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Research Article

Morphology Analysis of Hair Photoinduced and Chemical Damaged After Treatment with Sappan Wood (*Caesalpinia sappan* L.) Hair Tonic using SEM

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Abstract

Exposure to sunlight for a long time and repeatedly can also cause chemical and physical damage to human hair. Clinically, microscopic analysis using a scanning electron microscope (SEM) can assess hair damage by identifying the characteristic morphology of hair damage. This study aims to analyze the morphology of damaged hair chemically induced using 3% H_2O_2 and photoinduced UVB radiation before and after applying sappan wood (*Caesalpinia sappan*) hair tonic. The active ingredients used were ethanol extract, ethanol fraction, and chloroform-methanol fraction of *C. sappan*, which contains an antioxidant compound. *Caesalpinia sappan* simplicia was macerated using 96% ethanol and then partitioned using *n*-hexane. The ethanol fraction was then applied using vacuum column chromatography using chloroform : methanol (5 : 1) as eluent. The extracts and fractions were then formulated into hair tonic preparations. For SEM analysis, hair samples were coated with a sputter gold coater machine and divided into five treatments: undamaged hair, damaged hair with 3% H_2O_2 and UVB rays as a positive control, and treatment I, II, and III, in which the hair was damaged with 3% H_2O_2 and UVB rays respectively, then FI, FII, and FIII were applied, respectively (2000x magnification). Damaged hair with UVB induction shows moderate damage, and 3% H_2O_2 shows moderate to severe damage. The results in the treatment group show that the three hair tonics coated the hair cuticle, indicating an interaction with the hair fiber, and modified the cuticle by coating the cuticle.

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INTRODUCTION

Human hair functions to protect external factors, produce sebum and thermoregulate[1]. Hair consists of a follicle and a hair shaft. Hair follicles affect hair generation[2]. Using hair cosmetic products can harm the hair and scalp[3], affecting the smoothness of the hair surface, hair texture and thickness, and hair porosity[4]. Exposure to sunlight for a long time and repeatedly can also cause chemical and physical damage to human hair[5]. So, hair care products are needed to improve hair health and prevent further damage. Future hair care product developments emphasize treating damaged hair and maintaining hair morphology[6].

Sappan wood (*Caesalpinia sappan* L.) is a typical plant of West Kalimantan and several regions in Indonesia, such as East Java, West Java, Aceh, North Sumatra, and South Sulawesi. It contains the bioactive compounds brazilin ($C_{16}H_{14}O_5$) and

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brazilein[7,8]. Brazilin is the main component of *C. sappan*[9,10], which has the potential to develop as a medicinal compound with applications in cosmetics[11].

Sari *et al*.[12] state that hair tonic from ethanol extract, ethanol fraction, and chloroform fraction from *C. sappan* provide hair growth and antioxidant activity. The hair tonic formula was also proven safe after safety tests using the primary irritation test method and the Hen's egg test–chorioallantoic membrane (HET-CAM)[13]. In this research, an examination of hair morphology will be carried out using a hair tonic formula made from *C. sappan*.

Clinically, microscopic analysis such as the scanning electron microscope (SEM) can assess hair damage by identifying the characteristic morphology[14,15]. This method allows observations not only on the outer surface but also on the inside of the hair[16]. Hair damage methods can be done through exposure to UVB rays and chemical damage[17]. H_2O_2 is a chemical ingredient in hair styling cosmetics, such as curlers and hair dyes[18]. Meanwhile, UVB rays are sunlight radiation that causes hair damage[19]. However, based on a literature search, there needs to be more research data focused on hair morphology analysis as a qualitative test for hair care products, especially hair tonics. Therefore, this study aims to analyze the morphology of chemically induced hair using 3% H_2O_2 and photoinduced damaged hair using UVB radiation before and after applying hair tonic from *C. sappan* using SEM.

MATERIALS AND METHODS

Materials

This study used several materials, such as *C. sappan* from Sungai Pangkalan, Bengkayang Regency, West Kalimantan. 96% ethanol, *n*-hexane, and chloroform from Merck (Darmstadt, Germany), menthol, H_2O_2, propylene glycol, sodium benzoate, polysorbate 80, citrus oil, and butylhydroxytoluene (BHT) were purchased from Alkamid Co. (St. Tehran, Iran), distilled water was purchased from Bratacho (Banten, Indonesia), silica gel 60 (230-400 mesh) were purchased from Merck (Darmstadt, Germany). The tools used in this study were analytical balance scales (Sartorius BL 210S), filter paper, maceration vessel, rotary evaporator (Dragon LAB RE-10 Pro), vacuum column chromatography, UVB lamp 20W (Phillips), sputter coating machine, and SEM (Hitachi SU-3500).

Methods

Sample collection and processing

Caesalpinia sappan was determined at LIPI, Bogor, Indonesia (1333/IPH.1.01/If.07/V/2017). About 2 kg of *C. sappan* was carried out by wet sorting. After that, *C. sappan* was shaved, dried, and powdered. A total of 2 kg of *C. sappan* powder simplicia was macerated using 96% ethanol. Partitioning was carried out using *n*-hexane and ethanol (1 : 1), followed by separation using vacuum column chromatography using chloroform-methanol (5 : 1)[20]. Meanwhile, the hair tonic formula from our previous research[12] is presented in **Table I**.

Table I. Hair tonic formula[12].

Materials	Formula (%)			Use
	FI	FII	FIII	
Ethanol extract of *C. sappan*	0.1	-	-	Active ingredient
Ethanol fraction of *C. sappan*	-	0.1	-	Active ingredient
Chloroform-methanol fraction of *C. sappan*	-	-	0.1	Active ingredient
Menthol	0.5	0.5	0.5	Flavouring agent
Propylene glycol	7	7	7	Co-solvent
Polysorbate 80	1	1	1	Surfactant
BHT	0.1	0.1	0.1	Antioxidant
Natrium benzoate	0.5	0.5	0.5	Preservative
Isopropyl alcohol	60	60	60	Solvent
Citrus oil	0.1	0.1	0.1	Fragrance
Aquadest	Ad 100	Ad 100	Ad 100	Solvent

Hair morphology analysis

Hair sample preparation: This process used the method by Cao *et al*.[21] and Davis *et al*[16]. Hair samples were collected from a volunteer (ethical clearance No. 2796/UN22.9/TA/2021 from Universitas Tanjungpura) to ensure uniform hair condition and minimize differences in results due to volunteer variations. Hair samples were cut using stainless steel scissors, kept from light, and stored at room temperature. Immediately before treatment, the hair samples were washed using absolute ethanol for 20 minutes to remove oil on the hair surface, then dried at room temperature[22], cut separately using stainless steel scissors, and weighed accurately to 0.2 to 0.3 g.

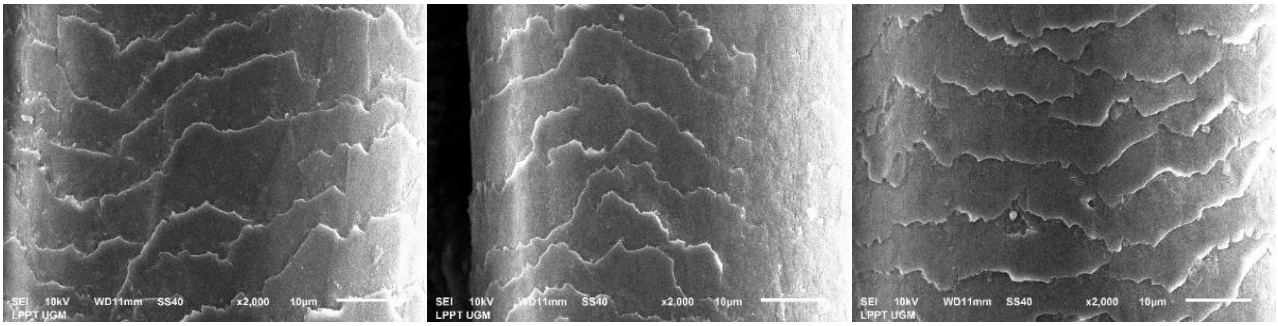
Hair samples morphology observation using SEM: Hair samples were divided into five treatments:

1. Negative control (C-): hair without damage.
2. Positive control (C+): damaged hair with 3% H_2O_2 and UVB light, respectively.
3. Treatment I (PI): damaged hair with 3% H_2O_2 and UVB light, respectively, then FI applied.
4. Treatment II (PII): damaged hair with 3% H_2O_2 and UVB light, respectively, then FII applied.
5. Treatment III (PIII): damaged hair with 3% H_2O_2 and UVB light, respectively, then FIII applied.

The UVB lamps simulated exposure to sunlight. Hair samples were irradiated using simulated 20W UVB lamps at a 20 cm distance for 24 hours. All experiments were carried out at room temperature[21]. Chemical hair damage was caused by bleaching using 3% H_2O_2 for four hours[23]. All treatment groups were given treatment every 24 hours for 12 times treatments. Before the SEM examination, the hair samples in all treatments were first coated with a sputter gold coater machine to improve the quality of the image samples. Each sample was visualized under x2000 magnification[16,23]. Then, it was examined under SEM for physical characteristics and hair morphology[14,24].

RESULTS AND DISCUSSION

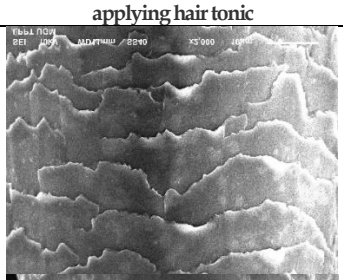
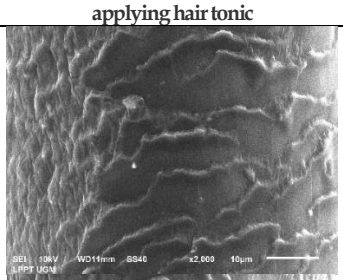
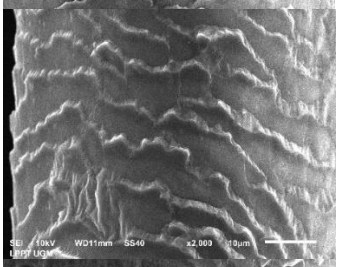
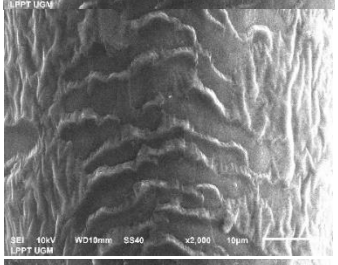
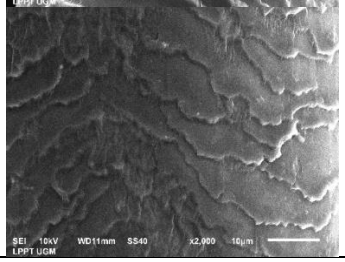
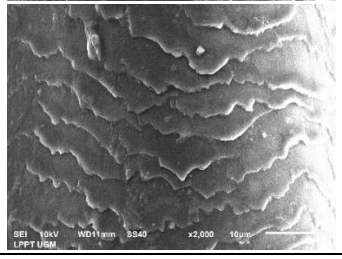
Hair characterization was performed with Hitachi SEM micrographs (type SU-3500) to generate microscopic images of hair[16]. A hair sample is prepared, washed, classified, and cut. The hair section attached to the conductive tape is coated with gold film coater using a vacuum sputtering machine[23] at 10 kV[16]. Scanning was carried out on several sides to ensure that the changes in the surface of the hair sample looked uniform[16]. Researchers used the degree of cuticle damage as a criterion for assessing hair damage. Weak damage occurs if there are obvious cracks in the cuticle, moderate damage if there are severe cracks and curvature, and serious damage if the cuticle is almost missing or completely missing[16]. Scanning uses SEM to assess hair damage by looking at the morphology of the hair surface[22]. The results obtained from the analysis using SEM are presented in **Figure 1** and **Table II**. The ends of the hair, as shown in C-, show a smooth, regular, and unraveled cuticle appearance despite the appearance of natural serrated edges. The C+ shows raised, torn, and sharply serrated cuticle edges. The cuticle almost disappears with C+ with 3% H_2O_2 induction[16]. For C+ with UVB induction, perimeter cracks develop on the cuticle scales, and the cuticle scales lift[21].



a **b** **c**

Figure 1. Hair cuticle morphology using SEM on C+ and C- with 2000x magnification: (**a**) undamaged hair (C-) shows a smooth appearance and no cuticles lifted, (**b**) chemically damaged hair using 3% H_2O_2 (C+) shows cuticles lifted, torn, and almost disappear, and (**c**) photodamaged hair using UVB (C+) shows cuticles lifted and torn edges.

Table II. Hair cuticle morphology using SEM in each hair treatment with 2000x magnification.

Treatment group	Hair morphology after chemical damaged using 3% H_2O_2 and applying hair tonic	Hair morphology after photodamaged using UVB and applying hair tonic
PI		
PII		
PIII		

Note: PI: damaged hair each with 3% H_2O_2 and UVB light respectively then applied FI; PII: hair damaged with 3% H_2O_2 and UVB light respectively then applied FII; and PIII: damaged hair with 3% H_2O_2 and UVB light respectively then applied FIII.

The hair surface for most of the samples shows a smooth cuticle, although it appears to have some serrated edges[25]. The smooth cuticle of the hair is shown with fringe scales that present a more regular, even, and unbroken contour[24]. The surface layer of the hair after application using hair tonic is visible and can be distinguished from SEM. No visible layers were on untreated hair (C-), which was damaged with UVB light and 3% H_2O_2[16]. However, after treatment with the hair tonic formula, the hair is completely coated. This correlates with the softness of the strands after applying the hair tonic[23]. Using 3% H_2O_2 as a chemical inducer represents using hair cosmetics such as dyes and hair curlers. As one of the components of permanent oxidative hair dye, 3% H_2O_2 can cause cuticle lifting and opening/eroding. As a result, it allows penetration of 3% H2O2 molecules into the cortex[16].

Hair consists of fibers composed of keratin (protein)[21]. Human hair usually has a cuticle located on the outermost layer of protein with a vital function: protecting the cortex so that the shine and texture of the hair are maintained[16,25]. Environmental factors such as UV irradiation and environmental pollution harm human hair[21]. UV irradiation is one of the factors that cause hair damage, both physically and chemically. Using cosmetics such as hair coloring and perm causes chemical hair damage[23]. Hair damage from UV irradiation is called photodamage. UV irradiation causes a decrease in the strength of the hair fiber structure, causing degradation of amino acids and proteins as well as lipid and melanin peroxidation[21]. Cystine plays an essential role in the decomposition of hair fibers, ultimately affecting their mechanical properties and causing the loss of cystine content[26]. It is indicated by the hair's physical appearance, which is rough and fades in color[21].

In our previous study[12] regarding the formulation of hair tonic from *C. sappan*, the pH of *C. sappan* hair tonic produced was 7. At an alkaline pH, hair care products can open hair's ability to clean oil, dirt, and dust tucked in the hair membrane (cuticle) so that hair can be clean[21]. Hair morphology using SEM in three treatments showed the presence of a hair tonic layer on the hair surface. This layer indicates the protection of the cortex and hair cuticle[23]. Antioxidant compounds contained in ethanol extract, ethanol fraction, and chloroform-methanol fraction of *C. sappan* can provide activity to prevent photo-oxidation of hair keratin due to UVB radiation. Prevention of photo-oxidation of cystine bonds into stearic acid in the hair cuticle can prevent hair damage[12]. **Figure 1** shows that chemical induction using 3% H_2O_2 allows the hair cuticle to erode, causing almost complete loss of the cuticle. Because the hair fiber consists of dead cells, if some of the substructures of the cuticle,

such as the A-layer and exocuticle, are eroded, it is difficult to carry out biological hair restoration[18]. However, we can repair the hair physically by using a hair tonic; one of its functions is to coat the hair cuticles, cover or fill in damaged cuticle areas[23], and fill the cuticle edges uplifted and torn[18]. The same thing is obtained by using shampoo and conditioner products, where the coating of the hair surface improves the feel of the hair[27].

CONCLUSION

Scanning electron microscope makes it possible to evaluate hair morphology and changes that occur on the hair surface after induction using 3% H_2O_2 and UVB rays as well as hair morphology that has been applied to hair tonic from *C. sappan*. Chemical-damaged hair using 3% H_2O_2 showed severe damage. Photodamaged hair using UVB rays showed moderate damage. The hair tonic application to the hair shows an interaction with the hair fiber so that it modifies the cuticle by coating the cuticle. However, to get a lighter layer of hair tonic, it is necessary to apply hair tonic intensively and regularly. It can repair damaged hair and allow the formation of a more uniform thin layer of hair tonic.

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AUTHORS' CONTRIBUTION

DYS: study conception and design; **DYS** and **IA**: data collection; **DYS**, **RW**, and **IA**: analysis and interpretation of results; **DYS**: draft manuscript preparation. All the authors approved the final version to be published.

DATA AVAILABILITY

The authors confirm that the data supporting the findings of this study are available within the article. Raw data supporting this study's findings are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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