The potential solvent for tannin extraction as a feed additive made of coffee husk (*Coffea canephora*) using Soxhlet Method

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Abstract. The purpose of this research was to determine the best of solvent for extracting tannins as a feed additive from coffee husks using the soxhlet method. The method used was quantitative with a completely randomized design experimental. The coffee husk used was a type of Robusta taken from the Kaliwates Region, Jember Regency, Indonesia. The material was the waste from the grinding of coffee cherries, namely the pericarp (parchment, pectin layer, pulp, and outer husk). This research consisted of 3 treatments (P1: Methanol, P2: Ethanol, P3: Acetone), each treatment consisted of 3 replications. The variables were the tannin extracts and residue of tannin extracts. The results showed that different types of tannin solvents had a significant effect on the tannin extracts yield and residue of tannin extracts (P<0.05). Methanol and ethanol solvents produced higher tannin than acetone solvents: 0.5392%, 0.6120%, and 0.2457% respectively. Coffee husk with ethanol solvent gave the least residue compared to methanol and acetone: 61.64%, 66.89%, and 85.26% respectively. The conclusion of this research was Ethanol is a solvent that produces the highest total tannin (0.6120%) and the lowest residue (61.64 ± 0.6688%) from coffee husks compared to methanol and acetone. The result of this research can be used as a consideration for the tannins extraction of other plantation waste.

1. Introduction
The availability of coffee waste in Jember Regency is quite abundant, which is 11,863 ton in 2017[1]. The husk composition of coffee is quite large, it is about 43.6% of the total fresh coffee fruit [2]. Coffee husk is one type of waste that has a quite high tannin. Tannin is nontoxic organic compounds that is classified as polyphenols commonly obtained from plant extracts. Tannin is beneficial as antioxidants, food additives, and drugs[3].

Tannins are phenolic compounds derived from secondary plant metabolism and develop complex bonds with proteins [4]. Physically, when dissolved into water, tannins will form colloids and give a sour and bitter taste. When tannins are mixed in alkaloids and gelatin, it will become sediment. Tannin is shiny yellow or white amorphous powder, almost colorless, with distinctive smell and taste [3]. Chemically, tannins are complex compounds in the form of polyphenol mixtures that are difficult to separate so that they are difficult to crystallize.

Tannin compounds can preserve proteins during the fermentation process, reduce ammonia levels, and increase the nutrient of silage products[5] Tannin is also known for its benefits as antioxidants, antidiarrhea, antibacterial and astringents. Tannins have strong bonds with protein molecules and have the ability to precipitate proteins.

For ruminants, tannin can be either advantageous or disadvantageous, depending on the amount consumed by the animal, its type and chemical structure as well as the composition of the rest of the feed, especially the concentration of CP feed. In temperate forage, in low to moderate concentrations
(<50 g / kg DM), it is beneficial in terms of increasing protein utilization without negatively affecting feed intake and digestion of nutrients. This shows that coffee husk waste can be used as a potential material for improving the quality of feed nutrients.

Tannins can be extracted using a single solvent or solvent mixture. The solvents that can be for tannin extraction are methanol, ethanol, or acetone solutions, and also ethyl acetate [6]. Extraction using Soxhlet with liquid solvents is one of the best methods used in separating bioactive compounds from nature. This is because the heating that occurs during the extraction process can increase the ability to extract compounds that are not soluble at room temperature, so that the activity of pulling compound is more maximal [7]. Therefore, tannins have an important role as feed additives, so this research was conducted to observe the extraction of tannins from coffee husk waste using different solvents. The purpose of the research was to determine the best solvent for extracting tannins as feed additives from coffee husks using the Soxhlet method.

2. Materials and Methods

2.1 Making coffee husk powder
Robusta coffee husk waste (from the husk coffee plantation, Jember, East Java Province, Indonesia). Dry coffee husk (<15%) water content is smoothed using the Whiley mill (2 mm screen). The results of the analysis of the Feed Technology Laboratory, Politeknik Negeri Jember (2021) showed that the coffee husk used contained the following nutrients: BK (91.0%); PK (11.3%); LK (1.6%); SK (16.6%).

2.2 Extraction of coffee husk waste tannins using Soxhlet method
Twenty gram of coffee husk powder wrapped in Whatman filter paper, is extracted with organic solvents according to treatment (methanol (99.8%), ethanol (70%), acetone (70%) as much as 375 ml. The extraction process uses a Soxhlet tool with a temperature of 90°C or characterized by the spinning of the solution inside the pumpkin. Extraction ends until the solution is clear. Filtrate extraction method Soxhlet inserted into rotary vacuum evaporator (60°C) until obtained concentrated extract and dried into the oven (60°C) [7].

2.3 Qualitative analysis of tannins.
The concentrated extract obtained is taken as much as 10 g and added with 3-4 drops of ferrite chloride solution (FeCl3). The formation of bluish black indicates the presence of phenols (tannins) [8]. Observed parameters: 1) Results of coffee husk extract and tannin yield; 2) residue from extraction

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X\% = \frac{The \ amount \ of \ tannin \ produced}{The \ amount \ of \ coffee \ husk} \times 100\%
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2.4 Data Analysis
Data obtained from the extraction of Soxhlet coffee husk tannins analyzed variance with Complete Random Design (CRD). Each treatment is repeated three times, significant results are followed by Duncan’s Multiple Range Test (DMRT).

3. Results and discussion
The tannin content of coffee husk by soxhlet method using methanol and ethanol as solvents were significantly different. Both of them produced quite high tannins compared to tannin extracts using acetone as a solvent (Table 1). From these results showed that the results of tannin extract were influenced by the kind of solvent. The highest content of coffee husk tannin was obtained from ethanol solvent by 0.612%, then the methanol solvent was 0.5392%, and the smallest one was the acetone solvent by 0.2457%.
Table 1. Tannin content of coffee husk using the soxhlet method on different types of solvents

| Number | Solvent Types | Coffee Husk Extraction Results (%) | Total Tannin (%) |
|--------|---------------|------------------------------------|------------------|
| 1      | Methanol      | 29.53 ± 1.639\(^a\)                | 0.5392\(^b\)     |
| 2      | Ethanol       | 28.17 ± 1.268\(^a\)                | 0.6120\(^a\)     |
| 3      | Acetone       | 6.33 ± 0.5118\(^b\)               | 0.2457\(^c\)     |

\(^{a,b,c}\) = Means were significantly different in each column (P<0.05)

The effectiveness of the extraction of a compound by a solvent depends largely on the solubility of the compound, that a compound will dissolve in a solvent of the same polarity. Tannins are polar compounds that will dissolve in polar solvents. Polar solvents include methanol, ethanol, and acetone. The dielectric constant measures the solvent's ability to reduce the strength of the electric field around the charged particles. Organic solvents based on their dielectric constants can be divided into two: polar and non-polar solvents. The dielectric constant is expressed as the resisting force between two electrically charged particles in a molecule. The higher the dielectric constant, the solvent is more polar. The dielectric constants of methanol, ethanol and acetone each have a dielectric value of 33.64; 25.16; and 21.13 respectively [9].

The results of coffee husk extract with organic solvents showed that coffee husk tannin extract could be done using polar solvents with a range of dielectric constants around 25.16 to 33.64 which found using methanol and ethanol solvents. Different solvents showed significant different of tannin production (total tannin). The largest content of coffee husk tannins is obtained from ethanol solvents by 0.612%; then methanol solvent was 0.5392%; and the smallest one was acetone solvent by 0.2457% (Table 1). The high total tannins of coffee husk extracts using ethanol showed that ethanol was the best solvents. This was happened because the acquisition of extraction products is based on the similarity of polarizing properties to solvents. It was also stated by Mandal [10] that ethanol heats up faster so that it can improve the extraction process. This was seen during the soxhlet process, ethanol solvents boiled first from other solvents indicated by the rotation of the solution in the soxhlet vessel. The type of solvent produced a different extraction residue, the largest being acetone at 85.26 ± 0.3921 followed by methanol a 66.89 ± 1.8619 and ethanol at 61.64 ± 0.6688 (Table 2).

Table 2. Residual levels of coffee husk using the soxhlet method in different alcohol solutions

| Number | Solvent type | Residue (%) |
|--------|--------------|-------------|
| 1      | Methanol     | 66.89 ± 1.8619\(^b\) |
| 2      | Ethanol      | 61.64 ± 0.6688\(^c\) |
| 3      | Acetone      | 85.26 ± 0.3921\(^a\) |

\(^{a,b,c}\) = Means were significantly different in each column (P<0.05)

An extraction with ethanol solvents, it produced the highest tannins so that it would produce the lowest residues (Table 2). This condition was because the coffee husk that could be extracted (as tannins) was the highest. In contrast, extraction using acetone solvent produced the lowest amount of tannin with the highest residue. Polar compound not only releases tannins from the coffee husk, but also dissolves other components that are polar such as carbohydrates and minerals [6], so that the extracted tannin contains different types of impurities. Das [11] mentions that the selection of the right type of solvent and temperature can release carbohydrates from tannins so that more tannins are produced, which is indicated by fewer residues. Because of extraction process, the extracted tannin contains different types of impurities including minerals, stilbenes and sugars.

4. Conclusion

Ethanol is a solvent that produces the highest total tannin (0.6120%) and the lowest residue (61.64 ± 0.6688%) from coffee husks compared to methanol and acetone.
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