The exploration of banana bunch as a new vegetable tanning agent

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Abstract. Recently, the green tannery is an issue that has been developed because it is known as a contributor of hazardous waste. The substitution of mineral tanning agents, such as chrome, with vegetable tanning agent is the best choice. Banana bunch has the potential to be an environmentally friendly tanning agent. This study aims to explore the banana bunch as a vegetable tanning agent for rabbit skin. The banana bunch was extracted using ethanol. The tannin quality of banana extract was determined by both qualitative and quantitative methods. The quality of leather tanned by the banana bunch was determined by chemical and physical properties. The qualitative results of the tannin content showed that the banana bunch had the tannin compounds, while quantitatively the tannin content was 4.1%. Banana bunch has an inhibitory potential, indicating that the banana bunch can be an anti-bacterial. Besides, the chemical analysis showed that the tanning by banana bunch had a water content of 14.90±0.01%, a water-soluble content of 3.55±0.01%, the pH value of 3.42±0.03, and the degree of tannage of 52.47±2.29%. Also, the physical quality of rabbit skin showed the tensile strength of 302.00 kg/cm² and the elongation of 174.12±6.44%. Based on these results, it shows that the extract of the banana bunch has the potential as a vegetable tanning agent.

1. Introduction
The leather industry is included in the sustainable industry. Wu [1] stated that this industry is one of the most effective ways to recycle raw hides for a by-product of animal husbandry, such as skin and hide. The tannery and leather industry is one of the development industries in Indonesia, along with the Indonesian growing fashion industry. Based on BPS [2], the growth of the leather, leather goods, and footwear industry has recently increased by 18.78%. However, this industry is not without problems. The tannery is a conventional industry which contributes hazardous waste and harms the environment [3]. It is due to the tanning agent derived from dangerous materials, such as chromium. Environmentally friendly tanning agents have begun to develop to overcome these problems. One of the best ways is to use a vegetable tanning agent. The requirement is to have tannin as a crosslinking agent in the hydroxyl group on the skin. Generally, vegetable tanning material commonly used in the tanning industry is mimosa, which is derived from acacia wood. Unfortunately, these chemicals are still imported and expensive. Also, acacia wood is an endangered plant [4,5].

Recently, the supply of vegetable tanning resources is imported from abroad and does not meet the local needs, so that the development of the leather industry is limited. Hence, finding more tannin resources to meet future industrial production needs is an essential goal [6]. The banana bunch contains tannin that can be used as a tanning agent. Banana bunch is known as a market waste that has not been
handled properly. The banana bunch is easy to obtain, inexpensive, sustainable, and environmentally friendly so that it can reduce the use of hazardous chemicals in the tanning process for leather products [7]. This study aims to investigate banana bunches as a vegetable tanning agent. Therefore, the use of the banana bunch as a vegetable tanning agent is interesting to explore.

2. Materials and methods

2.1. Extraction of tannins
Three banana species (Kepok, Raja, and Ambon) were extracted to get the tannins according to China [8]. The collected banana bunches were dried under the shed for seven days and cut into small chips. The chipped pieces were ground in a milling machine. About 10 g of milled barks were soaked in 200 mL of ethanol 70% in a glass beaker covered with an aluminum foil. The mixture was placed on a water bath maintained at 70°C for 60 minutes. The sample mixture was stirred using an overhead stirrer connected to the beaker’s opening through a small hole made on the aluminum foil. The extraction process continued for 4 hours, and then the filtrates were collected.

2.2. Qualitative analysis of tannins
A qualitative analysis of tannin in the banana bunch extract was done according to Ezeonu [9] by using ferric chloride, gelatine protein, ammonia, and chloric acid as the indicators. 1 mL of banana bunch extract was taken and put into each of three different test tubes and 10 mL of distilled water was added to each tube, followed by the addition of 2 drops of ferric chloride. The appearance of green coloration which disappeared on standing showed the presence of tannins.

The principle of the gelatine test for tannin quality was carried out by taking 1 mL of banana bunch extract and putting it into each of the 3 test tubes, then 1% gelatine solution was added and diluted with distilled water. The sedimentation formation indicated the presence of tannins. Besides, the principle of the tannin test with ammonia was carried out by taking 1 mL of banana bunch extract and putting it into each of the 3 test tubes. The ammonia was added then exposed to the air. The appearance of green coloration which disappeared on standing showed the presence of tannins. Next, the principle of the chloric acid test for tannin quality was carried out by taking 1 mL of banana bunch extract and putting it into each of the 3 test tubes, then 3 drops of chloric acid were added to each tube.

2.3. Total tannins (quantitative analysis)
The total tannin was determined quantitatively using Guo [6]. The extract of banana bunch was diluted with distilled water 20 times. 1 mL of the sample was added to 0.5 mL of Follin Denis reagent and 1 mL of 20% sodium carbonate then homogenized. The results were measured with a spectrophotometer with a wavelength of 720 nm. The total tannin content was expressed as the number of mg of tannic acid equivalent per gram of extract.

2.4. Antibacterial activity assay
The antibacterial assay method was administered according to Udkihuyati [10]. Besides, Mueller Hinton Agar (MHA) was prepared for the Staphylococcus aureus growth medium in a concentration of 1.5 x 108 CFU/mL. Fifty microliters of banana bunch extract and 50 μL of chloramphenicol (as the positive control) were inoculated in a petri dish. The petri dish was incubated in an incubator for 24 hours at 37°C. Next, antibiotic chloramphenicol as a positive control was dissolved in dimethyl sulfoxide (DMSO) with a concentration of 100 ppm. The measured zone of inhibition is the radius in mm, which is the clear area around the well.

2.5. Tanning process
The dried banana bunch tannin extracts were used in the tanning of rabbit skins, and the tanning process is displayed in table 1. The steps in the tanning process: soaking to restoring the water content in the skin; liming-unhairing to remove the hair and soluble protein; deliming to remove the lime; bating to
remove the globular protein; pickling to prepare the skin to enter the tanning process by decreasing the pH; tanning to make the skin more stable; neutralizing to remove some of the remaining free acids that came from acidification or when aging; fatliquoring to lubricate hydrated leather fibers during the tanning process; and the last was fixation.

**Table 1.** Technological parameter in tanning process of rabbit skins.

| Process                        | Materials            | Dosage (%) | Temperature (°C) | pH     | Time/min |
|-------------------------------|----------------------|------------|------------------|--------|----------|
| Soaking                       | Water                | 300        | 25               |        |          |
|                               | Alkyl sulfate        | 2          |                  |        |          |
|                               | Sodium hydroxide     | 1          | 12               | 90     |          |
|                               | Bactericide          | 0.03       |                  |        |          |
| Liming-unhairing              | Water                | 150        |                  |        |          |
|                               | Sodium sulfide       | 0.3        |                  |        |          |
|                               | Calcium hydroxide    | 1          | 25               | 60     |          |
| Deliming                      | Water                | 150        | 25               |        |          |
|                               | Ammonium sulfate     | 1          | 7                | 30     |          |
| Drain wash                    | Water                | 100        |                  |        |          |
| Bating and degreasing         | Pancreatic enzyme    | 0.3        |                  |        |          |
|                               | Degreasing agent     | 1          |                  |        |          |
| Pickling                      | Water                | 100        |                  |        |          |
|                               | Salt                 | 8          |                  |        |          |
|                               | Formic acid          | 1.5        | 3.8-4.0          |        |          |
| Tanning                       | Treatment 1: Banana bunch extract | 25 | 70               | 120    |          |
|                               | Treatment 2: Mimosa (Commercial vegetable tanning agent) | 25 | 70               | 120    |          |
| Neutralizing                  | Water                | 100        |                  |        |          |
|                               | Alkyl sulfate        | 1          |                  |        |          |
|                               | Sodium bicarbonate   | 0.3        | 25               | 6      | 30       |
| Retanning                     | Water                | 50         | 50               |        |          |
|                               | Bleaching syntan     | 4          |                  |        |          |
| Fatliquoring                  | Water                | 100        | 60               |        |          |
|                               | Synthetic oil        | 10         |                  |        |          |
| Fixation                      | Oxalit acid          | 1          | 3.8-4            | 40     |          |
|                               | Anti-fungi           | 0.03       |                  |        |          |

2.6. **Chemical characteristics of tanned skin**

The chemical characteristics of tanned skin consist of water content, water-solubility, and degree of tannage [11]. The water content was determined by weighing 5 grams of skin, then the skin was dried in a drying cabinet at 100°C ± 2°C. The resulting moisture content was expressed as a percent of the skin. The water solubility was determined by putting 50 ml of the extract in a glass cup and evaporated with a water bath until dry. Next, the sample was heated in a drying cabinet at 100°C ± 2°C until its weight remained. The result was a water-soluble substance and was expressed as a percent of the skin. The degree of tannage was the ratio between the content of tannin bound and the content of raw leather substances in %.
2.7. Physical characteristics of tanned skin
The physical analysis consisted of the tensile strength and the elongation. The tensile strength test was conducted by pulling the skin to reach the desired force or until the skin sample broke, expressed in N/mm². Elongation is the increase in skin length when the skin is stretched to break divided by its original length, expressed in % [12].

3. Results and discussion

3.1. Characteristics of banana bunch
The qualitative analysis results of tannin are shown in Table 2. This qualitative analysis is the initial stage to identify the tannin characters from natural materials, such as the banana bunch. The tannin identification was done by adding ferric chloride that resulted in a change in color from brown to dark green and murky. It was estimated that the phenol group in tannins could bind with ferric chloride to form the green color. Ezeonu [9] stated that the formation of dark green or dark blue in the extract after adding to ferric chloride causes tannin to form complex compounds with Fe³⁺ ions. The tests were done by adding gelatin solution that resulted in the sediment. Further, Thomas [13] and Zhang [14] pointed out that tannins can precipitate proteins, such as gelatin. The identification of banana bunch tannin extracts including condensed tannins or catechol with the addition of concentrated hydrochloric acid and the heating process formed a reddish-brown color. The catechol tanning agent is usually reddish-brown and will enlarge its molecules when heated in an acidic solution [15,16].

| Banana species | Qualitative Indicators for tannin | Tannin content (%) |
|----------------|----------------------------------|-------------------|
| Kepok          | Dark Green, Sediment, Green, Reddish brown | 4.10              |
| Ambon          | Green, Sediment, Green, Reddish brown | 1.34              |
| Raja           | Green, Sediment, Green, Reddish-slight brown | 0.44              |

Table 3. Physicochemical properties of tanned leather.

| Properties                      | Leather tanned by banana bunch | Leather tanned by mimosa |
|---------------------------------|--------------------------------|--------------------------|
| Water content (%)               | 14.90±0.01                     | 12.09±0.01                |
| Water-soluble content (%)       | 3.55±0.01                      | 7.74±0.04                 |
| pH value                        | 3.42±0.03                      | 4.41±0.02                 |
| Degree of tannage (%)           | 52.47±4.27                     | 57.00±1.27                |
| Tensile strength (kg/cm²)       | 302.00±2.29                    | 239.55±6.09               |
| Elongation (%)                  | 174.12±6.44                    | 173.68±4.88               |

Meanwhile, the quantitative analysis of tannin aimed to confirm and quantify the tannin content in natural ingredients. This analysis was carried out through the spectrophotometric method using the Folin denis reagent. The principle of the Folin denis method is the formation of blue complexes whose absorption can be measured in the visible area. According to Ezeonu [9], the formation reaction that occurs is the reduction of oxidation in which tannin as a reducing agent and Folin denis as an oxidizer. The oxidation reaction will form a blue color that can be read at maximum waves. In this study, there were three species of banana tested (Kepok, Raja, and Ambon). The total values of tannin content in each Kepok, Raja, and Ambon banana bunch consecutively were 4.10, 1.34, and 0.44%. The tannin content of the banana bunch is almost the same as Salix folium extract (4.0%) but smaller than that of
Acacia seyal bark extract (28.9%) [17]. Zhang [14] reported that the phenolic component in bananas varied depending on the banana variety. According to Griyanitasari [18], the tannin content of a tanning agent is considered good if the value is higher than 0.4% so that the tannin content in the banana bunch is considered suitable for use as a new vegetable tanning agent.

As a tanning agent, the banana bunch can also function as an anti-bacterial. This supports the aim of tanning the skin, which is protecting the skin from physical, chemical, and biological disorders, including leather damage by bacteria. The ability of banana bunch as an anti-bacterial can be seen from the presence of clear zones on the medium containing Staphylococcus aureus (figure 1). Staphylococcus aureus is the majority of the microbial species isolated from hides/skins [19]. Based on the observation results shown in figure 1, there is a clear zone with a diameter of 11.52 mm. It shows that the banana bunch was successfully inhibited bacterial growth. It is almost the same as Udkhiyat’s [10] results who used chitosan as an antibacterial agent (10-15 mm). This can be caused by the presence of secondary metabolites such as flavonoids, tannins, saponins, and alkaloids which act as anti-bacterial and influence the inhibition of bacterial growth.

3.2. Physicochemical properties of rabbit skin tanned by banana bunch
The chemical properties of the rabbit skin tanned by the banana bunch consisted of moisture content, water-soluble content, pH value, and degree of tannage. The moisture content of rabbit skin was 12.09%. Based on the Indonesian National Standards or SNI, this rabbit skin tanned by banana bunch was classified as a leather standard because the requirement of moisture content in leather is a maximum of 18% [11]. High water content (>18%) causes the quality of vegetable tanned skin to be easily overgrown by mold and inhibits the penetration of chemicals at a later stage. Nurbalia [19] explained that the water content of the skin is influenced by the nature of penetration of tanning agent material into the skin, wherein the tannin content begins to diffuse into the skin and attaches itself to the fibers. At the same time, it will release a certain amount of water.

Water-soluble content is a tanning material that is bound or not bound to collagen but is still soluble by water expressed in percent (%). The water-soluble substances in the treatment with banana bunch were 8.74%. This value approaches the skin standard in Indonesia based on SNI [11]. This obtained value is higher than skin tanned by Acacia xanthophloea extract (4.4%). Low water solubility indicates that leather tanned with tannins from the studied plants has good water resistance [8]. Covington [3] also stated that low solubility in water is shown by the level of water-soluble content. Besides, the pH value on the skin was 3.42. This obtained value approaches the skin standard based on SNI, which requires the skin to have a pH value ranging from 3.5 to 7 [11]. The pH of the skin tanned with vegetable material is acidic with a low pH, which can prevent the growth of bacteria [20]. In the meantime, the
degree of tannage of leather tanned with banana bunch was 52.47%. The result from this sample was almost similar to that of tanned leather with Accacia xanthophloea, 52.6% [8]. The value of the degree of tanning will affect the physical properties of the skin [21]. Table 3 shows the obtained elongation value by the tanned leather with the banana bunch of 174.12%. The high elongation is influenced by the process carried out that results in the stretch quickly, increase in length, and not easily torn, damaged, or changing shape [21]. The tensile strength of tanned leather with this banana bunch is 302 kg/cm². This value meets the Indonesian leather standard based on SNI, which is a minimum of 75 kg/cm²[11].

4. Conclusion
Based on the qualitative and quantitative tannin tests, the extract derived from Kepok banana had the best characteristic than other species (Raja and Ambon). Banana bunch extract could inhibit bacteria, such as Staphylococcus aureus. The rabbit skin tanned by banana bunch extract had almost the same chemical and physical properties as the skin tanned by the commercial tanning agent (mimosa) and is classified as the Indonesian leather standard (SNI). The banana bunch extract is considered suitable for use as a new vegetable tanning agent.

Acknowledgments
This work was jointly sponsored by the UP2M (Unit for Research and Community Services) Research Grant from Politeknik ATK Yogyakarta, Ministry of Industry, Republic of Indonesia.

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