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Study on a potentiality of plant aqueous extracts as natural dye fixatives in traditional dyeing processes using natural indigo from *Strobilanthes cusia* (Nees) Kuntze

Waranya Tharawatchruk, Supinan Janma, Watee Panthuwat, Sirachat Pitakrajpong, Tanongsak Sassa-deepaeng, and Pat Pranamornkith*

Agricultural Biochemistry Research Unit, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Lampang 52000, Thailand

***Corresponding author**: *p.pranamornkith@rmutl.ac.th*

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ABSTRACT

This research investigated the use of five local plants in Northern Thailand which were *Camellia sinensis* (CS), *Moringa oleifera* (MO), *Oroxylum indicum* (OI), *Schefflera leucantha* (SL), and *Tinospora cordifolia* (TC) as stabilizing agents for indigo dyed fabric. The selected plants were extracted and examined for phytochemical compositions. It was found that CS-extract revealed the highest content of total phenolic compound, flavonoid and tannin of 2.40±0.46 GAE/mg DW, 3.31±0.29 QE/mg DW, and 0.23±0.01 ECGC/mg DW, respectively. MO total phenolic compound, flavonoid and tannin of 0.96±0.04 GAE/mg DW, 1.81±0.15 QE/mg DW, and 0.15±0.02 ECGC/mg DW, respectively. For antioxidant properties, CS and MO exhibited the most efficiency of 6564.4±55.6 mM Fe^{2+}/g DW and 6512.6±220.6 mM Fe^{2+}/g DW, respectively. Consequently, these might be responsible for the ability for color fixation. Particularly, MO with the excellent properties resulted in a good color fastness score at 3-4. In contrast, CS seemed to be less fixative ability than MO.

Keywords: *Strobilanthes cusia* (Nees) Kuntze, *Camellia sinensis*, *Moringa olifera*, indigo dyed fabrics, natural fixative agents

INTRODUCTION

Natural indigo dyeing is a longstanding cultural practice in Northern Thailand, particularly in the provinces of Chiang Mai, Phrae, Nan, and Lampang. Among the natural dye sources, *Strobilanthes cusia* (Nees) Kuntze (indigo plant) has been traditionally used for generations, aligning with both local cultural heritage and the contemporary demand for eco-friendly and sustainable textile products. The northern region of Thailand provides a favorable environment for the cultivation of *S. cusia*, owing to its relatively cooler temperatures and higher humidity compared to other regions. These climatic conditions not only support optimal plant growth but also contribute to the distinctive aesthetic quality of indigo-dyed fabrics produced in this area.

The traditional method of indigo extraction and vat dye preparation is a complex biochemical process influenced by multiple parameters, including the material-to-liquid ratio, fermentation time and temperature, lime ($Ca(OH)_2$) quality as resulted an optimum pH, and dissolved oxygen concentration. These factors critically affect the yield of the extracted pigment and the quality of the vat dye (Pattanaik *et al*., 2021; Li *et al*., 2019). Typically, the indigo leaves are naturally fermented in water for approximately 24 h The solution without debris is normally added with lime water and oxygenated using air pump for only 10 to 15 min at ambient temperature to extract the pigment. The insoluble form of the dye, Thai known as Hom-Peag, is precipitated and later collected. For indigo to be rendered soluble and suitable for textile application as a vat dye, it must first be reduced to its leuco form. This is traditionally achieved using *Tamarindus indica* (tamarind) as a natural reducing agent. However, the price and availability of tamarind are subjected to seasonal fluctuations, particularly during the rainy season, as a result of fungal infections and reduced fruiting associated with adverse weather conditions.

In response to these limitations, the present study explores alternative locally sourced reducing agents that may exhibit properties equivalent to tamarind in the *S. cusia* dyeing process. This study considers *Spondias mombin* as an alternative reducing agent for the vat dye process. *S. mombin* is a member of the Anacardiaceae family. Its extract has been shown to possess high levels of phenolic compounds and ascorbic acid, contributing to strong antioxidant properties (Akanda *et al*., 2021;

Oladunjoye *et al*., 2021). *S. mombin* is also widely available in local of Northern Thailand, offering a cost-effective alternative.

This study also investigated the use of plant-based dye fixative, which was essential for natural dyeing of cotton fabrics. Dye fixative enhances the affinity of the dye to fabric fibers, improving color fastness and durability. In the absence of an effective dye fixative, many natural dyes result in poor color retention. Moreover, the choice of dye fixative can influence the final hue of the dyed fabric, thus expanding the achievable color palette.

To identify eco-friendly alternatives to synthetic dye fixative, five plant species with characteristic bitter taste were selected for investigation as potential bio-mordants: *C. sinensis* (CS), *M. oleifera* (MO), *O. indicum* (OI), *S. leucantha* (SL), and *T. cordifolia* (TC).

C. sinensis (tea), a member of the Theaceae family, is rich in tannins, catechins, and flavonoids—particularly thearubigins and theaflavins—which act as dye fixative and contribute to its reddish-brown and yellowish-brown pigmentation (Sarker *et al*., 2024; Xu *et al*., 2025). These compounds enhance dye fixation and make tea an effective and environmentally friendly option for natural fabric dyeing.

M. oleifera, a species in the Moringaceae family, also contains high levels of polyphenols and tannins. These compounds are known to promote bonding between dye molecules and cotton fibers, thereby increasing color fastness (Benli, 2024). In addition to its dye-affinity potential, moringa is a rich source of antioxidants such as ascorbic acid, flavonoids, and carotenoids (Stohs & Hartman, 2015).

O. indicum (Bignoniaceae) has a long history of medicinal and culinary use in Southeast Asia. Its bark has traditionally been used as a dye, and its young pods contain flavonoids such as baicalin, oroxylin A, and 5-hydroxymethylfurfural, which may function as bio-dye fixative in cotton dyeing processes (Rojsanga *et al*., 2023).

S. leucantha (Araliaceae) is a less-studied species with over 200 identified triterpenoid saponins. Although it has not been previously reported as a dye fixative, its bitter taste and phytochemical composition warrant investigation into its potential bio-dye fixative activity (Wang *et al*., 2021).

T. cordifolia (Menispermaceae) is a herbaceous vine known for its rich phytochemical profile, includs phenolics, alkaloids, glycosides, and clerodane-type diterpenes (Sharma *et al*., 2019). Although there are no previous reports regarding its use as a dye fixative, the chemical composition of this plant suggests its potential applicability in dyeing processes.

This study aims to investigate the phytochemical properties of five selected plant species—*C. sinensis*, *M. oleifera*, *O. indicum*, *S. leucantha*, and *T. cordifolia*—and evaluate their efficacy as natural mordants in cotton fabric dyeing using *S. cusia*. Additionally, the potential of alternative reducing agents such as *S. mombin* is assessed to support sustainable practices in traditional indigo dyeing.

MATERIALS AND METHODS

Extraction of Indigo dye from Strobilanthes cusia (Nees) Kuntze

Fresh *S. cusia* leaves were collected early in the morning. One kilogram of the leaves was submerged in 10 L of distilled water and fermented naturally for 24 h at ambient temperature. All the debris was then removed. The amount of 120 g $Ca(OH)_2$: Maelao Chiang rai lime kiln, commercial grade, was gently added. After that the solution was stirred vigorously for 20-30 min or until the blue dye was precipitated to form an insoluble substance. The dye paste was then filtrated using cheesecloth and collected for the next studies.

Preparation of reducing agent solution

In this study, *S. mombin* (SM-) was used as a reducing agent compared to *T. indica* which was used in a traditional method. SM-extract was prepared at a concentration of 50, 100, 150, 200 and 250 g/L. Meanwhile, *T. indica* (TI-) extract was prepared at a different concentration of 15, 20, 25, 30 and 35 g/L. The two plants flesh were squeezed in hot water and removed waste.

Preparation of fixative agent

The different parts of herbal plants such as leaves of *S. leucantha*, *M. oleifera*, *C. sinensis*, stem of *T. cordifolia* and bark of *O. indicum* were freshly selected, chopped and air oven dried at 60°C for a few days. All dried plant parts samples were extracted in boiling water at a concentration of 10 g/L.

Characterization of reducing agent

The UV-Vis spectral analysis of the mordants was performed using a UV-Vis spectrophotometer: Libras70, Biochrom, across a wavelength range of 200 to 800 nm.

RESULTS AND DISCUSSION

Reducing property investigation

Reducing agent extracts of Spondias mombin and Tamarindus indica (TI-) appeared in a smoky pale yellow, and muddy brown color, respectively as shown in Figure 1.

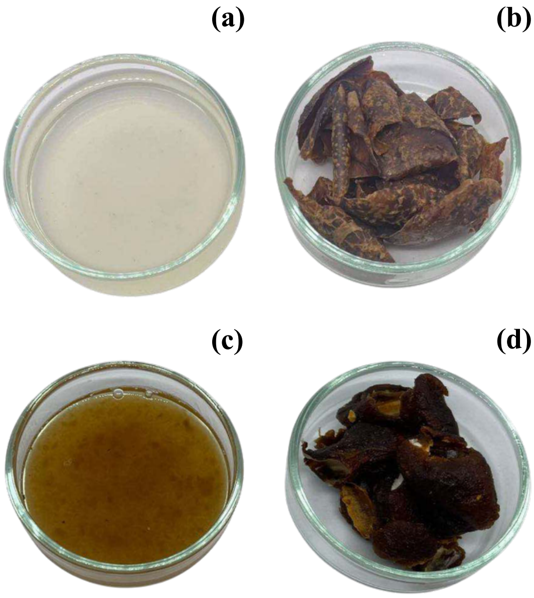


Figure 1. The appearance of (a) *S. mombin* extract, (b) *S. Mombin* dried flesh, (c) *T. indica* extract and (d) *T. indica* dried flesh.

The SM-extracts consists of plant pigments such as carotenoids, particularly β-cryptoxanthin which provides a pale-yellow color, Tiburski *et al*. (2011). The TI-extract was however presented the color of light brown which caused by enzymatic browning and Maillard reaction as indicated by Obulesu & Bhattacharya (2011).

To investigate the reducing properties, the extracts were used in various concentrations for fabric dyeing process and the results are shown in Figure 2.

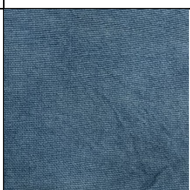
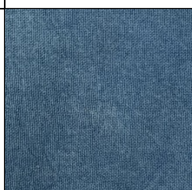
Reducers	Reducing agent concentration				
S. mombin extract	50 g/L	100 g/L	150 g/L	200 g/L	250 g/L
					
T. indica extract	15 g/L	20 g/L	25 g/L	30 g/L	35 g/L
					

Figure 2. Color obtained from fabrics dyed with *S. cusia* using *S. mombin* and *T. indica* extracts in various concentrations as the reducers.

According to Figure 2, using SM-extract and TI-extracts at various concentrations of 50-200 g/L and 15-35 g/L, respectively showed the optimal concentration of reducing agents. Applying SM-extract to vat dye at the concentration of 150 g/L provided a blue color with brighter than other concentrations. However, adding 25 g/L TI-extracts presented the brightest blue color among other concentrations. A comparison between the two reducing agents revealed that the use of TI-extracts resulted in a more pronounced blue hue compared to SM-extracts. This observation suggests that SM-extracts may be less effective as a reducing agent in the fabric dyeing process. Consequently, TI-extracts were deemed more suitable and were selected for subsequent experiments.

Fixative agent phytochemical composition and its ability

Fixative agent extracts of *T. cordifolia* and *S. leucantha* introduced pale yellow color. On the other hand, *O. indicum*, *M. oleifera*, and *C. sinensis* produced a color range from yellow to yellow-brown. The results showed in Figure 3.

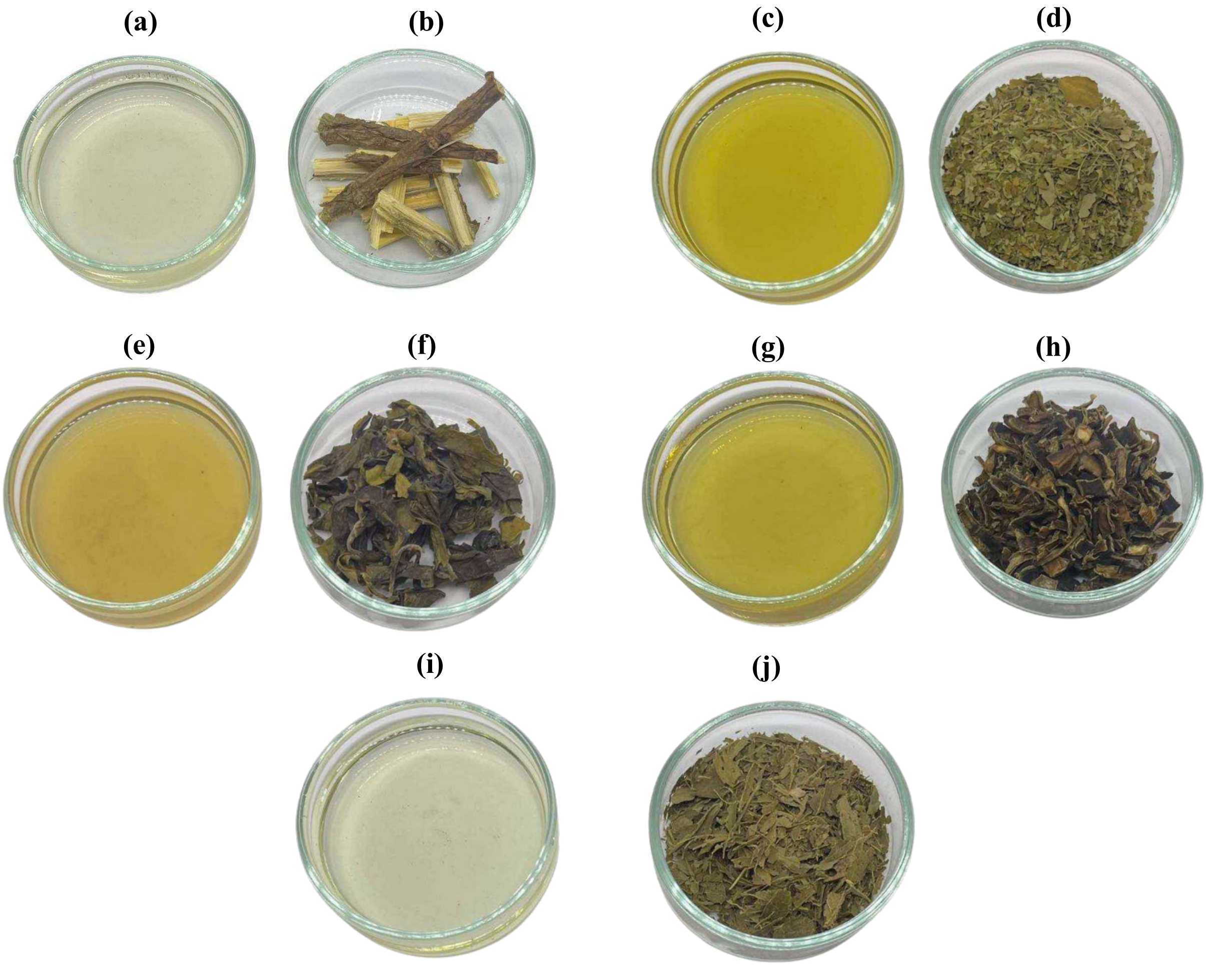


Figure 3. The appearance of (a) *T. cordifolia* extract, (b) *T. cordifolia* dried stem, (c) *M. oleifera* extract, (d) *M. oleifera* dried leaves, (e) *C. sinensis* extract, (f) *C. sinensis* dried leaves, (g) *O. indicum* extract, (h) *O. indicum* dried bark, (i) *S. leucantha* extract and (j) *S. leucantha* dried leaves.

The TC- and OI- extracts contain plant pigments mainly flavonoids, as reported in Tiwari *et al*. (2010) and Panomai *et al*. (2024), respectively. On the other hand, Quercetin was found in SL-extracts by El-Hagrassi *et al*. (2022). The CS- and MO- extract provided chlorophyll and carotenoid followed Aboulwafa *et al*. (2019) and Toscano *et al*. (2021).

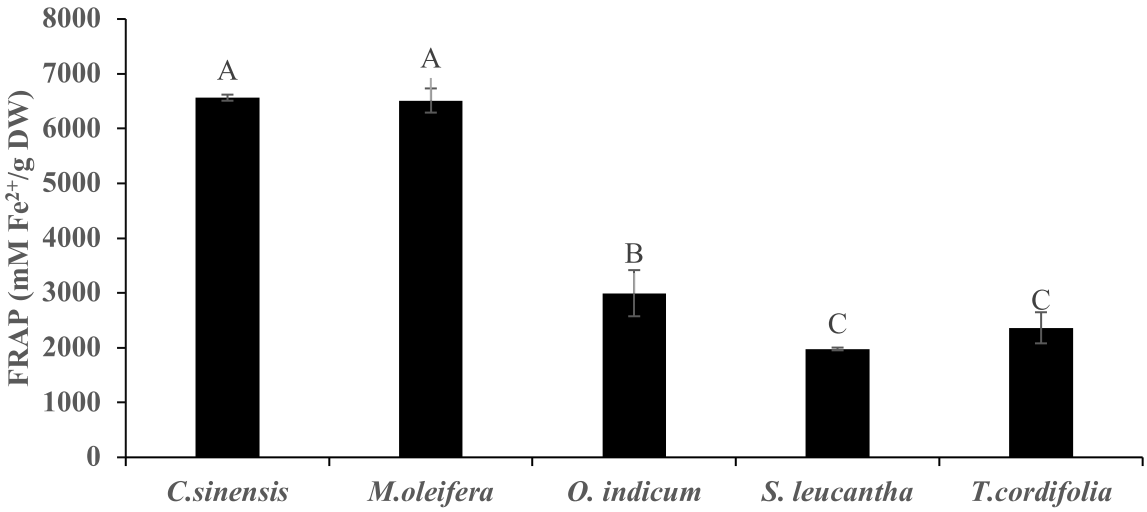


Figure 4. FRAP value of aqueous extracts expressed as mM Fe^{2+}/g of DW. Columns labeled with different letters were significantly different, $p < 0.05$ (n = 3).

Antioxidants serve a pivotal function in the natural dyeing of cotton textiles, especially in conjunction with bio-mordants. The presence of these compounds enhances color strength and improved color fastness, while also imparting bio-functional properties such as anti-bacterial and antioxidant activities to the dyed fabrics. Figure 4 illustrates the antioxidant activity of various extracts as assessed through the FRAP assay. The data indicate that the aqueous extracts of *C. sinensis* and *M. oleifera* exhibited the highest antioxidant properties (6564.4±55.6 mM Fe^{2+}/g DW and 6512.6±220.6 mM Fe^{2+}/g DW), which might be attributed to their significant polyphenol content, particularly in their phenolic and flavonoid fractions (Sassa-deepaeng *et al*., 2019; Baldisserotto *et al*., 2023). These extracts were followed by *O. indicum*, which demonstrated moderate antioxidant activity. In contrast, the extracts of *T. cordifolia* and *S. leucantha* displayed the lowest antioxidant activity (6512.6±55.6 mM Fe^{2+}/g DW). Numerous studies have demonstrated a positive correlation between antioxidant activity and the levels of phenolic compounds, flavonoids, and tannins presented in plant extracts (Muniyandi *et al*., 2019; Cosme *et al*., 2025). To further substantiate these findings, the phenolic, flavonoid, and tannin contents were also analyzed, with the results for phenolic content presented in Figure 5.

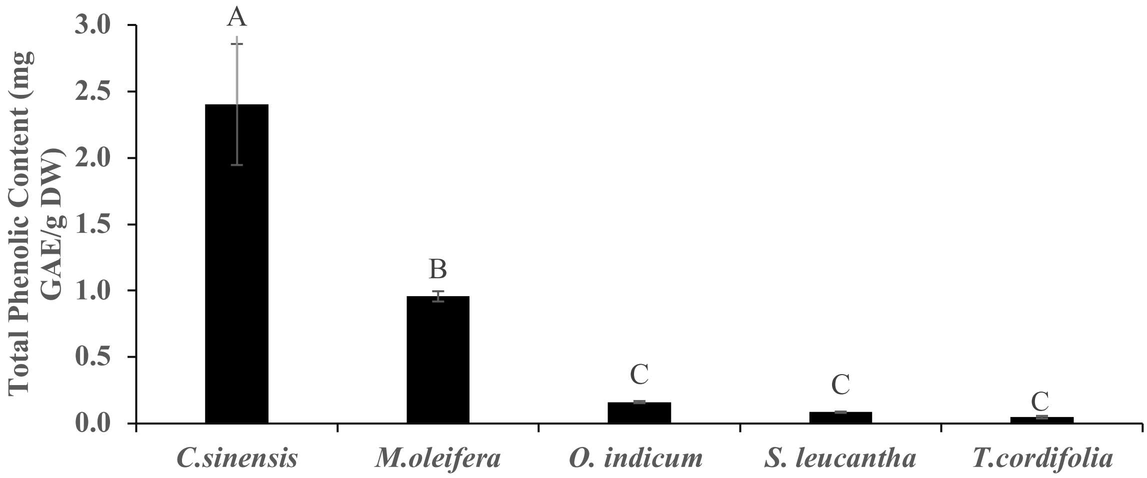


Figure 5. Total phenolic content of aqueous extracts expressed as mg GAE/g of DW. Columns labeled with different letters were significantly different, $p < 0.05$ (n = 3).

Phenolic compounds possess strong antioxidant properties, meaning they can donate electrons. In the dyeing process, particularly with vat dyes (like indigo) or natural dyes requiring reduction, phenolic compounds can act as natural reducing agents, converting dye precursors to their soluble (leuco) forms that can penetrate the fabric fibers. Thus, the total phenolic content (TPC) of the aqueous extracts is presented in Figure 4. Among the extracts, the highest TPC value was observed in the leaf extracts of *C. sinensis* (2.40±0.46 mg GAE/g dry weight), which is consistent with the findings reported by Sassa-deepaeng *et al*. (2019) and Murokore *et al*. (2023). This was followed by *M. oleifera* (0.96±0.04 mg GAE/g dry weight), which exhibited a moderate TPC. In contrast, the extracts of *O. indicum*, *S. leucantha*, and *T. cordifolia* demonstrated the lowest TPC values. Furthermore, the subclass of phenolic compounds, specifically flavonoids, was also examined, and the results are presented in Figure 6.

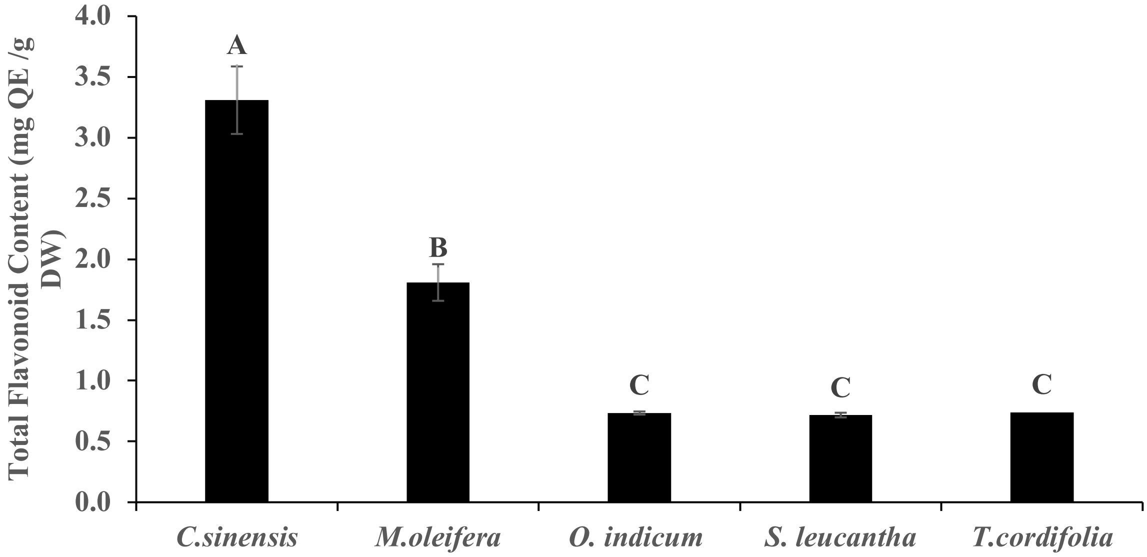


Figure 6. Total flavonoid content of aqueous extracts expressed as mg QE/g of DW. Columns labeled with different letters were significantly different, $p < 0.05$ (n = 3).

The total flavonoid content (TFC) in plant extracts is a crucial factor influencing the fabric dyeing process. Flavonoids, a diverse group of polyphenolic compounds, contribute to the dyeing process through various mechanisms, including their antioxidant and reducing properties, mordanting and fixation abilities, color development and hue alteration, and their role in enhancing dye stability. Moreover, flavonoids offer significant environmental and health benefits by reducing the use of harmful chemicals, thus making the process safer for workers and lessening environmental pollution.

The TFC of the aqueous plant extracts is presented in Figure 5. Among the extracts, the leaf extract of *C. sinensis* exhibited the highest TFC value of 3.31 ± 0.29 mg QE/g dry weight, which is consistent with previous studies by Sassa-deepaeng et al. (2019) and Murokore et al. (2023). This was followed by *M. oleifera*, which showed a moderate TFC value of 1.81 ± 0.15 mg QE/g dry weight. These results align with findings from the FRAP assay and total phenolic content analysis, further supporting the role of flavonoids as important contributors to the dyeing process. In contrast, the extracts of *O. indicum*, *S. leucantha*, and *T. cordifolia* exhibited the lowest TFC values.

In addition to flavonoid content, the tannin content of the extracts was also assessed, with the results presented in Figure 7.

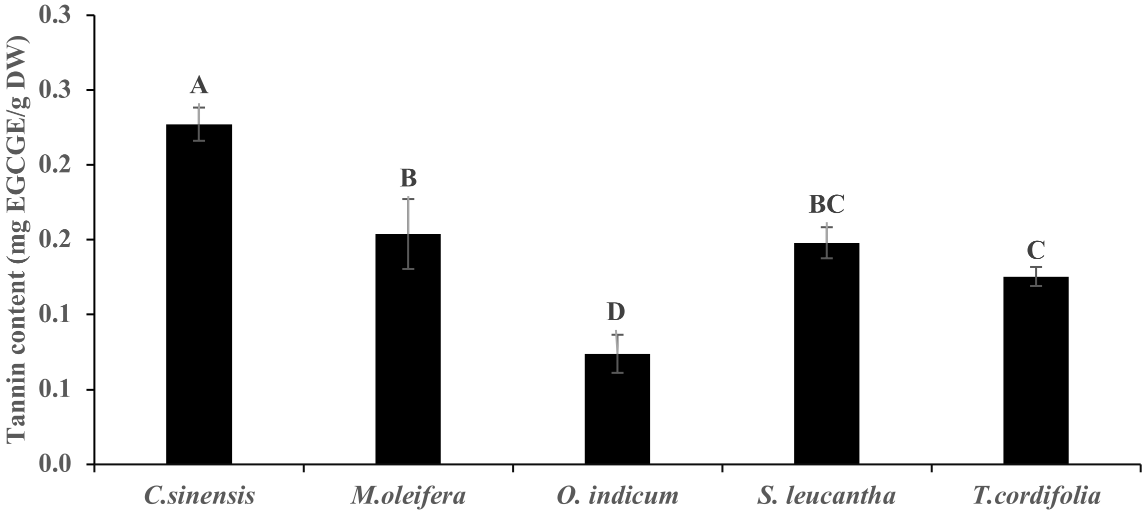


Figure 6. Tannin content of aqueous extracts expressed as mg EGCGE/g of DW. Columns labeled with different letters were significantly different, $p < 0.05$ (n = 3).

Tannin represents a key natural mordant in the dyeing of cellulose-based fibers such as cotton. Functioning as a binding agent, it facilitates the interaction between dye molecules and fiber substrates, thereby enhancing dye uptake, fixation, and overall colorfastness. Additionally, tannins contribute to pH stabilization during the dyeing process and can intensify the color of certain natural dyes. The tannin content (TC) of the aqueous plant extracts is presented in Figure 7. Among the samples analyzed, the leaf extract of *C. sinensis* exhibited the highest TC value (0.23 ± 0.01 mg EGCGE/g dry weight), consistent with the findings of Lambrecht *et al*. (2023). This was followed by *M. oleifera* and *S. leucantha*, both of which demonstrated moderate TC values of 0.15 ± 0.02 mg EGCGE/g dry weight and 0.15 ± 0.01 mg EGCGE/g dry weight, respectively. These variations in tannin concentration may contribute to differences in the shade and depth of coloration observed in dyed cotton fabrics. In contrast, the extracts derived from *O. indicum* and *T. cordifolia* displayed the lowest TC values among the samples tested.

The observed ferric ion reduction in the FRAP assay supports the antioxidant activity of the extracts, which is indicative of the electron-donating capacity of polyphenolic compounds (Khiya *et al*., 2021). Among the tested plant extracts, *C. sinensis* exhibited the highest antioxidant activity, followed by *M. oleifera*, *S. leucantha*, *O. indicum*, and *T. cordifolia*, respectively. Notably, *M. oleifera* demonstrated a high potential as a natural mordant, comparable to the traditionally used *C. sinensis*.

In addition to its antioxidant capacity, *M. oleifera* extract contained the highest levels of phenolics, flavonoids, and tannins among the tested plants, which are key contributors to its reducing properties. These phytochemical constituents are recognized for their ability to enhance dye fixation on natural fibers by forming stable complexes with both dye molecules and fabric substrates. Therefore, *M. oleifera* emerges as a promising alternative bio-mordant for traditional cotton fabric dyeing using *S. cusia*.

To further characterize the chemical composition of the extracts, UV–Vis spectrophotometric analysis was conducted, and the resulting spectra are presented in Figure 8.

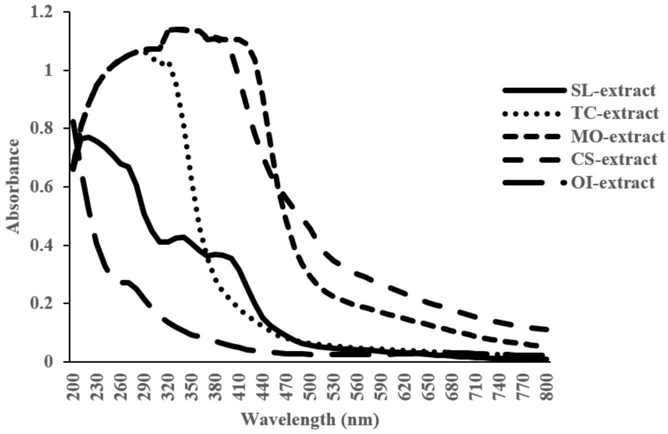


Table 6. UV-VIS spectra of 10 g/L crude extracts in distilled water.

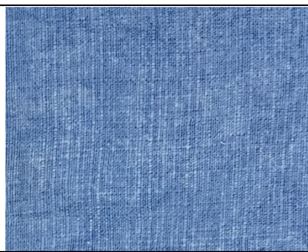
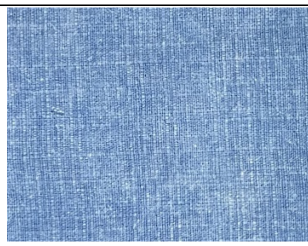
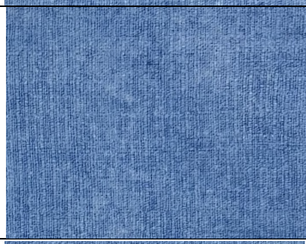
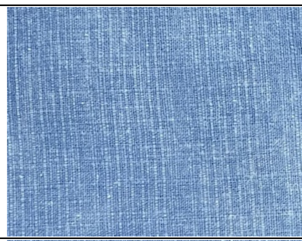
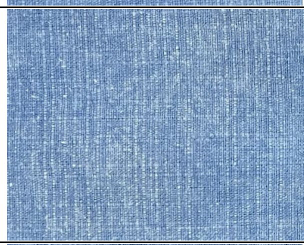
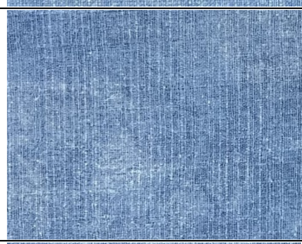
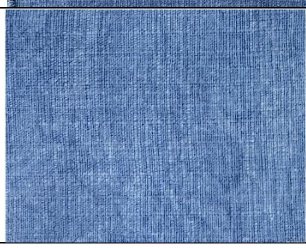
Fixative agents	Color before washing	Color after 5 times of washing	Wash fastness value
S. leucantha extract			3-4
T. cordifolia extract			3-4
M. oleifera extract			3-4
C. sinensis extract			3
O. indicum extract			3

Figure 9. The appearance of dyed fabrics after 5th times of dyeing using different fixative agent and wash fastness value.

Figure 8 was illustrated the characteristic spectra of the aqueous extract of fixative agents at wavelength of 200-800 nm. The profile suggested the broad peak at 350-440 nm for MO- and CS-extract. Kusmita, *et al*. (2015) introduced the peak of pigments in CS-extract such as pheophytin at 408 nm, pheophorbide at 409 nm, and violaxanthin at 417 and 438 nm. MO-extract provided the peak at 412 nm of the yellow color pigment supported by Aouf *et al*. (2024) which could be β-carotene, Dănilă & Lucache (2016). The TC-extract displayed a peak at 260 nm of phycocyanin pigment, Puri & Patil (2022). The SL-extract peak was at 220, 260, 340 and 410 could represent phycoerythrin and phycocyanin pigments, Dănilă & Lucache (2016). The OI-extract provided a similar UV-Vis profile pattern with SL-extract and could contain both phycoerythrin and phycocyanin pigments.

To investigate the fixative property, five fixative agents was applied to the final step. The 10% (w/v) bio-fixative agents were used. Later, the dyed fabric samples were tested for color fastness to washing. All fabric samples were washed 5 times and assessed the change in color with the grey scale. Color obtained before and after 5 times of washing were presented in Figure 9. As the results in Figure 9, it is revealed that fixing the fabrics color with 10% (w/v) of fixative agents mostly showed rating scale from 3. Color changes of indigo dyed fabrics with CS and OI was visible and staining was also noticeable. The fabrics color fixed with the rest of fixative agents, SL, TC, and MO, provided a wash fastness value number of 3-4 which defined a slight color change with a minimal staining.

CONCLUSION

Among the plant extracts tested, *M. oleifera* demonstrated the highest potential as a natural mordant, comparable to the traditionally used *C. sinensis*. This is attributed to its superior levels of phenolics, flavonoids, and tannins, which are critical for the mordanting process. The results indicate that *M. oleifera* extract can effectively fix dye to fabric, achieving a color fixation comparable to *C. sinensis*, which has been traditionally used for this purpose. Additionally, fabric treatments with 10% (w/v) fixative agents yielded satisfactory results, with a rating scale of 3 observed for *O. indicum* extract. Notably, visible staining was also apparent for the positive control using *C. sinensis*, further supporting the potential of *M. oleifera* as an effective mordant.

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