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A STUDY ON THE EFFECTIVENESS OF ANTIFUNGAL AGENTS ON COTTON FABRICS COLORED BY GAMBIR NATURAL DYES (*UNCARIA GAMBIR*)

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ABSTRACT. *The increasing interest in the use of natural dyes is not accompanied by sufficient information and method for the application due to broad natural dyes resources existence. Gambir (Uncaria gambir), one of promising favorable natural dyes, has been used as batik coloring for decades. However, the application of gambir extract as a textile colorant faces a severe problem to be encountered which is the microbial growth such as fungi, mold, and bacteria. This study proposed several antifungal agents to suppress the growth of the fungus such as chitosan, aloe vera, and formaldehyde. To conduct the fungus inhibition concentration of every antifungal agent, Aspergillus niger was introduced on the colored cloth medium with the addition of Potato Dextrose Broth (PDB) growth media. Three methods of antifungal addition during the coloring process were investigated namely pre-coloring, post-coloring, and mix coloring process. The growth of Aspergillus niger was observed and measured the diameter and thickness of the colony. One-way ANOVA was used to analyze the treatment significantly. The result showed that all concentration of chitosan and formaldehyde addition, significantly, could suppress the fungus growth. However, formaldehyde is a toxic ingredient and harmful to the environment which becomes a limitation in the application. Meanwhile, from the significance level, Aloe vera could not inhibit the Aspergillus niger growth with the addition of 2 and 5 g/L, but it could suppress the Aspergillus niger growth with the addition of 8 g/L. Furthermore, the optimum condition was observed on the addition of 5 g/L chitosan at post-coloring method because of the minimum growth area of the Aspergillus niger.*

Keywords: Natural dye, Uncaria gambir, Antifungal activity, Chitosan

ABSTRAK. *Indonesia memiliki sumber daya alam yang melimpah sebagai bahan baku pewarna alami. Untuk itu sejalan dengan meningkatnya minat dalam penggunaan pewarna alami, perlu disertai dengan informasi cara penggunaan yang memadai. Gambir (Uncaria gambir), adalah salah satu pewarna alami yang sangat menjanjikan, karena telah digunakan sebagai pewarna batik selama beberapa dekade. Namun, aplikasi ekstrak gambir sebagai pewarna tekstil menghadapi masalah serius, yaitu pertumbuhan mikroba seperti jamur dan bakteri. Penelitian ini mengusulkan beberapa bahan tambahan sebagai antijamur untuk menekan pertumbuhan jamur. Beberapa bahan antijamur yang diteliti adalah: kitosan, aloevera, dan formaldehid. Untuk mengetahui kemampuan masing-masing jenis dan konsentrasi antijamur dalam penghambatan pertumbuhan jamur pada media kain, dilakukan penambahan Aspergillus niger pada kain tersebut dengan media pertumbuhan Potato Dextrose Broth (PDB). Pada penelitian ini diamati tiga metode penambahan antimikroba yaitu: sebelum kain diwarnai, setelah kain diwarnai, dan dicampur pada proses pewarnaan. Pertumbuhan Aspergillus niger diamati dengan mengukur diameter dan ketebalan koloni. One-way anova digunakan untuk analisis data. Berdasarkan analisis tersebut dapat dinyatakan bahwa penambahan kitosan dan formaldehid pada berbagai konsentrasi, secara signifikan dapat menekan pertumbuhan jamur. Formaldehid adalah bahan beracun dan berbahaya bagi lingkungan, untuk itu merupakan pertimbangan dalam aplikasinya. Penambahan Aloevera dengan konsentrasi 8 gr/L baru dapat menghambat pertumbuhan Aspergillus niger. Berdasarkan penelitian ini dapat*

ditetapkan bahwa aplikasi kitosan pada kain setelah diwarnai dan dengan konsentrasi 5 gr/L dapat menekan pertumbuhan Aspergillus niger paling optimum.

Kata kunci: pewarna alami, Uncaria gambir, antijamur, chitosan.

PENDAHULUAN

Natural dyes are commercial products which become popular recently although it has been used for decades. The arising awareness of environment notably becomes the primary aspect of popularity. This advantageous due to the properties of natural dyes such as harmless, biodegradable, profusion number, and environmental friendly(Shahid & Mohammad, 2013a, 2013b). Another useful feature is that some types of natural dyes have antimicrobial properties which will be helpful in the coloring application as antimicrobial activity. Furthermore, the strict regulation applied by governments across the world regarding environmental issues makes natural dyes demand higher (Kiumarsi, Parvinzadeh Gashti, Salehi, & Dayeni, 2017; Parvinzadeh Gashti, Katozian, Shaver, & Kiumarsi, 2014).

There are many feedstocks in nature as natural dyes such as leaves, flower, branch, fruit, extract, animals, and insect. Derived from many natural components, natural dyes have several obstacles for the development ranging from the extraction process to the application. The development is also obstructed from the availability in each region because each region has its own natural dyes source. In every area, including Indonesia, the extraction and application of natural dyes have rapidly developed from its resource concerning economic factor. Gambir (*Uncaria gambir*), mostly exported around 80%, is one of Indonesia's plantation commodities which is a promising substituent for natural dye (Nazir, 2001).

Gambir extract consists of tannins of 24.56% that is potentially used as tanneries and fabric dyes (Dhalimi, 2015). As a tannins compound, catechin is the most chemical found in Gambir which consists of catechin acid (catechin) and Tannat catechin acid (catechin an-hydrate). Catechin can be used as a textile dye which produces brownish color (Webster & Gove, 1966). When catechin heated in basic solution, it will quickly form Tannat catechin and easily dissolve in water or polar solvents (Hayani, 2003). Although theoretically tannin is a resistant substance for microbial decomposition and a toxic for particular microorganism, the application of catechin extract from gambir as natural dyes arises several complaints from batik and weaving artisans. When catechin extract is dissolved in water and applied as natural dyes, it is quickly overgrown by a microorganism such as fungi, mold, and bacteria after the coloring process occurred. The growth of the fungi and mold in the dyed-fabric potentially degrades the fabric strength, so the fabric is easily ripped and damaged, while the growth of bacteria causes terrible smell (Paul, 2014). The growth of fungi, mold, and bacteria can be prevented by applying antifungal agents. The mechanism of antifungal agents can kill the microbe called a biocidal or inhibit microbial growth called biostatic effect at the cellular level (Gao & Cranston, 2008; Rahman, Ahsan, & Islam, 2010). The mechanism varies depending on the type of antifungal agents used. The antifungal agent mechanism immobilizes microbes by attacking the cell wall directly causing the cell leaks and cannot perform its biological function.

The antimicrobial property is an essential nature of natural dyes when natural dyes application occurred. During usage and storage of natural colored fabric, microorganisms such as fungi, mold, and bacteria, can grow on textile fabrics. Oil and sweat from a human being can improve microbial growth in the fabrics which leads to a decrease in the function, hygiene, and aesthetics of the textile material during the use while humidity will affect the microbial growth during the storage (Gao & Cranston, 2008). Fungi, mold, and bacteria take the responsibility of

these problematic issues. Fungi and mold can cause discoloration, stains, and damage to fabric fiber. In addition, bacteria can cause unpleasant smells and stickiness when using cloth. To meet customer satisfaction, the product is optimized by suppressing the microorganism growth on the fabric, either on the coloring process or post-coloring process.

As mentioned previously, several natural dyes have antibacterial properties from its components, such as gambir and neem leaves. However, this antibacterial component has low purity in the natural dyes extract. It is compounded by the presence of impurities that can lead to the growth of microorganism (Paul, 2014). As an example of gambir natural dyes, even though it has tannin content which has antimicrobial properties, the inhibitory properties are less effective. To study the addition of antifungal agents, several established antifungal agents are investigated namely chitosan, *aloe vera*, and formaldehyde. The antifungal mechanism in the presence of chitosan is strongly believed as fungistatic rather than fungicidal (Raafat, Von Bargen, Haas, & Sahl, 2008). It involves the interfering the fungal growth by cell wall morphogenesis mechanism which is similar to the bacteria cell (El-Ghaouth et al., 1994). Furthermore, a microscopic observation that is impeding on the enzyme's activity which is responsible for fungus growth had been reported with the diffusion of chitosan oligomers inside the hyphae (Eweis, Elkholy, & Elsabee, 2006). Chitosan has many benefits in pharmacy and biomedical, and its biological activity is antioxidant, antitumor, antifungal, and antibacterial so can be used as antifungal additive (Younes & Rinaudo, 2015). Aloe vera contains both mono and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins, and mineral (Saniasiaya, Salim, Mohamad, & Harun, 2017). The studied by Arunkumar, S. et al. (Arunkumar & Muthuselvam, 2009) had discovered 26 kinds of bioactive phytochemical compounds in aloe vera extract using several solvents. Another study conducted by Cock I.E. (IE, 2008), anthraquinones, dihydroxyanthraquinones, saponins, acemannan, and aloe-emodin is responsible for direct and indirect antimicrobial properties. *Aloe vera* is rich in enzymes, amino acids, mineral, vitamin, polysaccharides, and other compounds that efficient as an anti-inflammatory, antifungal, antibacterial and support cell regeneration (Sharrif Moghaddasi & Res, 2011). Formaldehyde which known as a mono-aldehyde has sporicidal and virucidal activity, but it works slower than other aldehyde compound used as antimicrobial, glutaraldehyde, (Rubbo, Gardner, & Webb, 1967). Formaldehyde is a disinfectant that can inhibit microbial activities. In this study, the antimicrobial capability would be presented by the antifungal ability of the substances because *Aspergillus niger* was used as the microorganism. *Aspergillus niger* is well known for its growth ability in a wide range of climatic and environmental conditions. This fungus is widespread throughout the world biome such as land, agro-ecosystem, plants, animals, rocks, water, fossils, to humans (Abdel-Azeem et al., 2016). Because of its nature, Aspergillus niger is the most discovered fungus on the cloth (Lang, 2001). Therefore, to improve the quality of the fabric, an attempt was performed to add antifungal additives as finishing agents in this study.

METODELOGI PENELITIAN

Bahan

The materials used in this study were purchased and obtained from several places as seen in Table 1.

Table 1. Materials used in this study

Material	Source	Brand	Purity
Gambir powder	Local market - Yogyakarta	Bulk	-
Aloe vera	Local market - Yogyakarta	Bulk	-
Chitosan	Chem-mix Pratama - Yogyakarta	Bulk	-
Formaldehyde	Workshop Batik Indigo - Yogyakarta	Bulk	35%
Alum ($Al_2(SO_4)_3$)	Workshop Batik Indigo - Yogyakarta	Bulk	-
Cotton fabric	Workshop Batik Indigo - Yogyakarta	Bulk	-
Acetic Acid	Chemical Engineering Laboratory - UGM	Merck	96%
Distilled water	Chemical Engineering Laboratory - UGM	Bulk	-
Aspergillus niger (LMB01)	Microbiology Laboratory - UGM	-	-
Potato Dextrose Broth (PDB)	Microbiology Laboratory - UGM	Sigma-Aldrich	-

Metode

Several steps were conducted in this study. Those steps were natural dyes preparation from gambir powder, antifungal addition, and bacterial growth analysis. Natural dyes preparation was prepared by dilute gambir powder in distilled water with concentration 6 g/ 125 mL at 100°C for 2 hours. Afterward, the solution was filtered to obtain the liquid phase as natural dyes. The coloring process was performed at 95°C for 30 minutes by immersing the cloth in natural dyes solution. Antifungal agent addition was observed in three different steps, namely post-coloring, pre-coloring, and mixed-coloring using those natural dyes, the suffix of the method indicated the addition of antifungal agent during the coloring process. The weight of cloth was measured both before and after antimicrobial treatment to calculate the % color fastness according to this equation:

$$\%\ color\ fastness = \frac{IW - FW}{IW} \times 100 \qquad (1)$$

where *IG* and *FG* describe initial weight and final weight of cloth after treatment

The addition of antifungal agents were varied depend on the substance addition, chitosan varied in 1, 3, and 5 g/L in 2% acetic acid solution; aloe vera gel concentration was 2, 5, and 8 g/L in water; and formaldehyde used 5, 15, and 25 ppm in water. Coloring process was held for 30 minutes at 95°C with the addition of alum afterward as much as 20 g/L. Chitosan and aloe vera concentration as much as 3 g/L and 5 g/L respectively were found to be sufficient to inhibit fungus growth [23]. Thus, the concentration of chitosan and aloe vera have varied those value. Furthermore, the limitation of formaldehyde usage in the cloth is below 75 ppm [24].

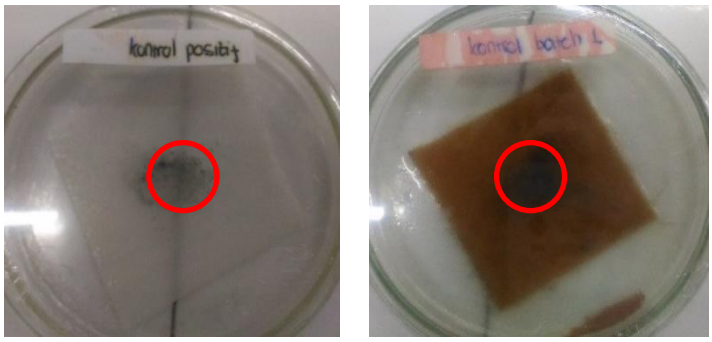
The initial weight of cloth was measured before the application of the antimicrobial agent for the implementation of the antimicrobial agent. The process was carried out at a temperature of 95°C which contains a solution of antimicrobial reagent. Fabric samples and antimicrobial additive solutions performed at 1:100 to liquor ratio (MLR) material, i.e., for antimicrobial treatment 1 gram of cotton cloth needs to be used for 100 mL antimicrobial additives. The process was carried out for 30 minutes at the pH 5 with the addition of 2% acetic acid solution. The solution was cooled at 30°C and washed using warm water followed by rinsed using cold water. In the mixed-coloring process, the same amount of natural dyes solution was mixed with the antimicrobial reagent in the presence of the cloth sample. The cloth was dried and weight to identify the antifungal agent addition.

The fungal growth was analyzed using Potato Dextrose Broth (PDB) growth medium with addition of Aspergillus niger. Twenty four grams of PDB was diluted into 1 L of erlenmeyer flask and autoclaved at 121ºC and 2 atm for 30 min. Furthermore, the inoculum of Aspergillus niger was made by suspending spores into a sterile ringer solution, NaCl 0.85% v/v solution with distilled water solvent which was sterilized using an autoclave. The cotton fabrics with diameter 8 cm which had been prepared were dripped with

PDB medium as much as 0.75 μL and Aspergillus niger suspension using inoculation needle. The colony analysis of fungal Aspergillus niger was conducted after three days of the inoculation process. In this study, the diameter of the fungal colony was fully measured. Furthermore, the study of the significance test was conducted by ANOVA® software to describe the antifungal activity of each antifungal agent.

Hasil Penelitian dan Pembahasan

As the basis, both dyed (batch control sample) and undyed cotton fabric (positive control sample) using gambir extract were analyzed the fungal growth without the addition of antifungal agents. The image of the inoculation plate for positive and batch control sample are shown in Fig. 2 (a) and (b), respectively. Fungal colony diameter measurement for positive and batch control sample was presented in Table 2 with a qualitative observation on the fungal growth. The thickness of the fungal colony should be measured to differentiate the spread and dense growth. Even though the measurement was performed qualitatively, the thickness of the fungal colony was comparable on every treatment. The fungal colony was increased in the batch control sample compared to the positive control sample. Hypothetically, the increasing in nutrition amount of batch control sample would cause higher fungal growth by dyeing process using gambir extract. Catechin in gambir extract belongs to the tannins group and *Aspergillus niger* which can produce tannase enzyme. Tannase or tannin acyl hydrolase can catalyze the hydrolyzation process for hydrolyzable tannins like Tannat acid, methyl gallate, ethyl gallate, n-propyl gallate, and isoamyl gallate into glucose and gallic acid (Rodrigues, 2016). Gallic acid catalyzes the second step for tannic acid degradation.



(a) Positive control sample (b) Batch control sample

Figure 2. Fungal growth test result in control sample

Table 2. Antifungal test

Control Sample	The diameter of the fungal colony, mm			Spore thickness*
	Test 1	Test 2	Average	
positive control sample	16	15	15.5±0.7	++
dyeing batch control	17	17	17.0±0.0	+++

*spore not so thick (+), thick (++), very thick (+++)

Table 3. Fungal growth test, variation in type and antifungal concentration in post-colored method

Antifungal agent		The diameter of the fungal colony, mm			Spore thickness*	% Color Fastness
Type	Concentration	Test 1	Test 2	Average		
Formaldehyde	5 ppm	12	10	11.0±1.4	++	0.73

	15 ppm	12	9	10.5±2.1	++	0.90
	25 ppm	12	9	10.5±2.1	+	0.36
	1 g/L	15	10	12.5±3.5	+++	1.01
Chitosan	3 g/L	11	10	10.5±0.7	++	1.04
	5 g/L	10	8	9.0±1.4	+	1.40
	2 g/L	16	12	14.0±2.8	+++	1.39
Aloe vera	5 g/L	17	12	14.5±3.5	++	1.03
	8 g/L	14	11	12.5±2.1	+	1.65

*spore not so thick (+), thick (++), very thick (+++)

Fungal growth tests have been carried out in the same way for control of samples and other samples that have been added to antifungal agents. The results of variations in the amount and agent concentration of antifungals are shown in Table 3 and for variations in the method of adding antifungals in Table 4. Compared with the control diameter of 17 mm, all types and concentrations for antifungal agents showed a tendency to reduce the fungal colony. The treatment using antifungal agents such as formaldehyde, chitosan, and *aloe vera* can generally inhibit the growth of *Aspergillus niger* fungi. In this case, *Aspergillus niger* can still grow in all samples and produce spores for reproductive purposes. It is caused by the concentration of antifungal agents attached to the fabric which is very small due to the small adsorption ability of cotton fabrics and competes with the absorption of natural dyes. For the record, *Aspergillus niger* has great growth ability in all environmental conditions, so antifungal agents in cotton cloth samples cannot work according to their function.

Percentage of color fastness, seen in Table 3, briefly explains the amount of antifungal concentration which was added at dyed cotton fabrics. It can be seen that the larger the antifungal agent concentration, the higher the percentage of color fastness occurred. It could be occurred by the dissolved of natural dyes into the solution due to the acidic condition applied when the addition of the antifungal agent performed. Furthermore, the diffusion of the antifungal agent was believed to occur from the liquid phase into the solid phase, cotton fabrics, because of the reduction of dyes. The higher % color fastness gives a better antifungal addition. Hence, the antifungal addition should be examined using fungal growth test.

The mass difference is shown in Table 4 also represents the amount of antifungal concentration addition. This amount contains two factor namely natural dyes and antifungal agent addition. For post-colored method, there was an addition of 0.1461 grams of gambir natural dyes, but after undergoing treatment for antifungal addition, a mass reduction of 0.0643 grams occurred. The pre-coloring method had the mass of antifungal and gambir natural dyes as much as 0.0170 grams and 0.1166 grams, respectively. The decay of antimicrobial substances during the fabric coloring process is assumed to be ignored because chitosan has resistance to dissolve when had been bound with the fabrics as stated by (Ammayappan & Moses, 2009). In the last method, mixed-coloring, the total amount of antifungal and gambir natural dyes was 0.0894 grams. The absorption of the antifungal agent is believed lower than gambir natural dyes due to the data of post- and pre-coloring method.

Table 4. Fungal growth test for cotton fabric with antifungal agent, variation in method of application

Antifungal agent's method of application	The diameter of the fungal colony, mm		Spore thickness* The mass difference on the cotton fabric
	Test 1	Test 2	

Post-coloring	11	10	++	0.0818
Pre-coloring	27**	12	+	0.1336
Mixed-coloring	15	12	+++	0.0894

*spore not so thick (+), thick (++), very thick (+++) ** excluded in the ANOVA analysis

For each type of antifungal agent used there was a trend of data for reducing in the fungal colony for increasing in antifungal agent concentration. It supports the hypothesis in which the higher the antifungal agent concentration, the higher antifungal agent concentration would be attached to the cotton fabric. Thus, the ability to inhibit fungal growth would be better. Qualitatively, increasing in antifungal agent concentration also results in reducing spore thickness. From three types of antifungal agents, chitosan shows the best of inhibition activity. The ability as antibacterial and antifungal of chitosan has been proven in many kinds of product (Alonso et al., 2009). Afterward, chitosan as much as 3 g/L would be used as an antifungal agent to evaluate the effective method. Post-coloring addition was the best method to replenish antifungal ability on the cotton fabrics due to a minimum diameter of spore obtained which can be seen in Table 4.

Table 5. Significance test using One-Way ANOVA and LSD calculation

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*Fcrit*
Between Groups	134.02	13.00	10.31	2.37	0.07	2.58
Within Groups	56.50	13.00	4.35			
Total	190.52	26.00				

Table 6. Significance test using One-Way ANOVA and LSD calculation

MS	4.35
t table	2.16
alpha	0.05
DFE	13.00
r	2.00
LSD value	4.50

A one-way ANOVA compiled with Least Significant Difference (LSD) calculation was being used to analyze the significance of fungus diameter statically. The significance of the diameter could be used to evaluate and optimize the best parameter as an antifungal agent. The output from the one-way ANOVA test and LSD calculation is presented in Table 5 and Table 6.

The LSD value, developed by Fisher in 1935, is the difference to know the significance of every compared value. The significance group can be seen in Table 7. There are two significance group, namely subscript (a) and (b). Group (a) has a significant difference against a group (b). It indicates that most treatments have a significant difference with the control except the addition of 2 and 5 g/L *Aloe vera*. Although the significance of the colony diameters lies on the same group, the addition of chitosan 5 g/L on the post-coloring process would be the best condition to be applied as an antifungal agent as statistically the lowest value. Furthermore, the addition of chitosan is more effective in the same concentration used compared with *Aloe vera* addition. Formaldehyde also has

an excellent antifungal activity, but formaldehyde has a limitation of the usage because of its toxicity. The effect of chitosan addition as an antifungal agent should be further examined for another natural dye to give a generalization of chitosan utilization, although, many utilization of chitosan as antifungal had been discovered by many researchers.

Table 7. Significance tesult of bacterial growth in the fabrics

Antifungal agent	Concentration	Avg. The diameter of the fungal colony, mm
Positive control	–	15.5[b]
Dying batch control	–	17.0[b]
Formaldehyde	5 ppm	11.0[a]
Formaldehyde	15 ppm	10.5[a]
Formaldehyde	25 ppm	10.5[a]
Chitosan	1 g/L	12.5[a]
Chitosan	3 g/L	10.5[a]
Chitosan	5 g/L	9.00[a]
Aloe vera	2 g/L	14.0[b]
Aloe vera	5 g/L	14.5[b]
Aloe vera	8 g/L	12.5[a]
Post-coloring	3 g/L*	10.5[a]
Pre-coloring	3 g/L*	12.0[a]
Mixed-coloring	3 g/L*	13.5[a]

*chitosan as an antifungal agent

Kesimpulan

To conclude, three antifungal agents, namely chitosan, *Aloe vera*, and formaldehyde, were successfully investigated on the application of gambir as natural dyes. Statistically, only 2 and 5 g/L of a*loe vera* were not adequate to suppress *Aspergillus niger* growth proved by the significance value of colony diameter of all conditions based on a one-way ANOVA and LSD calculation. Furthermore, the optimum condition was reached in addition of 5 g/L chitosan on post-coloring addition of antifungal agent application with a diameter of the fungal colony as much as 9.00 mm with the statistically lowest value. This work hopefully could help the utilization of gambir as natural dyes by addition chitosan as a significant antifungal agent, but further study should be constructed regarding on chitosan as an antifungal agent for natural dyes utilization to compromise with the generalization of chitosan.

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