. This variety of indigo plants is widely found in the northern region of Thailand. These dues to the suitable weather, which is colder and has a higher moisture content than the other parts of Thailand. For this reason, this type of indigo plant makes the indigo-dyed fabrics of northern Thai more charming and unique.

However, the critical steps of conventional dyeing, such as water-to-material ratio, fermentation time, fermentation temperature, lime quality, pH, and dissolved oxygen concentration, are influent related to indigo yield and qualities (Pattanaik et al., 2021; Li et al., 2019). In the traditional method of indigo dyeing preparation, indigo leaves were immersed in lime hydrate, $Ca(OH)_2$, in water and thoroughly stirred for 48 hours at ambient temperature to extract pigment indigo. The supernatant and debris were removed, and precipitated indigo extracted (Hom-Peag) was collected. Afterward, the insoluble extract was reduced into leuco-white form, more solubilized using the reducing agent tamarind (*Tamarindus indica*). Unfortunately, tamarind costs constantly fluctuate in the rainy season due to fungal disease and rain deficit during peak flower and fruit-bearing stages. Therefore, two new reducing agents in the local area were investigated using equivalent tamarind properties for the *S. cusia* dyeing process.

Amaranthus viridis (Thai; Pak-Khom), an annual herb in the family *Amaranth ceae*, is widely grown and consumed in northern and northeastern Thailand at a low cost. It was reported that total phenolic contents, total flavonoid contents, tannins, saponins, alkaloids, and glycoside derivatives were rich in leaves. These components contributed to reducing properties measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reducing ability assay (Ahmed et al.,2013). Thus, it might be the new challenged plant for use in this experiment as a reducing agent. Another material such as *Carissa carandas* Linn. is also documented as a reducing agent by Khunchalee and Charoenboon (2019). Its extract possessed a high content of total phenolic contents which contributed to high antioxidant properties evaluated by using DPPH, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), Ferric reducing antioxidant power (FRAP) assay.

Furthermore, the *Carissa carandas* Linn was found abundantly in northern Thailand as an ornamental plant with its low-cost fruit.

In this work, these local plants were taken for phytochemicals investigation and used in traditional cotton fabrics dyeing as reducing agents with *Strobilanthes cusia* Nees.

MATERIALS AND METHODS

Dye extraction from aerial parts of indigo plant

One kilogram of aerial parts from the indigo plant was chopped, immersed in 10 L of distilled water, and naturally fermented for 24 hours at ambient temperature. Afterward, the residual was removed, and 120 g of $Ca(OH)_2$ was added to a solution. Then, the solution was mixed vigorously to oxidize the mixture for 20-30 minutes or until the solution turned to blue color. Afterward, the supernatant was discarded by using cheesecloth, and the dye paste was collected for further studies.

Indigo dye solution preparation

Sixty grams of dye paste and 30 g of $Ca(OH)_2$ were mixed and dissolved in 1.4 L of water. The mixture was boiled at a controlled temperature of 40°C with gentle and continuous stirring. After heating for 10 min, the 250 mL of 10% reducing solution agent was added and gently stirred for 40 min to remove oxygen. When the foam decreased, the solution turned yellowish green, and the foam color was bluish purple.

Reducing solution agent preparation

As a traditional method, the 100g of *Tamarindus indica* (TI-) extract was prepared in 1 L of hot water (90 °C). The samples, *Carissa carandas* Linn. (CC-) and *Amaranthus viridis* (AV-) extracts, were also conducted similarly.

Characterization of reducing agent

The UV-Vis spectral analysis of the reducing agent was conducted using a UV-Vis spectrophotometer in the range of 200-1000 nm.

Determination of reducing capacity

DPPH assay

The reducing ability of antioxidants toward the DPPH radical was measured in triplicates, according to Yodthong et al.(2020a). The purple DPPH working solution (oxidized form) was prepared in absolute ethanol to achieve the final absorbance of 1.0 ± 0.2 at 495 nm measured using a microplate reader (BIOBASE, China). Ten microliters of appropriately diluted sample were mixed with 200 µL of the working solution. After incubation for 30 min at the ambient temperature in the dark in the microplate, the yellow oxidized form DPPH was generated; then, the absorbance was determined at 495 nm using a microplate reader. The percentage of inhibition of reducing ability was calculated using the equation:

$$\% \text{ Inhibition} = [(OD_{max} - OD_{sample})/OD_{max}] \times 100$$

The linear curve was generated by plotting the percentage of inhibition against the concentration, and the results were expressed in microgram/mL (R^2 = 0999). After the trend line was plotted with the regression equation in Microsoft Excel 2019 software, put y=50, and calculated the x value in appropriated dilution to get the IC_{50} of DPPH.

Ferric reducing antioxidant power assay

FRAP assay was performed in triplicated according to the protocol to 96-well microplates of Carrasco-Sandoval et al. (2022) with some modifications. FRAP working solution consisted of 0.3 M of acetate buffer (pH 3.6), 0.01 M of HCl (Labscan, Thailand), and 0.02 M of $FeCl_3 \cdot 6H_2O$ (Merck, Germany) in the ratio of 10:1:1 (v/v). Twenty microliters of appropriately diluted sample were mixed with 280 µL of working solution. After incubation for 30 min at the ambient temperature in the dark in the microplate, the tripyridyltriazine complex was formed; then, the absorbance was determined at 630 nm by using a microplate reader (Biobase EL-10A, China), and the results were expressed in microgram Gallic acid equivalent (GE) per g of dry weight (DW).

Determination of total phenolic content

As indicated by Sassa-deepaeng et al. (2019), the total phenolic content (TPC) was determined by using Folin-Ciocalteu (FC) colorimetric method in triplicates. Twenty µL of TI-, CC-, or AV- extracts were shaken rigorously with 100 µL of FC (Merck, Germany) reagent in 1,980 µL of DI water. After incubation for 5 min at ambient temperature, 300 µL of 7% Na_2CO_3 (Univar, Ajax Finechem, Australia). The mixtures were incubated in the dark for 60 minutes before measurement at 765 nm using a spectrophotometer. The content of TPC was expressed as microgram gallic acid equivalent (GAE) per milligram dry weight.

Determination of flavonoid content

The flavonoid content (FC) was examined by the aluminum trichloride ($AlCl_3$) colorimetric method with some modifications by Yodthong et al.(2020b) in triplicates. Twenty-five µL of various

extract concentrations was added to 75 µL of DI water followed by 25 µL of 5% $NaNO_2$ (Univar, Ajax Finechem, Australia) on a microplate and incubated for 5 min at the ambient temperature. Afterward, the mixture was mixed with 25 µL of 10% $AlCl_3$ (Lobachemie, India) and then incubated for 6 minutes under the same condition. At the final step, 100 µL of 1M NaOH (Merck KGaA, Germany) was added and then incubated for 30 min in the dark. The mixtures were incubated again in the dark for 60 minutes before measurement at 405 nm using a microplate reader. The flavonoid content was expressed as microgram quercetin (Sigma–Aldrich, Germany) equivalent (QE) per milligram dry weight.

Determination of tannin

The content of tannin affected the reducing properties of the sample. To measure the tannin content (TC), the vanillin assay was performed in triplicates according to the protocol of Sassa-deepaeng and Yodthong (2019). The 250 µL of the extract was mixed with 450 µL of 1% of vanillin (Merck, Germany) reagent and incubated for 5 min at the ambient temperature in the dark. Afterward, the mixture was shaken with 300 µL of concentrated HCl (Labscan, Thailand) and then incubated for 30 minutes under the same condition. Finally, the color of the solution turned red, and the absorbance was measured at 500 nm. The tannin content was expressed as microgram Epigallocatechin gallate (Sigma–Aldrich, Germany) equivalent (EE) per milligram dried weight.

Dyeing

The clothing samples with the dimension of 10×10 cm (W×D) were prepared. Pre-dyeing was conducted by washing the fabrics with detergent and water to remove coated starch. Damp fabrics were dyed in a working solution for 2 min at ambient temperature and then exposed to oxygen for 2 min. After the oxygenation process, the dyed fabrics turned blue. To intensify the fabrics, the dyeing was repeated five times. The dyed cloth samples were washed in water, twisted tightly until damp, and soaked in warmed 10% tea solution (60°C) for 2 min. The samples were then washed and twisted again before soaking in 10% potassium alum solution (60°C) for 2 min. The clothes were cleaned and damped repeatedly. To finish the dyeing process, the sample was soaked in 10% NaCl solution (60°C) for 2 min, followed by washing and drying.

Color analysis

An UltraScan VIS Spectrophotometer (HunterLab, USA) was used to obtain average values for L*a*b*. Fabric sample colors were generated by the software automatically.

Statistical analysis

The obtained data were analyzed using Microsoft Excel 2016 for Windows. The data were analyzed by one-way ANOVA and Duncan's mean comparison test at the 5% significance level (Steel et al., 1997).

RESULTS AND DISCUSSION

It was found that the 10% (w/v) of *Amaranthus viridis* (AV-), *Carissa carandas* Linn. (CC-), and *Tamarindus indica* (TI-), extracts revealed the color of pale yellow, light pink, and light brown, respectively as indicated in Figure 1.

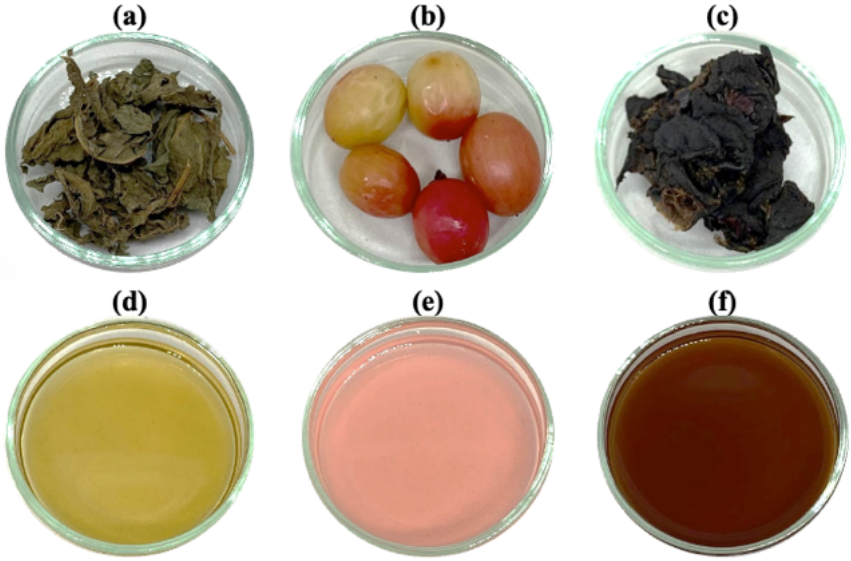

Figure 1. The appearance of (a) *A. viridis*, (b) *C. carandas*, (c) *T. indica* and the extracts of (d) AV-extracts, (e) CC- extracts, and (f) TI- extracts.

The color of the AV- extracts was pale yellow, as documented by Koyyati et al. (2014). It might consist of plant pigments such as carotenoids and soluble flavonoid derivatives, which were responsible for their yellow. In contrast, CC- extract was exhibited in light pink due to its composition of anthocyanin, as reported by Sarkar et al. (2018). However, the TI-extract was elicited in light brown, which resulted from enzymatic browning and Maillard reaction, as indicated by Obulesu and Bhattacharya (2011). A phytochemical was conducted to investigate these extracts' properties, and the results are in Table 1.

Table 1. Phytochemical properties of *A. viridis*, *C. carandas*, and *T. Indica* extracts.

Extract	pH	IC_{50} of DPPH (mg/mL)	FRAP (µg GAE/mg DW)	TPC (µg GAE/mg DW)	FC (µg QE/mg DW)	Tannin (µg ECGC/mg DW)
A. viridis	7.0±0.04^{c}	0	7.48±0.46^{b}	0.16±0.00^{c}	0.21±0.02^{c}	1.38±0.14^{b}
C. carandas	2.6±0.00^{b}	35.04±3.55	47.42±2.22^{a}	0.93±0.03^{b}	0.92±0.15^{a}	7.55±0.41^{a}
T. indica	2.2±0.01^{a}	163.07±9.74	36.63±4.68^{a}	1.03±0.04^{a}	0.66±0.01^{b}	6.85±0.03^{a}

^{abc}Within a column, means without a common superscript differ (P<0.05).

The crude extract prepared by dissolving in hot DI water showed the pH value of CC-extract was close to the control sample TI-extract. It might result from the high composition of organic acids of CC-extract, such as ursolic acid (Neimkhum et al., 2021; Bhosale et al., 2020). However, the AV- extract also consisted of organic acids such as oxalic acid (Kheyrodin, 2009) and many alkaloids (Sasikumar et al.,2015), which contributed to the increase of the extract pH. Unfortunately, the reducing activity IC_{50} of AV- extract possessed very low measured by DPPH assay, whilst the CC- extract showed more excellent reducing activity than control sample TI-extract. This phenomenon was insisted with the ferric ion reduction, an indicator of the electron donor, characteristic of the antioxidant action of the polyphenols (Khiya et al.,2021) measured by FRAP assay. However, to investigate the source molecules of reducing power, the total phenolic content (TPC) assay was conducted using Folin– Ciocalteu colorimetric method. The highest TPC of plant aqueous extracts was found in TI- extract following CC- extract and AV- extract. This indicates CC-extract had a high ability to reduce Folin–Ciocalteu reagent nearly to the control sample. In addition, CC-extract also exhibited the highest flavonoid tannin content, contributing to the reducing properties. Therefore, CC-extract might be an excellent tentative plant for using traditional cotton fabrics dyeing with *S. cusia* instead of TI- extract. To characterize the composition of the extracts, the UV-Vis spectra of the extract were recorded by spectrophotometry and shown in Figure 2.

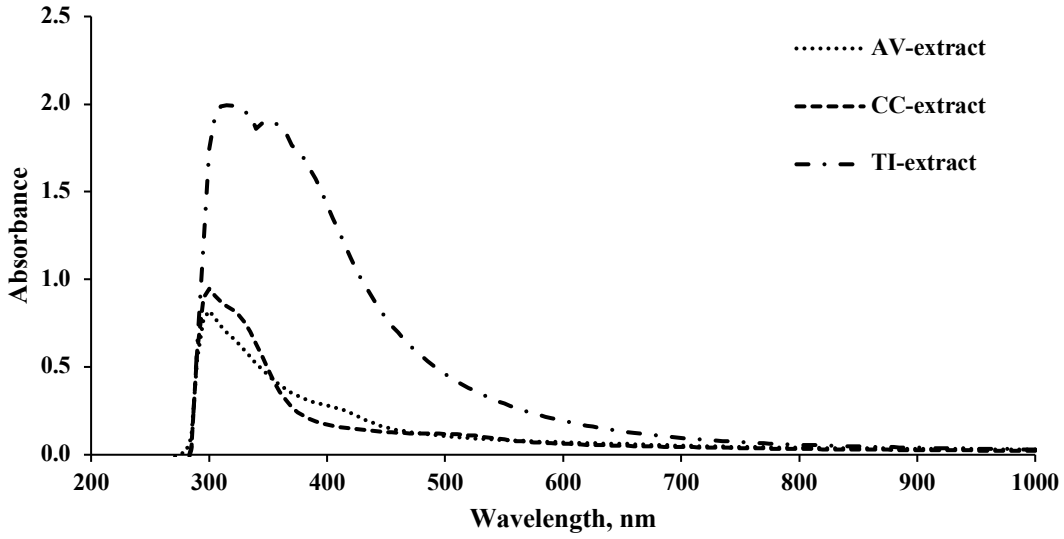

Figure 2. UV-VIS spectra of 10g/L crude extracts in distilled water.

As indicated in Figure 2, it was found that the characteristic spectra showed absorptions of AV-extract in the 290 nm to 360 nm, CC-extract in the 290 nm to 380 nm, and TI-extract in the 305 nm to 555 nm. The absorption range of 300-400 nm might be considered to originate from the $\pi \rightarrow \pi^*$ transitions in the B ring for the cinnamoyl system of flavonoids in the extract, as indicated by Mongkholrattanasit et al. (2011). However, to investigate the reducing properties, the extracts were employed in fabric dyeing, and the results are shown in Figure 3.

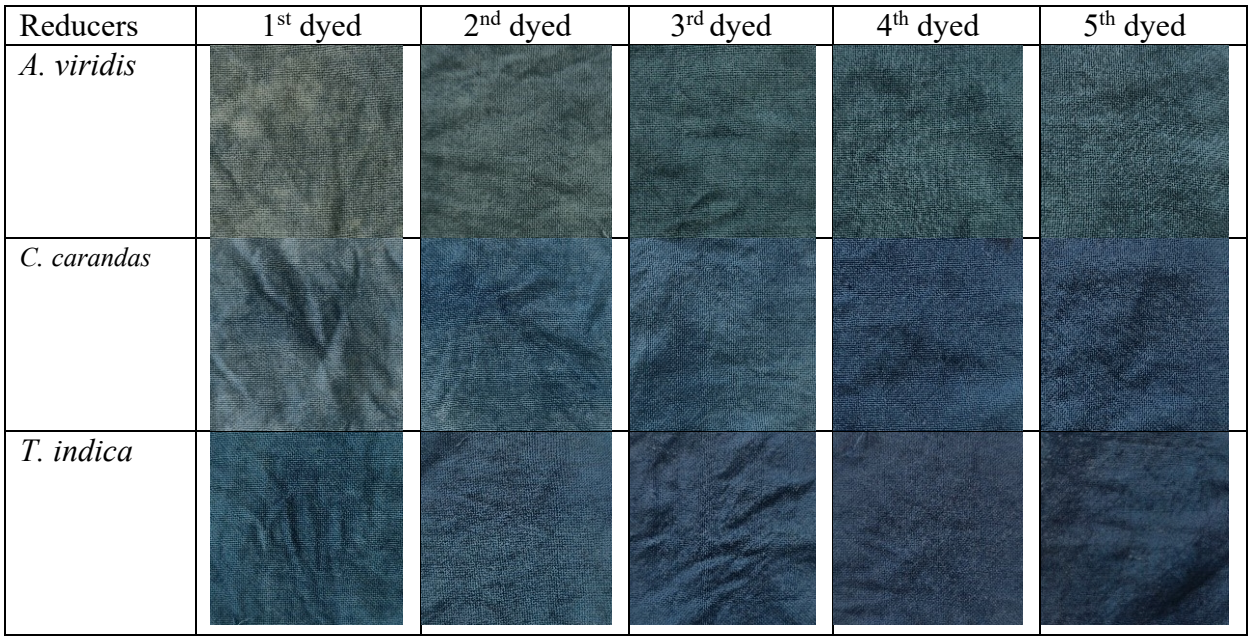

Figure 3. The color was obtained from dyed fabrics with *S. cusia* using *A. viridis*, *C. carandas*, and *T. indica* extracts as reducers.

The color appearance of using each reducing agent is presented in Figure 3. Fabric dyed with *S. cusia* and AV-, CC-, or TI-extracts showed light blue in 1st dyeing and increasing intensity in the following dyeing. The 5th dyed cotton fabric with *S. cusia* and AV-extract possessed pale blue, whereas the cotton fabric with *S. cusia* and CC-extract showed dark blue using control TI- extract. However, the proper mordant was applied as a dye fixative. In this experiment, the 10% (w/v) bio-mordant tea solution was used, and the color obtained was presented in Figure 4.

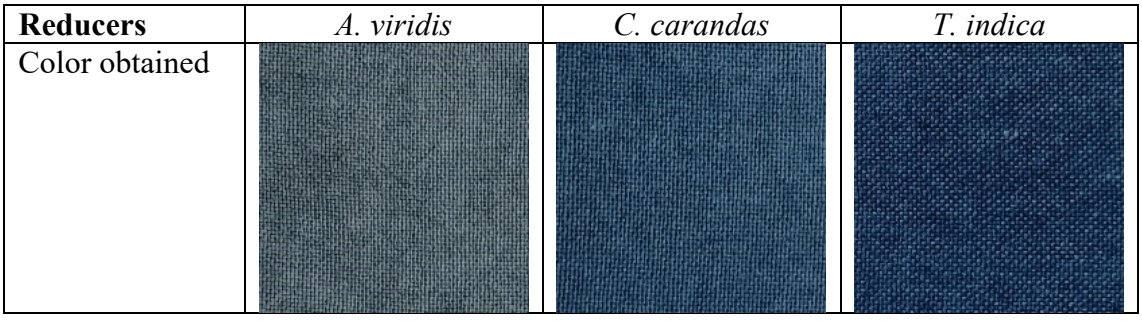

Figure 4. The appearance of dyed fabrics after 5th dyeing, mordanting with tea, and ironing.

It is clearly indicated that 10% (w/v) of CC-extract showed the blue color shade of the fabrics dyed, which is visually similar to TI- extract. Therefore, the dyed fabrics were colorimetrically observed, and the data were presented in Table 2. From Table 2, It is clear that the color shade fabrics dyed with 10% (w/v) of CC - extract were similar to that using 10% (w/v) of TI-extract but not 10% (w/v) of the AV-extract. Therefore, it can be concluded that the CC-extract had a high potential of reducing agent for *S. cusia* dyeing. To optimize the concentration of CC-extract in dyeing, the various concentrations of CC-extract were used before mordanting. The results are presented in Figure 5.

Table 2. The color values of 5th fabrics dyed with *S. Cusia* using AV-, CC-, and TI-extracts as reducers.

Reducers	L*	a*	b*
AV-extract	57.86±1.73[b]	-4.80±0.09[b]	-5.99±0.74[b]
CC-extract	39.27±0.47[a]	-3.26±0.13[a]	-17.26±0.58[a]
TI-extract	36.09±1.74[a]	-3.78±0.09[a]	-17.95±0.75[a]

[abc]Within a column, means without a common superscript differ (P<0.05).

Table 3. The color values of 5th fabrics dyed with various concentrations of CC-extract as reducers.

Con.	L*	a*	b*
15% dyed	40.95±2.75[a]	-3.91±0.25[a]	-15.54±1.59[a]
20% dyed	33.35±0.52[b]	-2.31±0.24[b]	-18.44±0.33[a,b]
25% dyed	29.97±0.81[c]	-1.37±0.30[c]	-19.30±0.21[a,b]
30% dyed	29.27±0.47[c]	-1.16±0.24[c]	-18.87±0.22[a,b]
35% dyed	28.27±0.54[c]	-0.89±0.19[c]	-19.27±0.54[b]

[abc]Within a column, means without a common superscript differ (P<0.05).

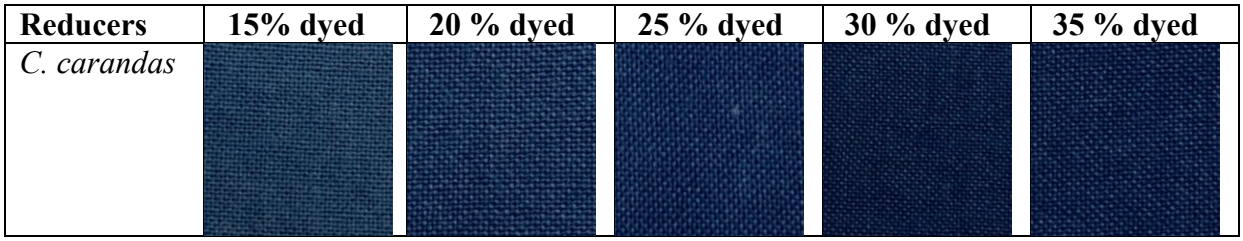

Figure 5. The **color** was obtained from dyed fabrics with *S. cusia* using various concentrations of CC extracts as reducers.

From Figure 5, It was found that the blue color shade of the fabrics dyed with various concentrations of CC-extract visually observed was intensified with a higher concentration. The data from the colorimetric analysis are shown in Table 3.

It was found that fabrics dyed with *S. cusia* and CC-extract showed a higher color strength and responded to a higher concentration of CC-extract. The optimal concentration for using equivalence to 10% (w/v) of TI-extract was between 15% and 20% (w/v) of CC-extract.

CONCLUSIONS

In summary, *C. carandas* extracted with hot water is suitable for use in the *S. cusia* dyeing process and *T. indica* extract. The 15-20% (w/v) of CC-extract possessed excellent reducing properties for blue color formation on cotton fabric. Therefore, CC-extract has a high potential reducing agent in traditional cotton fabric dyeing. This presents a promising low-cost reducing agent for green dyeing fabric in the near future.

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