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Extraction of natural dyes from tarum leaves (*Indigofera Tinctoria L*) using the Ultrasound-Assisted Extraction (UAE) method

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Abstract

The rapid development of the textile industry has led to increased use of synthetic dyes. Although synthetic dyes are more practical and economical, their use can have adverse effects on health and the environment. Natural dyes are utilized as alternatives with indigo leaves (*Indigofera tinctoria* L.) being one source known for producing a blue color. However, the commonly used extraction methods require long extraction times and significant solvent consumption. Given the limitations of current methods, innovation is needed for an efficient extraction method that produces safe and environmentally friendly natural dyes, one of which is ultrasound-assisted extraction (UAE). Therefore, this study aims to determine the extraction process of indigo leaves using UAE method, then identify the parameters influencing the extraction process using the UAE, determine the extraction yield using UAE, assess the dyeing results of the extracted dye from indigo leaves on cotton fabric, and compare the extraction results of indigo dye through the UAE method with the addition of CaO and NaOH solutions. The results of this study indicate that the natural dye from *Indigofera tinctoria* obtained the best conditions at extraction time of 90 min, feed-to-solvent ratio of 0.05 g/ml, and CaO-to-solvent ratio of 0.005 g/ml. Furthermore, the addition of CaO solution obtained the highest yield at 4.25 %, while the addition of NaOH solution resulted in 4.8 %. Additionally, the analysis and test results of the application of natural dye from *Indigofera tinctoria* on cotton fabric revealed a darker color with the addition of NaOH solution compared to the addition of CaO solution.

Keywords: *indigofera tinctoria l,* natural dyes, ultrasound-assisted extraction, yield

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1. INTRODUCTION

The dyes commonly used in the textile industry typically involve synthetic pigments. Synthetic pigments are chemical colorants derived from substances such as Fe_2O_3, TiO_2, and others (Al-tohany, et al., 2022). Synthetic pigments are chemical colorants with potential adverse effects on health and the environment. Waste from synthetic dyes can cause water pollution, indicated by changes in pH, BOD, COD, and

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DO, thus disrupting aquatic life (Berradi, et al., 2019). Furthermore, a substance can be termed a dye if it possesses color groups (chromophores) and can bind to textile fibers (auxochromes). Color arises due to the absorption of electromagnetic radiation with specific wavelengths in the visible light spectrum (Oliveira and Santos, 2016; Rabinowitz et al., 2021). Molecules of colorants are combinations of bonds from unsaturated organic substances with their constituent groups. Furthermore, the unsaturated organic substances in colorant formation consist of aromatic compounds such as aromatic hydrocarbons and their derivatives, phenols and their derivatives, as well as hydrocarbon compounds containing nitrogen (Dulo et al., 2021; Kumar Gupta, 2020; Ustin and Jacquemoud, 2020). The raw materials for synthetic colorant production are derived from coal or petroleum, which are aromatic hydrocarbon compounds, such as benzene, naphthalene, and anthracene, while natural colorants mostly originate from plants and microorganisms. One plant that can serve as a source of natural dyes is the indigo plant (*Indigofera tinctoria* L). The *Indigofera tinctoria* L plant has purple flowers, which can naturally produce blue color. In addition to blue, this plant can also yield green color, where the blue dye extracted from the indigo plant is combined with other natural yellow color-producing plants.

The blue dye produced by the tarum plant is caused by the compound of indigo. However, this compound cannot be directly extracted from tarum leaves; instead, the leaves contain precursors, compounds indicant and isatan B (indoxyl-β-ketogluconate), pivotal in forming indigo (Hsu et al., 2018; Speranza et al., 2020). The indicant compound has colorless glucoside soluble in water, easily hydrolyzed into glucose (dextrose, $C_6H_{12}O_6$) and (indoxyl, C_8H_7NO) due to the enzyme indimulase (Reningtyas et al., 2022). The indoxyl molecule is a colorless compound, easily oxidized to indigotin (blue indigo) in an alkaline environment (Rahayuningsih et al., 2022).

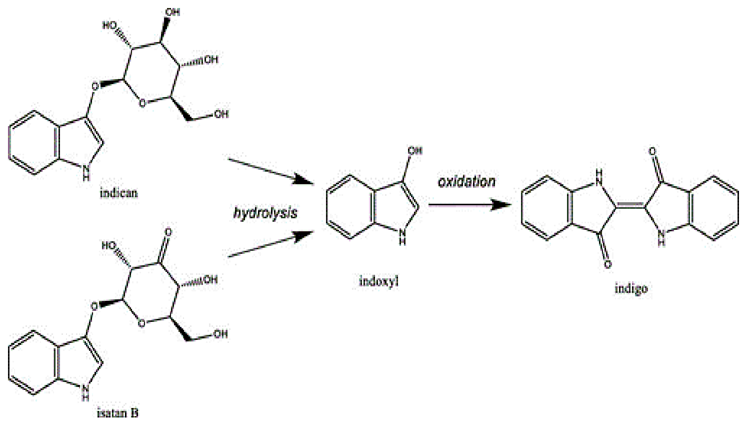

Figure 1. Indigo biosynthetic process (Fabara and Fraaije, 2020)

The process of extracting and separating indigo dye from a mixture of other compounds using a solvent (separating agent) is called extraction method. Furthermore, an extraction method can be considered ideal if its extraction rate is high, non-destructive, and time-saving. In previous studies, indigo extraction still largely employed conventional methods including maceration (Campos, et al., 2022; Pattanaik, et al., 2021), reflux, percolation, and Soxhlet method (Helmy, 2020; Rajan and Cindrella, 2019). Several previous research on the use of ultrasonic wave methods is a novel extraction for synthesizing natural dyes and bioactive components in plants (Mansinhos, et al., 2021; Syafaatullah, et al., 2021; Syafaatullah and Mahfud, 2021; Zahari, et al., 2020). Some methods have successfully extracted the substance using ultrasonic method. These methods are used to obtain high antioxidant content with relatively short time, higher yield, lower energy consumption, and solvent usage. The reason is related to the phenomenon of cavitation increasing the mass transfer between the solid-liquid surface (Çalışkan and Şayan, 2022; Carreira-Casais, et al., 2021; Kumar, Srivastav, and Sharanagat, 2021; Medina-Torres, et al., 2017; Wang, et al., 2022). Additionally, the advantage of ultrasonic methods in the extraction process is the cell walls becoming porous, allowing compounds to diffuse more quickly. However, there is limitation research related to dye extraction using UAE methods, and the quality of the resulting color is not yet clearly understood, necessitating further investigation. Certainly, research contributions are needed to explore the potential of ultrasonic extraction technology in plants for natural dye production as a novel, environmentally friendly, and non-toxic extraction method.

Therefore, this study aims to determine the extraction results of natural dyes from tarum leaves using the UAE method. Furthermore, the extracted results undergo analysis and evaluation of several influencing parameters, including the feed to solvent ratio used, the addition of alkali solution to the extract quality, and the extraction time selected. Additionally, the application of indigo dye in this research through mordanting process on cotton fabric can be determined.

2. MATERIALS AND METHODS

2.1. Material and Chemical

The materials used are tarum leaves (*Indigofera tinctoria* L) and distilled water as a solvent. The addition of NaOH and CaO (Merck) is used as the base-forming material in the extracted solution. Subsequently, Turkish Red Oil (TRO) solution is used as a fabric cleaner, and alum is used as a color fixative on the test fabric.

2.2. Tools

The main equipment used is the ultrasound cleaning bath and glass beakers, as shown in **Figure 2**. The main ultrasound equipment, KRISBOW model KW1801033, has specifications including ultrasound power of 100 W, voltage of 240 V/50 Hz, maximum frequency of 40 kHz, tank capacity of 2.8 L, and tank dimensions (length = 23.5 cm; width = 13.5 cm; and height = 10 cm).

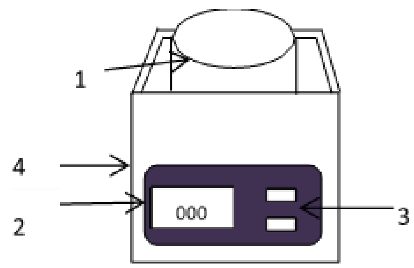

Figure 2. Scheme of the Ultrasound-Assisted Extraction (UAE)

1 = Beaker glass

2 = Digital display

3 = Mode button

4 = Ultrasonic cleaning bath

2.3. Research Procedure using the Ultrasound-Assisted Extraction (UAE)

Fresh indigo leaves are weighed according to the specified variables. The parameters in this study include the solvent used are obtained from distilled water with extraction times of 30, 60, 90, and 120 min. The ultrasonic frequency is set at 40 Hz, and the temperature is maintained at 60 °C. The feed to solvent ratio (F/S) is 0.02, 0.05, and 0.08 g/ml respectively, while for the addition of alkali, the CaO to solvent ratio (CaO/S) is 0.001, 0.005, and 0.009 g/ml, and the NaOH to solvent ratio (NaOH/S) is 0.005 g/ml. Furthermore, the operation is carried out at atmospheric pressure contained solvent volume of 200 ml. The apparatus is assembled as shown in **Figure 2**. Thus, the weighed indigo leaves are placed into a glass beaker. a 200 ml of distilled water is added to the glass beaker, which is covered with aluminum foil. Subsequently, the ultrasonic cleaning bath is set to a frequency of 40 Hz. The extraction process is carried out according to the specified time. After that, the extract is filtered from the indigo leaf residue. Then, solutions of CaO and NaOH are added to the extract solution according to their respective variables and heated using a water bath. The resulting solid extract is weighed, and the extraction yield is calculated.

2.4. Dyeing on Cotton Fabric

To assess the dyeing results on cotton fabric, an application test was conducted following the specified research design. The fabric dyeing process began by preparing a Turkish Red Oil (TRO) solution by mixing 2 g of TRO with 1 L of water. Then, the cotton fabric was immersed in the TRO solution for 30 min, removed, and dried. Afterward, the powdered dye extract was dissolved in warm water (± 50 °C). The cotton fabric was dried and then immersed in the dye solution, stirred for 15 min, and dried. Subsequently, a fixing solution was prepared by mixing 50 g of alum with 1 L of water. The cotton fabric was immersed in the fixing solution when it was semi-dry for ± 10 min. Afterward, the cotton fabric was rinsed thoroughly with water and dried.

3. RESULTS AND DISCUSSION

Based on Dhiya et al. (2017) and Sonya et al. (2019), the optimal extraction temperature using the UAE method is 60 °C. Therefore, this temperature was set, and the temperature increase profile was recorded at intervals of every 10 minutes.

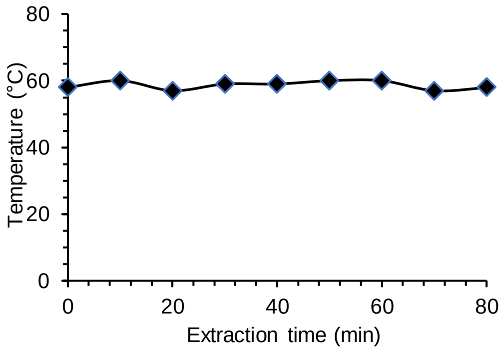

Figure 3. Tarum leaf extraction temperature profile

As the extraction time increases, ultrasonication will raise the temperature, so to maintain the extraction temperature at 60 °C, cooling is carried out for every increase beyond 60 °C. Figure 3 shows the temperature rise after 10 minutes reaching 1-2 °C, then during the cooling process, after reaching 60 °C, the decrease reaches 2-4 °C.

The ultrasound-assisted extraction (UAE) method operates with a mechanism involving liquid subjected to ultrasonic waves, producing specific vibrations as spontaneous microbubbles. These bubbles then move rapidly and collide with each other after attaining sufficiently high pressure. The transmission speed of ultrasonic waves occurs on the surface of the extracted material, leading to micro cavitation and shock waves. Subsequently, the solution containing particles experiences high micro-mixing, increasing heat and mass transfer rates, including diffusion from the content within the pores of the solid (Contamine et al., 1994).

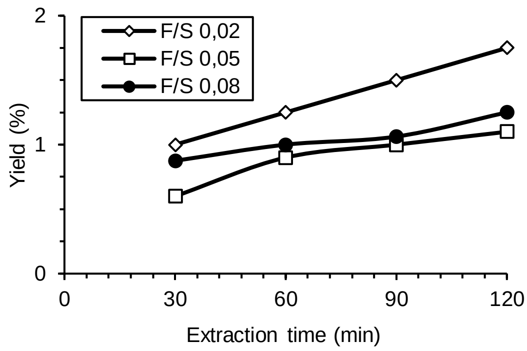

Figure 4. Effect of time on extraction yield with the addition of CaO/S 0.001 g/ml with variations in F/S

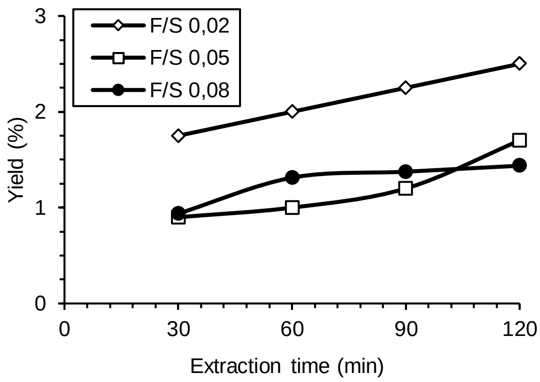

Figure 5. Effect of time on extraction yield with the addition of CaO/S 0.005 g/ml with variations in F/S

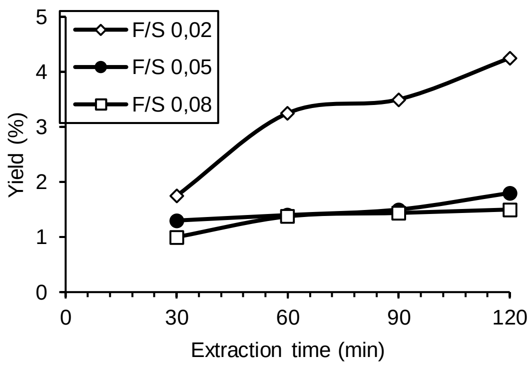

Figure 6. Effect of time on extraction yield with the addition of CaO/S 0.009 g/ml with variations in F/S

From **Figures 4, 5,** and **6**, it can be observed that the highest yield is achieved at an extraction time of 120 min. However, despite the highest yield obtained at extraction time of 120 min, for the F/S variables of 0.02 and 0.05 g/ml, there is a change in color in the extracted solution to a yellowish-green hue after exceeding 90 min. Research conducted by Delvitasari (2013) reported that the extraction time affects the color stability of the extracted solution, which decreases after reaching 90 min (Delvitasari, 2013). Furthermore, the increase in yield with the increasing extraction time is attributed to the cavitation effect. Additionally, a dark blue solution at F/S 0.08 g/ml is obtained after 120 min of extraction, and its yield tends to be smaller compared to the other two ratios. The reason is at F/S 0.08 g/ml, the extraction driving force is low, and solvent saturation is achieved more quickly, resulting in a lower yield (Prasetyo S, 2015).

Another factor influencing the yield of natural indigo dye extraction is the ratio of feed to solvent (F/S). From **Figures 4** and **6**, it can be observed that as the F/S ratio increases, the resulting yield decreases. This study reported that the highest yield was obtained at the smallest material-to-solvent ratio of 0.02 g/ml. Tarum leaves can be well extracted with a relatively low material density. The increasing ratio of feed to solvent leads to higher solvent viscosity, hindering cavitation formation. The higher viscosity of the solvent reduces the interaction between the solvent and the material, consequently resulting in a lower yield (Maran et al., 2015). Furthermore, the extraction yield indicate that F/S ratios of 0.02 and 0.05 g/ml are higher compared to the yield at 0.08 g/ml because the smaller the ratio of tarum leaves to solvent, the greater the driving force. It is connected by more solvent molecules come into contact with dissolved molecules, resulting in a higher extraction of solute; and the solvent saturation degree with solute increases, leading to an increase in yield.

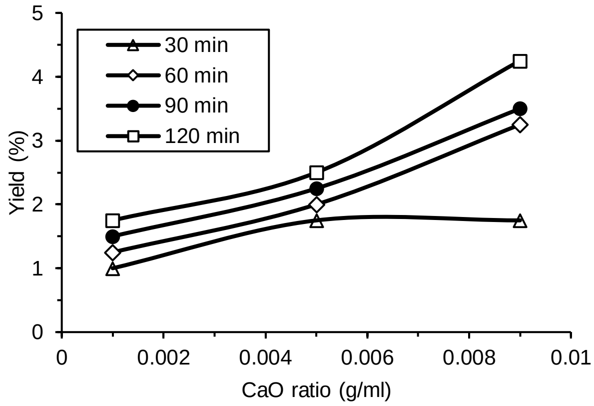

Figure 7. Effect of adding CaO/S on the extraction yield of indigo dye with F/S 0.02 g/ml

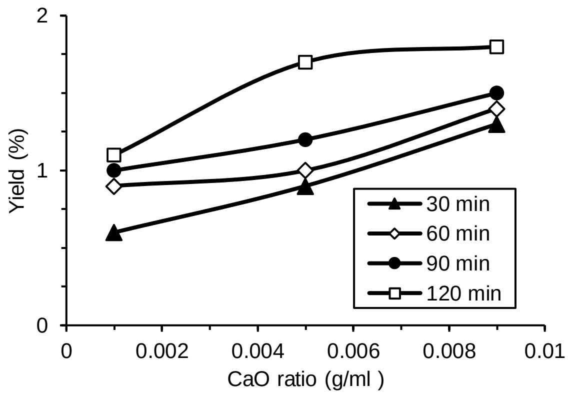

Figure 8. Effect of adding CaO/S on the extraction yield of indigo dye with F/S 0.05 g/ml

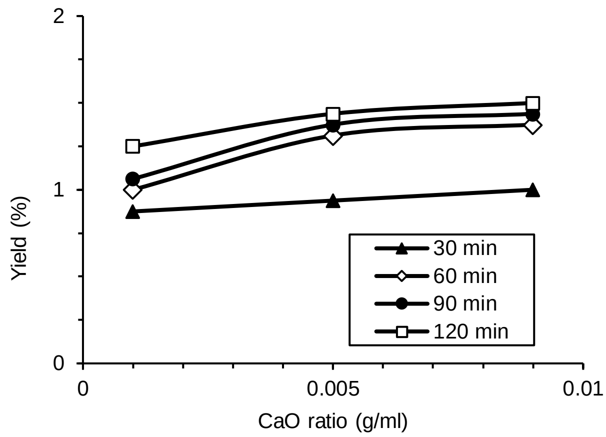

Figure 9. Effect of adding CaO/S on the extraction yield of indigo dye with F/S 0.09 g/ml

Based on **Figures 7, 8,** and **9**, the highest yield for tarum leaf extraction is obtained at a CaO/S ratio of 0.009 g/ml with an extraction time of 120 min, The extraction yield for the tarum leaf ratios of F/S are 4.25 % for 0.02, 1.8 % for 0.05, and 1.5 % for 0.08. As the concentration of CaO increases, a higher yield is also obtained (Lestari & Riyanto, 2004).

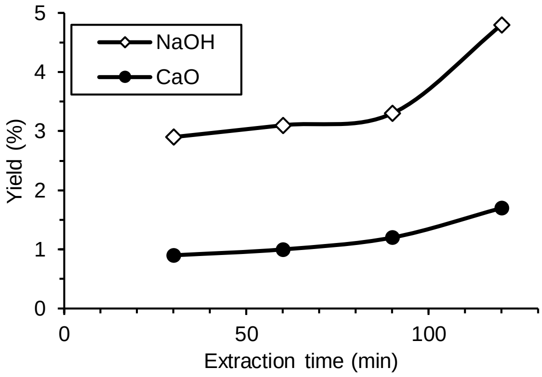

Figure 10. Effect of adding CaO and NaOH with 0.005 g/ml on the extraction yield of indigo dye with F/S 0.05 g/ml

The NaOH solution with a ratio of 0.005 g/ml is used as a comparison with the CaO solution. As shown in **Figure 10**, the yield for extractions over 30, 60, 90, and 120 min are 2.9 %, 3.1 %, 3.3 %, and 4.8 %, respectively. Similar to the results obtained with addition of CaO, the highest yield is obtained of 120 min, attributed to the cavitation process. Furthermore, it can be observed that extraction yield from adding the NaOH solution of 0.005 g/ml is greater compared to adding the CaO solution with the same mass ratio variable. In addition, NaOH exhibits greater solubility characteristics than CaO, thereby improving its capacity to dissolve tarum leaves (Paryanto, et al., 2012).

For dye extraction from tarum leaves in the community, CaO solution is commonly used as the alkaline agent, as it is more economical. Generally, CaO solution serves as a substitute for NaOH. The higher yield obtained using NaOH compared to CaO indicates that the stronger the base used, the more dye is extracted. Causing indoxyl is readily oxidized by air to form blue indigo pigment in an alkaline environment.

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

Figure 11. Staining results on fabric with F/S 0.05 g/ml and CaO/S 0.005 g/ml with time variables: (a) 30 min; (b) 60 min; (c) 90 min; (d) 120 min

From the coloring results in **Figure 11**, it can be observed from extraction with consistent material and CaO ratio variables, the color remains light blue at 30 min, transitions to dark blue at 60 min, achieves a deep blue hue at 90 min, and ultimately, after 120 min, degrades to a bluish-green tint. Therefore, the optimal time variable chosen is 90 minutes as it yields the most stable color with indigo pigment.

(a) (b) (c)

Figure 12. Results of dyeing fabric using indigo dye extract for 90 min and CaO/S of 0.005 g/ml with (a) F/S 0.02; (b) F/S 0.05; (c) F/S 0.08 g/ml

The color of the natural dye extract from tarum leaves, as depicted in Figure 12, indicates that the less solvent or the more leaves used for extraction, the more blue color is formed in the extract. It is evident that at F/S 0.02 g/ml, the resulting color is greenish-yellow due to the low ratio of material to solvent, resulting in a significant driving force and rapid degradation. Subsequently, indigo dye becomes apparent at F/S 0.05 g/ml, with the color progressively darkening to a deep blue at F/S 0.08 g/ml. The increase in dark blue color with the material ratio is caused by the increasing concentration of indigo dye. The higher the dye concentration, the brighter the hue, and vice versa. Research by Putri et al. (2015) and Dhaniar et al. (2016) also indicates that the higher the material-to-solvent ratio, the lower the brightness level, or the darker and more intense the color becomes (Putri and Nisa, 2016; Widoretno and Kunhermanti, 2016).

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

Figure 13. The results of dyeing fabric using indigo dye extract for 90 min and F/S 0.05 g/ml (a) CaO/S 0.001; (b) CaO/S 0.005; (c) CaO/S 0.009; and d) NaOH/S 0.005 g/ml

The use of excessive CaO ratios indicates a decrease in color intensity, notably after surpassing the CaO ratio of 0.005. Subsequently, at the CaO ratio of 0.009, the color of the natural dye extract from tarum leaves turns greenish-yellow (**Figure 13c**). Kun Lestari (2004) stated that the optimal CaO ratio for extracting indigo dye is 0.007 g/ml, and above this ratio, the extracted indigo experiences a decline. It is caused by excessive lime solution usage leads to excessively high alkalinity, resulting in a color change in the extracted solution to greenish-yellow. Furthermore, the dye extract using NaOH produce a darker color compared to the dye extract results using CaO (**Figure 13d**).

4. CONCLUSION

Based on conducted research, the optimal condition for extracting natural dye (indigo) from tarum leaves using the UAE method was determined to be at an extraction time of 90 minutes. The influence of the material-to-solvent ratio and the addition ratio of CaO to the solvent to achieve optimum conditions, respectively, were found to be 0.05 g/ml and 0.005 g/ml. Furthermore, the highest yield obtained from indigo extraction was observed at feed-to-solvent ratio of 0.02 g/ml and the addition of CaO solution at 0.009 g/ml, resulting 4.25 %. Additionally, the addition of NaOH solution to the indigo extraction resulted in the highest yield at 4.8 %. The application test of indigo dye using the mordanting method on cotton fabric revealed a richer color with the addition of NaOH solution compared to the addition of CaO solution.

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