

Ida Nuramdhani,¹ Nurfadilah Ikhsani,² Iceu Agustinisari³

¹ Department of Textile Chemistry, Politeknik STTT Bandung, Bandung, Indonesia

² Postgraduate Program of Master of Applied Science in Textile Engineering and Apparel Technology, Politeknik STTT Bandung, Bandung, Indonesia

³ Indonesian Centre for Agricultural Postharvest Research and Development, Bogor, Indonesia

Preparation of Nano-Sized Eucalyptus Leaves Extract and its Application on Cotton as Natural Dyes and Antibacterial Agents

Priprava izvlečka z nanodelci iz listov evkaliptusa in njegova uporaba kot naravnega barvila in protibakterijskega sredstva za bombaž

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Corresponding author/Korespondenčna avtorica:

Ida Nuramdhani

E-mail: ida.nuramdhani@stttekstil.ac.id

ORCID iD: 0000-0002-1034-3598

Abstract

Nano-emulsions of *Eucalyptus globulus* leaves extract in 96% ethanol and 96% methanol were successfully prepared using the method of homogenization at various speeds of 13,000 rpm, 15,000 rpm and 17,000 rpm, with the addition of non-ionic surfactant to prevent agglomeration. A phytochemical analysis showed the presence of tannin, phenol and flavonoids. The size of the resulting nano-emulsions was measured using a particle size analyser (PSA) prior to the dyeing of cotton fabrics using the two-dips-two-nips method. Antibacterial tests were carried out on the extracts and dyed fabrics. The results showed that nano-sized particles (< 100 nm) were obtained from all extracts in both solvents used at all speeds. All dyed fabrics showed good levelness in a pale-brown colour with K/S values < 1 for all samples under spectrophotometric measurement. The colour fastness of the dyed fabrics to washing and rubbing were good to very good, with grey scale values ranging between 4/5–5. Antibacterial tests in *Staphylococcus aureus* bacterium showed that the eucalyptus leaves extract solution exhibited antibacterial ability, as evidenced by the formation of clear zones around the wells. Much better antibacterial activity was demonstrated by the dyed fabrics. Changing the particle size to nano increased the effectiveness of the antibacterial activity of the *Eucalyptus globulus*. Results showed that the nano-sized eucalyptus leaves extract demonstrated a more significant antibacterial activity than dyeability.

Keywords: *Eucalyptus globulus*, nano-emulsion, antibacterial, natural nano-dye

Izvleček

Nanoemulzije z izvlečki listov rastline *Evkaliptus globulus* v 96-odstotnem etanolu in 96-odstotnem metanolu so bile uspešno pripravljene z metodo homogenizacije pri hitrostih 13.000 vrt./min, 15.000 vrt./min in 17.000 vrt./min



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in dodatkom neionske površinsko aktivne snovi za preprečevanje aglomeracije. Fitokemična analiza je pokazala prisotnost taninov, fenolov in flavonoidov. Velikost delcev v nanoemulzijah je bila izmerjena z analizatorjem velikosti delcev (PSA) pred barvanjem bombažnih tkanin z metodo dveh potopov-dveh stiskov. Opravljeni so bili protibakterijski testi na ekstraktih in obarvanih tkaninah. Rezultati so pokazali, da so vsi izvlečki pri obeh topilih in pri vseh hitrostih vsebovali nanodelce, velike manj kot 100 nm. Vsi proučevani vzorci tkanin so bili enakomerno obarvani v svetlo rjavi barvi in so pri spektrofotometričnih meritvah izkazali vrednosti $K/S < 1$. Barvna obstojnost pri pranju in drgnjenju je bila dobra do zelo dobra, z vrednostmi sive lestvice med 4/5 in 5. Protibakterijski testi na bakterijo *Staphylococcus aureus* so pokazali, da ima emulzija izvlečka iz listov evkaliptusa protibakterijsko aktivnost, saj so se na plošči agar tvorila inhibitorna območja okrog ločeno nacepljene testne bakterije. Obarvane tkanine so pokazale veliko boljše protibakterijske aktivnosti kot nebarvane. Velikosti delcev je dokazano povečala učinkovitost protibakterijske aktivnosti izvlečka iz listov *Evkaliptus globulus*. Rezultati kažejo, da je ekstrakt nanodelcev iz listov bolj izboljšal protibakterijsko aktivnost kot barvno sposobnost.

Ključne besede: Evkaliptus globulus, nanoemulzija, protibakterijski, naravno nanobarvilo

1 Introduction

In recent years, there has been increasing demand for the use of natural resources, driven by a global shift towards sustainability and environmental consciousness [1–2]. This trend emphasizes the importance of harnessing renewable and biodegradable materials for various applications, and reducing reliance on synthetic chemicals that often pose ecological and health risks. In this context, natural dyes and antibacterial agents derived from plant sources have garnered significant attention due to their eco-friendly nature and potential health benefits [3–4]. Research on the development of natural dyes therefore continues to increase and attract more attention because of the global requirements that tends to favour sustainable and environmentally-friendly materials [5–6].

Eucalyptus globulus, commonly known as the blue gum tree, is a well-known source of phytochemicals with a wide range of bioactive properties [7–8]. Traditionally, extracts from eucalyptus leaves have been utilized for their therapeutic properties, including anti-inflammatory, antioxidant, and antimicrobial effects [4, 8]. Recent studies have highlighted the potential of *Eucalyptus globulus* as a promising resource for natural dyes and antibacterial agents [9–10]. The rich composition of bioactive compounds, such as

tannins, flavonoids and essential oils, in eucalyptus leaves underpins their ability to serve as an effective and sustainable alternative to synthetic dyes and antimicrobial agents [11–12]. In previous studies, Eucalyptus extracts were made and applied in macro or micro measures [13]. In general, dyeing with natural dyes results in poor colour fastness due to their low affinity for textile fibre [13–14]. On the other hand, the manufacture of dyes in nanosize is quite promising for further exploration, particularly due to its ability to increase the effectiveness and efficiency of the application process, including its ability to increase colour fastness [15].

The development of nano-sized particles from natural extracts represents an innovative approach to enhancing their efficacy and applicability. Nanosizing refers to the reduction of particle size to the nanometre scale, which significantly alters the physical and chemical properties of a material [15–16]. For eucalyptus leaves extract, nanosizing can lead to increased surface area, enhanced solubility and improved interaction with target substrates. These modifications are particularly advantageous in the context of dyeing processes, where uniform colour distribution and penetration are crucial, and in antimicrobial applications, where higher surface activity can result in more effective bacterial inhibition.

Nano-sized particles tend to easily agglomerate. To prevent them from remaining in nano-size, it is therefore necessary to keep them in the form of nano-emulsions by adding an emulsifier to the solution during homogenization [17–18]. In nano-emulsion systems stabilized by non-ionic surfactants, the surface charge comes from the adsorption of ions present in the aqueous phase or due to friction between droplets and the dispersion medium. The adsorbed ions on the droplet surface generate a coulomb repulsive force between the particles that will hinder aggregation [19].

Ideally, dyeing using nano-sized eucalyptus leaves extract gives higher colour strength, colour fastness and antibacterial abilities [20]. The aim of this work was therefore to validate this concept by comparing the quality of dyeing results using normal-sized extracts with nano-sized extracts [21]. This study focused on the preparation of nano-sized eucalyptus leaves extract and its application as a natural dye and antibacterial agent. By leveraging the nanoscale properties of the extract, the research aimed to demonstrate the improved performance and sustainability of natural products derived from *Eucalyptus globulus*. The findings of this research are expected to contribute to the growing body of knowledge regarding natural resource utilization and offer practical solutions that are in line with the principles of green chemistry and sustainable development.

2 Experimental

2.1 Materials

Six samples of nano-sized eucalyptus leaves extract were prepared from extraction using two different solvents, i.e. 96% methanol and 96% ethanol, which were then homogenized at three different speeds, i.e. 13,000 rpm, 15,000 rpm and 17,000 rpm. Two other control samples prepared from the extraction of the leaves with 96% methanol and 96% ethanol were added. The leaves of *Eucalyptus globulus* used

for this research were obtained from an Indonesian state-owned forest plantation, Perum Perhutani KPH Jatirogo, East Java, Indonesia. Methanol (96%) and ethanol (96%) were purchased from Sigma Aldrich Indonesia. To prevent agglomeration during homogenization, two types of emulsifiers, i.e. polyethylene glycol 400 (Merck KGaA) and Tween 80 (Merck KGaA) were utilized in each process. The resulting nano-emulsions were then used for dyeing on 100% woven cotton (RFD or ready for dyeing) obtained from the stock in the Laboratory of Textile Physical Chemistry in Politeknik STTT Bandung.

2.2 Preparation of nano-sized eucalyptus leaves extract

The preparation of nano-sized eucalyptus leaves extract and the characterization thereof were carried out in the Nanotechnology and Microbiology Laboratories of the Indonesian Centre for Agricultural Postharvest Research and Development, Bogor, Indonesia. Fresh eucalyptus leaves that had been cut into small pieces were washed under running water three times, and then dried in an oven at 70 °C for 30 minutes. A total of 500 g of eucalyptus leaves (the total amount used for this research) were divided into two and macerated in each corresponding solvent (200 ml 96% methanol or 96% ethanol) for 48 hours. Samples were then filtered and evaporated at 70 °C to obtain pure extracts [22]. Eighty ml extracts were obtained as the yield from the total amount of leaves used.

Phytochemical analysis on the presence of the total tannin, phenol and flavonoid content was performed to the resulting extracts. For the tannin content analysis, a blank solution containing 0.5 ml of Folin Ciocalteu reagent and 1.25 ml of saturated Na_2CO_3 in distilled water (up to 25 ml) was prepared. Incubation at room temperature for 20 minutes was performed. Absorbance was measured using a UV-Vis spectrophotometer at $\lambda = 725$ nm. A stock solution of tannic acid was made by homogeneously dissolving 50 g of tannic acid in distilled water (up to 50 ml). A standard curve was developed from the

measurement of absorbance of a series of four different concentrations of the mixture of tannic acid, Folin Ciocalteu and saturated Na_2CO_3 in distilled water, with the same incubation procedure. Total tannin content in each eucalyptus leaves extract was carried out by the same procedure, replacing tannic acid with sample eucalyptus leaves extract. Tannin content was expressed as mg of tannic acid equivalent/gram of extract, which was calculated from a standard solution regression curve. For phenol content analysis, a standard solution was also made by dissolving 1 ml of Folin Ciocalteu reagent in 10 ml distilled water, which was vortexed for 1 minute. Up to 0 ml of 7% Na_2CO_3 and distilled water were added to reach 25 ml. The mixture was incubated at room temperature in a dark room for 90 minutes. Absorbance measurements were carried out at $\lambda = 750$ nm. A stock solution of gallic acid (1000 ppm) was made, followed by the same procedure to create a standard curve. Total phenol content in each eucalyptus leaves extract was expressed as mg gallic acid equivalent/gram extract. Finally, the determination of flavonoid content was carried out by making blank solution of 3 ml of 5% AlCl_3 in 10 ml distilled water. The mixture was incubated at room temperature in a dark room for 90 minutes after being vortexed for 1 minute. Absorbance was measured at $\lambda = 420$ nm. A standard solution containing 50 g of quercetin in 50 ml distilled water was prepared in a 50 ml measuring flask. A standard curve was also made, and the content of total flavonoid was expressed as mg of quercetin equivalent/gram of extract.

As previously mentioned, eight different extracts were prepared at different variations, as presented in Table 1. Each sample of the nano-sized eucalyptus leaves extract was prepared under high-speed stirring in an Ultra-turrax homogenizer with the following chemical composition: 2% polyethylene glycol, 5% Tween 80, 46.5% Eucalyptus extract and 46.5% water. This composition was determined based on previously performed experiments.

Table 1: Variations of eucalyptus leaves extract prepared

Name of samples	Type of solvent	Speed of homogenization (rpm)
A	Methanol (96%)	No homogenization (control)
B		13,000
C		15,000
D		17,000
E	Ethanol (96%)	No homogenization (control)
F		13,000
G		15,000
H		17,000

In the initial stage, two mixtures, referred to as Mixture 1 and Mixture 2, were prepared. To make Mixture 1, the exact amounts of polyethylene glycol and Tween 80 needed for each sample were mixed and stirred for 10 minutes at 600 rpm. Mixture 2 was prepared from the eucalyptus leaves extract and water with the aforementioned composition under the same conditions with constant stirring. Mixture 1 was then carefully dripped into Mixture 2, still with constant stirring. After the last drop, homogenization was started using an Ultra-turrax homogenizer at each corresponding speed (13,000 rpm, 15,000 rpm and 17,000 rpm) for 10 minutes. The two control samples were mixed without homogenization.

Particle size analysis (PSA) was immediately carried out for each sample of the resulting nano-emulsion. The principle of the PSA test is to measure Brownian motion between particles and the medium (water). Measurements were made under light scattered by a collection of particles. The angle of light scattering is inversely proportional to the size of the particles. The greater the scattering angle, the smaller the particle size.

2.3 Dyeing on cotton and evaluation of dyed fabrics

The dyeing process and the evaluation of dyeing results were carried out in the Laboratory of Textile Physical Chemistry, Politeknik STTT Bandung using the continuous two-dip-two-nip method. The wet pick up (WPU) of the padder was set at 80%. Up to 100 ml of each dyeing solution (solution A to H)

was prepared in a saturation bath and the fabric was soaked in the solution for 10 minutes. The fabric was then padded without any additions of other auxiliaries. The fabric was then subjected to the drying process at room temperature. Pre-mordanting with aluminium sulphate and ferro sulphate was carried out to improve the dyeing procedure.

The colours of all dyed fabrics were then evaluated using a Minolta CM-3600d visible light spectrophotometer (Minolta Singapore (Pte), Singapore). The colour depth was expressed in the K/S value obtained from reflectance measurements using the Kubelka-Munk reflectance theory [23], while the colour shades are expressed using L^* , a^* and b^* values. To ensure the accuracy of the data, measurements were made at three different positions of the fabric. In addition, colour fastness to washing of the dyed fabric was tested according to SNI ISO 105-C06-2010 (Textiles – Tests for colour fastness – Part C06: Colour Fastness to Domestic and Commercial Laundering) [24]. To do this test, the sample cloth was covered with cotton and wool (according to sections F01 to F08 of ISO 105-F:1985) on two different sides before being put into a vessel that already contained washing liquor (4 g/l ECE phosphate reference detergent, without an optical brightening agent) and 10 steel marbles. The process was carried out in a Launder-O-meter machine at 40 °C for 45 minutes. The fabric was then rinsed under cold running water and dried. The change in colour of the sample and staining of the adjacent fabrics were evaluated using the grey scale, based on ISO 105-A03. In addition, the colour fastness to rubbing of the dyed fabrics was tested according to SNI ISO 105-X12(E):2016 (Textiles – Tests for colour fastness – Part X12: Colour fastness to rubbing) [25].

2.4 Antibacterial test

Antibacterial tests were carried out on a solution of eucalyptus leaves extract before and after homogenization, and on the dyed fabric. For testing the extract, the well-known Broth or agar dilution method was applied by using agar as a medium for bacterial growth and 0.85 NaCl as a medium for bacterial

dilution. The process of making agar was carried out first by mixing 0.13 gram of sodium broth and 0.18 gram of bacteriological agar in an Erlenmeyer flask. A total of 1,000 ml of distilled water was added and the mixture was homogenized. The resulting solution was then covered with aluminium foil and heated using a hot plate at 100 °C with constant stirring at 80 rpm until the solution was clear, and then cooled. On the other hand, the preparation of 0.85% NaCl as the bacterial diluent medium was carried out by homogenizing 8.5 gram of NaCl in 1,000 ml of water. The resulting sodium agar and NaCl solutions were stored in a heat-resistant basket covered tightly with aluminium foil and cotton to prevent the lid from opening during the sterilization process. All tools, including tips, petri dishes and well moulds, as well as materials (sodium agar and NaCl solution) were sterilized in an autoclave at a temperature of 121 °C and pressure of 4 atm for 15 minutes. After cooling, all materials were stored in a refrigerator to keep them sterile.

For the process of inoculating the bacteria, a *Staphylococcus aureus* bacteria pellet was placed in 10 ml of sterile NaCl solution, and then covered with cotton. The work was carried out in a laminar air flow that had been sterilized with 70% ethanol. The solution was then incubated at 37°C for 48 hours and observed to determine whether bulk was formed. Turbidity in the solution indicated that the bacteria was successfully grown and developed. The resulting bacteria solution was stored in a refrigerator to keep them alive.

Finally, and antibacterial test using the agar dilution method was performed. The bacterial solution was diluted with sterile NaCl solution up to six times, which was vortexed for 30 seconds to ensure a homogeneous solution in each dilution process. Next, 1 ml of bacterial and 25 ml of diluted sodium agar solution were placed sequentially on a petri dish, and then homogenized by rotating the dish and left to stand until hard. A well with a sterile mould in the middle of the petri dish was made. Ten microliters of the test sample or the blank was placed

in the well. The cup was covered and incubated at 37 °C for 48 hours. Observation was performed to check whether a clear zone formed. The larger the diameter of the clear zone, the better the activity of the antibacterial substance. For antibacterial test on fabric, the ISO 20743-2011 standard was followed using basically the same method used for the extract, only that the sample was in the form of fabric.

3 Results and discussion

3.1 Phytochemical analysis of the eucalyptus leave extracts

Phytochemical analysis was conducted on eucalyptus leaves extracts to assess the level of total phenols, tannins and flavonoids, all of which are natural contents that are expected to contribute to the dyeing and antibacterial properties of the extract. Table 2 presents the phytochemical analysis results of the extract.

Table 2: Results of phytochemical analysis of the eucalyptus leaves extract

Eucalyptus leaves extract solution	Concentrations (µg/ml) of		
	tannin	phenol	flavonoid
In 96% ethanol	35.97	232.69	60.12
In 96% methanol	31.68	238.76	63.51

The data in Table 2 show approximately the same amount of tannin, phenol and flavonoid in both extracts (96% ethanol and 96% methanol). This means that in both solvents, the dyeing and antibacterial properties of the extracts are expected to be only very slightly different. Tannin is a complex organic compound comprising phenolic components that contribute to the yellow to brown pigmentation observed. The resulting extract showed a brown hue that confirmed the existence and quantity of total tannins obtained through spectrophotometric analysis with tannic acid as the reference standard. The extract was then subjected to reaction with Folin-Ciocalteu reagent and sodium carbonate until a blue coloration. This change in colour signified a redox reaction where tannins function as reducing

agents and Folin-Ciocalteu as oxidizing agents [17]. *Eucalyptus globulus* leaves are already known to contain tannin as a compound that is easily soluble in organic solvents such as methanol, ethanol and acetone. Ethanol and methanol were selected as solvents used in this research because they were easy to obtain and relatively cheaper than other organic solvents, while the maceration process at room temperature was used because the process was easy and it was expected to maintain high levels of leaves extraction yield. In excessive heat, tannin will easily break down into pyrogallol, pyrocatechol and phloroglucinol, causing the loss of its colouring function. Therefore, the maceration process at room temperature was used to maintain the content of tannin in the extract. From the observation during the experiment, a deep brown solution was obtained, indicating the successful extraction of tannin, which acted as a weak acid because of its content of several phenolic OH-groups, indicated by the pH 5.7 of the extract solution. The extraction using methanol and ethanol was intended to dissolve not only tannin, while other important compounds in the Eucalyptus leaves, such as essential oil containing 1,8-cineol, demonstrated antibacterial properties. The 1,8-cineole compound, with its fresh aroma of camphor, demonstrates good bioactivity for some gram-positive and some-gram negative bacteria, as well as anti-fungal, anti-inflammatory and antioxidant properties [4, 12].

The extract also contained phenolic compounds that function as antioxidants [18]. A total phenol analysis was conducted using gallic acid as the standard. Similar to the tannin analysis, the solution turned blue due to redox reactions. A blue colour solution indicated the presence of phenolate ions, which were directly proportional to the phenol content in the solution. Finally, a total flavonoid test was conducted using aluminium chloride as a reagent and quercetin as the standard. Aluminium chloride can form a stable complex with mostly hydroxyl groups presented in flavonoid compounds. The configuration of this stable complex compound

was shown by a yellow-coloured solution that was observed during the test. Flavonoids are secondary metabolite compounds that are most often found in plants that function as antioxidants and can be used as antibacterial agents.

3.2 Size of the nano-emulsion

As evident from Table 3, the results of the particle size analysis showed that the target size of the nano-emulsion (in the range of 1–100 nm) for eucalyptus extract was achieved from all the extracts in 96% ethanol homogenized at speeds of 13,000 rpm, 15,000 rpm and 17,000 rpm, which resulted in sizes of 22.84 nm, 52.40 nm and 32.21 nm, respectively. At a speed of 15,000 rpm, the size of the nano-emulsion was slightly higher than the other emulsions. This could be because of the aggregation process of the resulting nanoparticles during the homogenization process that resulted in a slightly larger size [9], although it was still in an acceptable nanosize. This could have occurred when the additional speed stimulated nanoparticles that successfully formed to collide and then reaggregate. At a speed of 17,000 rpm, the higher stirring speed was able to again separate the aggregating nanoparticles, so that the

size of the emulsion could still be formed at a slightly lower nanosize of 32.21 nm. Likewise, the results of the nano-emulsion measurements of eucalyptus leaves extract with a 96% methanol solvent showed that nano-size targets were achieved at speeds of 13,000 rpm, 15,000 rpm and 17,000 rpm with sizes of 83.95 nm, 16.85 nm and 64.90 nm, respectively. Here, we can see the opposite trend of results, where the nano-emulsion produced from the homogenization process at a speed of 15,000 rpm demonstrated the smallest size compared to the other two samples. From the test results it can be concluded that the speed of homogenization and the types of solvent were not directly proportional to the reduction in the size of the resulting particles. However, it is noteworthy that the results of this work can be a reliable reference in carrying out similar work in the future because the results presented in this study are those of works that were validated through repeating the exact same method at least once for each process condition. The PSA data of each sample was taken from the average of three different measurements of the same sample, which was then averaged further, taken from the two different samples homogenized under the same condition.

Table 3: Results of the particle size analysis of the eucalyptus leaves extract solutions

Eucalyptus leaves extract solution	Speed of homogenization (rpm)	PSA results	
		Diameter (nm)	Polydispersity index (PDI)
In 96% ethanol	0	153.73	0.192
	13,000	22.84	0.470
	15,000	52.40	0.489
	17,000	32.21	0.685
In 96% methanol	0	1,472.17	0.836
	13,000	83.95	0.350
	15,000	16.85	0.402
	17,000	64.90	0.467

The Polydispersity index (PDI) value from the test results shows a fairly good value, which is below 0.5 for all homogenized solutions. PDI is an index value used to estimate the range of particle size dis-

tribution present in a sample. The smaller the PDI value, the more uniform the particle size [26]. From the test results, it can be concluded that the formed nano-emulsion is quite homogeneous.

3.3 Dyeing results

The dyeing of cotton fabrics with the eucalyptus leaves extract may occur because of the interaction between cellulose and the tannin contained in the extract, which also have numerous hydroxyl groups that can interact to form hydrogen bonds with the

hydroxyl groups of cellulose, as depicted in Figure 1 [13]. In this case, where the tannin was not isolated, interaction with other polyphenols contained in the extract was also possible. However, from a visual analysis of the dyed fabrics, the colour of tannin was dominant.

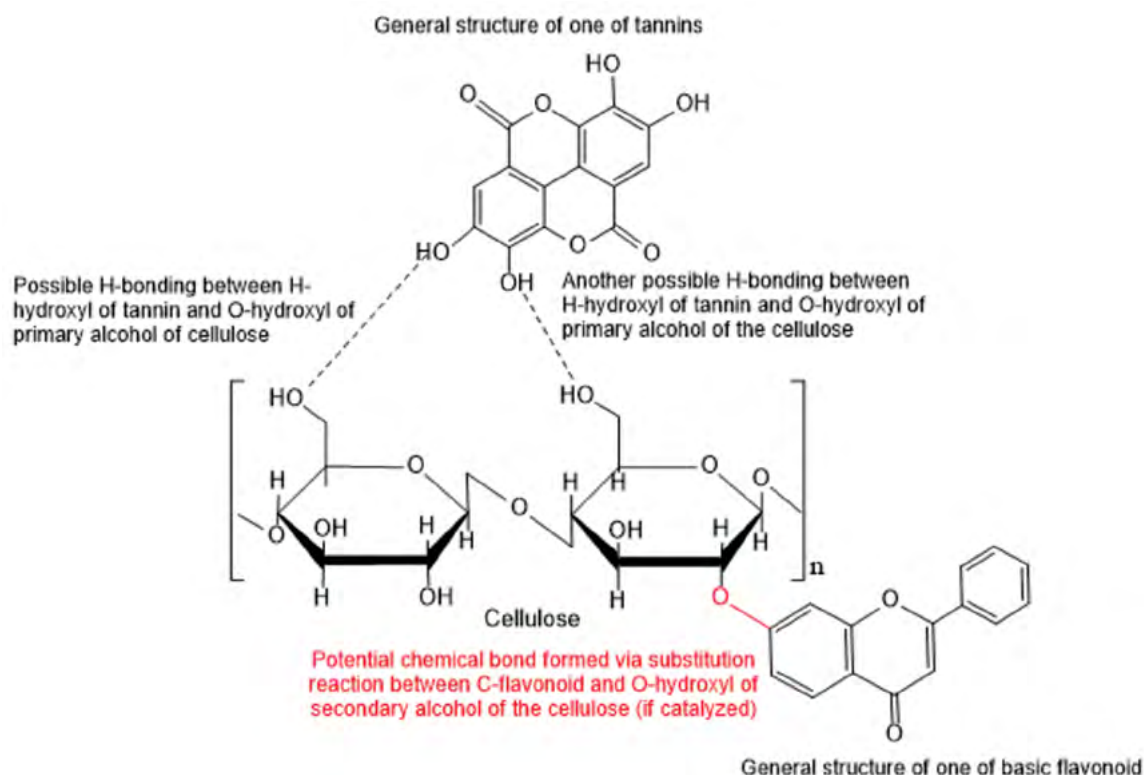


Figure 1: Schematic interaction between tannin and flavonoids and cotton fabric

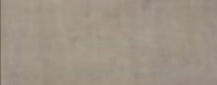
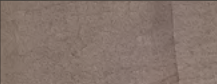
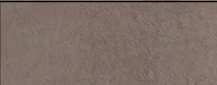
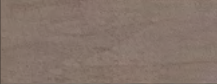
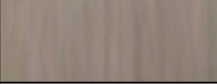
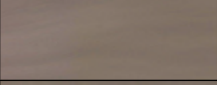
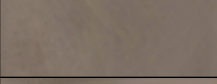
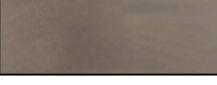
Potential interactions between tannin and flavonoids contained in the extract and cellulose, as illustrated in Figure 1, shows that at least two possible strong interactions could occur: (i) through a hydrogen bond between the H-hydroxyl of tannin and O-hydroxyl of the primary alcohol of the cellulose; and/or (ii) through a strong covalent bond between the C-flavonoid and O-hydroxyl of the secondary alcohol of the cellulose, which can be formed through a substitution reaction mechanism. Of course, the reaction needs to be catalysed. In addition to the two-example mechanism, phenol that was confirmed to be contained in the extract can also create a physical or chemical interaction with the cellulose [27]. Colour measurement results

of the dyed fabric using visible-light spectrophotometer and photographed images of the fabric are presented in Table 4. It is evident from the table that the fabric dyed with nano-sized eucalyptus leaves extract in ethanol and methanol, respectively with speed variations of 13,000 and 17,000 rpm, quantitatively demonstrated better penetration of the dyes into the fabric represented by higher K/S values, as seen by a higher colour strength than the other samples. This was consistent with the size of their nano-emulsions. Kinetically, smaller-sized particles can adsorb and diffuse much more easily and quickly into the fabric, and with more particles, which results in higher colour intensity. However, the K/S values of all samples were not significantly different,

meaning that the colour difference between each dyed fabric was not visually identified. The K/S value is an important parameter in the study of coloured materials, particularly in the context of the Kubelka-Munk theory, which is used to describe the reflectance and transmittance of diffuse materials, where K represents the absorption coefficient and S represents the scattering coefficient, which both represent how much light is absorbed and scattered respectively per-unit distance. This theory provides a mathematical model to relate the reflectance of a diffusely reflecting sample to its absorption and scattering properties. The K/S value can be derived from reflectance measurements using the formula: $K/S = (1 - R)^2/2R$, where R represents the reflectance of the material. It is also important to explain here that all K/S values were taken from their maximum wavelengths because those wavelengths represent the point of highest absorption, providing critical and consistent information about a material's co-

lour properties and ensuring accuracy in various practical applications. It is widely understood that coloured materials have specific wavelengths based on their colour, known as the maximum wavelength (λ_{max}) of absorption. The K value is highest at λ_{max} because this is where absorption reaches its peak. The K/S ratio at this wavelength thus gives the most significant information about the colour strength of the materials. Taking the K/S value at the maximum wavelengths ensures that the measurement reflects the most intense interaction of light with the material. This also helps in accurately determining the concentration of colorants in a sample, understanding its colour properties, and predicting its behaviour under different lighting conditions. By standardizing the measurement at the maximum wavelength, it becomes easier to compare the colouring efficiency and properties of different samples or batches of the same materials. This is important for consistency in industries such as the textile industry [28].

Table 4: Colour test results of the dyed fabrics (without mordant)

Eucalyptus leaves extract solution	Speed of homogenization (rpm)	Photo of dyed fabrics with eucalyptus leaves extract	Colour test results (measured at maximum wavelength of 430 nm)					
			K/S value	Reflectance (%)	Standard deviation	L*	a*	b*
In 96% ethanol	0		0.870	26.023	0.444	78.64	2.77	14.15
	13,000		0.909	18.107	0.122	60.01	3.18	11.44
	15,000		0.870	26.023	0.444	72.84	0.81	14.41
	17,000		0.887	22.667	0.272	72.70	1.82	13.67
In 96% methanol	0		0.868	26.473	0.359	80.07	2.27	11.98
	13,000		0.888	22.327	0.229	75.28	1.20	12.88
	15,000		0.898	20.453	0.127	65.61	1.00	13.10
	17,000		0.882	23.693	0.206	73.83	1.57	13.14

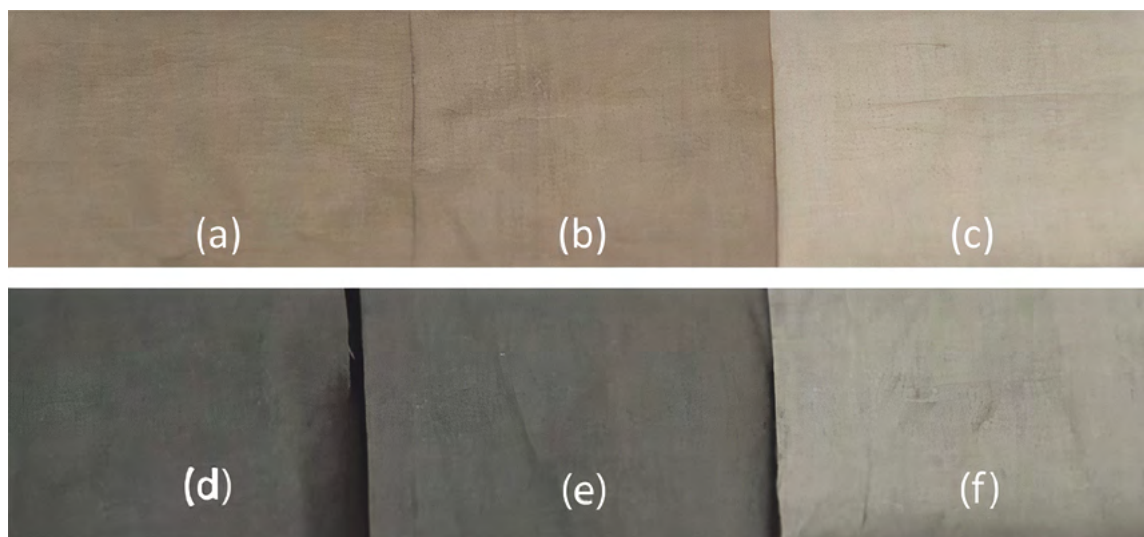


Figure 2: Visual appearance of fabrics dyed used nano-sized eucalyptus leaves extract, mordanted with aluminium sulphate in (a) ethanol at 13,000 rpm, in (b) methanol at 13,000 rpm and in (c) methanol without homogenization; mordanted with ferro sulphate in (d) ethanol at 13,000 rpm, in (e) methanol at 13,000 rpm, and in (f) methanol without homogenization

Moreover, with the repetition of the same measurements on the same sample at five different sites, the levelness of the dyeing results was also analysed, and all samples demonstrated a very good levelness of dyeing, represented by their low standard of deviation value (< 1). Colour difference ($\Delta E_{L^*a^*b^*}$) was not separately calculated here because there were no samples taken as the standard, and the L^* , a^* and b^* values of each sample were found to be significantly different. The value of K/S of each sample was relatively low, showing the pale shade of the dyed fabric. This was confirmed by the high L^* values of all dyed fabrics. In terms of colour shades, it is evident that all samples demonstrated positive a^* and b^* values, meaning that the all-brown coloured fabrics had a strong yellowish shade with slightly reddish colours. The pale shade of the dyed fabric was also the reason that the effect of homogenization speed on colour strength could not be clearly observed. It has been suggested in the other work that the exhaust dyeing method is used with a higher concentration of the dye to achieve a more clearly different colour of the dyed fabric. Mordanting, however, increased the

colour strength of the dyed fabrics and shifted the colour shade, as seen by those fabrics mordanted using ferro sulphate (FeSO_4). Figure 2 presents some selected images of photographed dyed fabrics that were pre-mordanted with aluminium sulphate ($\text{Al}_2(\text{SO}_4)_3$) and ferro sulphate (FeSO_4).

In addition, Table 5 presents the colour fastness to washing and rubbing of the dyed fabrics. It is evident that the fabrics dyed with nano-sized eucalyptus leaves extract showed slightly better fastness. However, in general, all samples showed very good fastness to washing and rubbing. This was indicated by the grey scale values of staining and the colour change obtained from the fastness test, which varied between 4/5 and 5. The same results can also be observed from the testing of dry and wet rubbing fastness, which also varied between 4/5 and 5. The good colour fastness of the dyed fabrics could be because of the very strong interactions between the nano-sized dyes and because of the very bright and light colour showed by all dyed fabrics, which was confirmed by the high L^* values of all samples, i.e. ranging between 60.01 and 80.07.

Table 5: Colour fastness to washing and rubbing of the dyed fabrics

Dyed fabrics with eucalyptus leaves extract	Speed of homogenization (rpm)	Colour fastness to washing		Colour fastness to rubbing		
		Grey scale for staining on		Grey scale for colour change	Grey scale for staining on dry rubbing cloth	Grey scale for staining on wet rubbing cloth
		cotton	wool			
In 96% ethanol	0	4/5	4/5	4/5	5	4/5
	13,000	5	5	5	5	5
	15,000	5	4/5	5	5	5
	17,000	4/5	4/5	4/5	5	5
In 96% methanol	0	4/5	4/5	4/5	5	4/5
	13,000	4/5	4/5	4/5	5	4/5
	15,000	5	5	5	5	5
	17,000	5	5	5	5	4/5

3.4 Antibacterial properties

Antibacterial tests were carried out to check the antibacterial properties of the *Eucalyptus globulus* in the form of extract and the dyed fabric. The results are presented in Figures 3 and 4. The test results showed that in a solution of eucalyptus leaves extract with 96% ethanol and methanol solvents, shown as B and C in the Figure 3, respectively, clear zones were formed, at distances of 1.76 cm and 1.96 cm, respectively. This demonstrated that the antibacterial substance made from the eucalyptus leaves extract was successfully extracted with both solvents, and the ability to inhibit the bacteria was slightly higher in the extract with methanol solvent than in the extract with ethanol solvent. According to Rahayu [29], the inhibition zone activities of antimicrobials are classified into four categories: weak activity (<5 mm), medium activity (5–10 mm), strong activity (> 10–20 mm), very strong activity (> 20–30 mm). Antimicrobial inhibitory activity is expressed based on the clear zone generated around the well. The diameter of the bacterial growth inhibition zone is measured in mm. Thus, the antibacterial activity of the *Eucalyptus globulus* extract in 96% ethanol and methanol solvents were both strong.

The homogenization process using an Ultra-turax homogenizer on the extracts provided an even

better antibacterial effect of the formed *Eucalyptus globulus* nano-emulsions. This can be seen by the results presented by images D and E in Figure 3, which shows nano-sized *Eucalyptus globulus* in the ethanol and methanol, where both demonstrated very strong antibacterial activity with inhibition zones of 2.34 cm (23.4 mm) and 2.36 dm (23.6 mm), respectively. The results indicated that the nano-sized particles in the nano-emulsion demonstrated higher antibacterial activity than those without homogenization or in a micro-sized emulsion. This confirmed that the nano-sized emulsions could diffuse very well on agar that has been inoculated with bacteria, thus improving the antibacterial activity [26]. All extracts measured in this study showed strong to very strong antibacterial activity, while the distance of their inhibition zones is still under the positive control of *Eucalyptus globulus* essential oil (sample A). These results are reasonable because in essential oil, the eucalyptus globulus content could reach maximum purity. On the other hand, under the extraction process in 96% methanol or ethanol, there might be some impurities that cannot be isolated or separated from the extract, thus decreasing the overall strength of the antibacterial activity of the extracts.

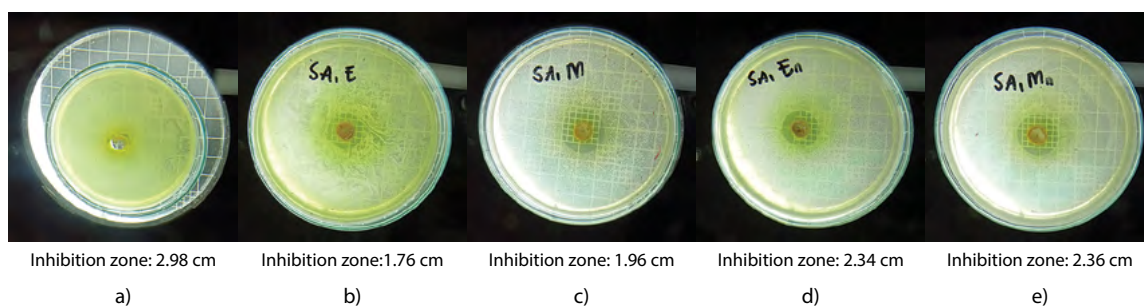


Figure 3: Antibacterial properties of the eucalyptus leaves extracts: a) positive control of *Eucalyptus globulus* essential oil, b) in 96% ethanol, c) in 96% methanol, d) homogenized at 13,000 rpm in 96% ethanol, and e) homogenized at 15,000 rpm in 96% methanol

An antibacterial test on fabric dyed with nano-sized and normal size eucalyptus leaves extract was carried out using the same method as those on the extract solutions, but referencing SNI ISO 20743-2011 (SNI ISO 20743-2011 – Antibacterial) [30] in the case of samples on the dyed fabrics. The aim of the test was to confirm whether after passing the dyeing process, the antibacterial properties

of the *Eucalyptus globulus* in the dyed fabrics still survived. Images of the test results are presented in Figure 4. It is evident from Figure 4 that all dyed fabrics showed higher antibacterial ability compared to the eucalyptus leaves extract solutions. These are shown by the clear zone formed in all images with inhibition zones at a greater distance than those in the extract solutions.

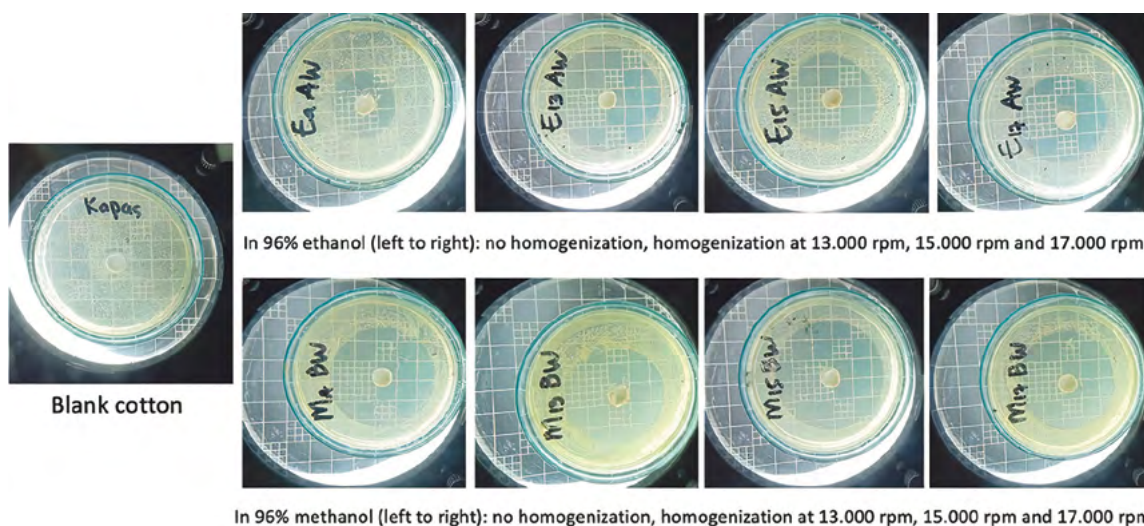


Figure 4: Antibacterial properties of the dyed fabrics

For further clarity, Table 6 presents the quantitative results of the tests. The measurements on the dyed fabrics were taken at three positions, and the average distance of the inhibition zones was calculated. It is evident from the data that all dyed fabrics that were dyed by normal or nano-sized *Eucalyptus globulus* extract demonstrated very strong antibacterial activity. In contrast, the blank cotton

fabric showed very weak antibacterial activity. These results confirmed that dyeing cotton fabric with *Eucalyptus globulus* extracts had a significant effect on the formation antibacterial properties. The stages of dipping and nipping of the fabric during the dyeing process did not decrease or nullify the antibacterial effect. On the other hand, that effect increased significantly.

Table 6: Inhibition zones of the dyed fabrics

Fabrics dyed by extracts	Speed of homogenization (rpm)	Distance of inhibition zones (mm) from three measurements	
		Average	Standard deviation
Blank/standard cotton	-	0.33	0.47
In 96% ethanol	0	38.33	1.25
	13,000	45.00	0.82
	15,000	40.33	0.47
	17,000	43.00	0.00
In 96% methanol	0	40.00	0.82
	13,000	45.33	0.47
	15,000	42.00	0.82
	17,000	44.33	0.47

Similar to the results of the test on the extract solutions, the antibacterial test on fabric dyed with nano-sized eucalyptus leaves extract showed a larger clear zone than those dyed with the normal size of the Eucalyptus extract. Distribution of the nano-sized eucalyptus particles in the fabric was more compact and thus gave a much higher surface area or surface activity. Therefore, much higher amount of the particle could penetrate the fabric, and result in higher antibacterial activity. Moreover, the size does matter for the case on a solid surface such as fabric. The smaller size distribution on the surface of the fabric could also physically prevent penetration of the bacteria into the fabric because the size of the pore between particles might be much smaller than the size of the bacteria itself. This phenomenon might occur apart from the chemical effect of the antibacterial properties coming from the *Eucalyptus globulus* extract used in the dye-

ing process. To confirm the durability of antibacterial properties, two fabrics dyed with nano-sized eucalyptus leaves extract homogenized in 96% ethanol and methanol at 13,000 rpm were washed according to the ISO-6330:2012 standard, IDT (Textiles – Domestic washing and drying procedures). The antibacterial activity of the washed fabrics was then tested. The results showed that there was no significant decrease in antibacterial activity after washing. As indicated by the average distance of inhibition zones of the washed fabrics, the antibacterial activity of the two selected fabrics was maintained. The decreases in the inhibition zone distance were only 0.87 mm or 1.93% (from 45.0 mm to 44.13 mm) and 1.36 mm or 3.00% (from 45.33 mm to 43.97 mm) for fabrics dyed with extract homogenized in ethanol and methanol, respectively. Measurement results are presented in Table 7.

Table 7: Comparison of antibacterial activity of selected dyed fabrics before and after domestic washing and drying

Fabrics dyed by extracts		Distance of inhibition zones (mm) from 3 times measurements	
		Average	Standard deviation
In 96% ethanol at 13,000 rpm	Before washing	45.00	0.82
	After washing	44.13	0.25
Difference		0.87 (1.93%)	
In 96% methanol at 13,000 rpm	Before washing	45.33	0.47
	After washing	43.97	1.81
Difference		1.36 (3.00%)	

In general, the antibacterial activity of the fabrics was still very strong (> 20-30 mm) after the domestic washing and drying treatment. This confirms that

the polyphenols contained in the extract, i.e. tannin, phenol and flavonoid, played an important role in providing a significant antibacterial activity of the

dyed fabrics. The durability test also showed that strong interaction between the polyphenols and cotton fabric had occurred. This is also confirmed by the good-very good washing fastness of the dyed fabrics to washing and rubbing, as presented in Table 5. From the dyeing results, it can also be concluded that antibacterial activity demonstrated by the nano-sized eucalyptus leaves extract was more significant than its dyeability because the visual appearance of the dyed fabrics only showed the typical colour of most natural dyes. They did not demonstrate a distinctive shade or a more intense colour.

4 Conclusion

Nano-sized *Eucalyptus globulus* extracted in 96% ethanol and methanol solvents, using Tween 80 and polyethylene glycol surfactants, were successfully made through homogenization at various speeds of 13,000 rpm, 15,000 rpm, and 17,000 rpm in this research. The content of tannins, phenols and flavonoids in the extract was determined. A significant decrease in the size of Eucalyptus particles in the extract homogenized at the aforementioned speeds was observed from the results of size analysis using a PSA (particle size analyser). The dyeing of cotton fabric with the extracts using the two dips-two nips method showed a pale brown shade at the maximum wavelength of 430 nm. All dyed fabrics showed good and very good fastness to washing and rubbing. The antibacterial activity of the *Eucalyptus globulus* leaves extracts were all strong and very strong, which was demonstrated very well by their inhibition effect. On the other hand, the dyeing of cotton fabric with the extracts demonstrated the typical colour of most natural dyes. A typical phenomenon discovered was that nano-sized Eucalyptus particles in the extract demonstrated higher antibacterial activity than those in normal size. Moreover, the antibacterial activity of dyed fabric was significantly higher than those in the form of extract solutions.

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