Tannin characteristic from *Hevea brasiliensis* and *Durio zibethinus* with pressure and hot water extraction

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**Abstract**: The aim of this study was selected the best bark, temperature and pressure for extraction phenol and tannin with hot water solvent. The extract was characterised into condensed tannin and hydrolysable tannin with spectrophotometer. Tannin are plant secondary compound that influence feed metabolism in the rumen and pasca-rumen organ. Tannin have ability to bounding feed protein and other nutrient. Tannin classified into condensed and hydrolysable tannin, condensed tannin is a bypass agent to decrease deamination and other nutrient degradation in the rumen organ, furthermore hydrolysable tannin may divide as phenol and sugar group in the rumen. Phenol group form HT may become antioxidant in the blood and influence rumen ecology. The result for this study is *Hevea brasiliensis* has phenol and tannin content more than *Durio zibethinus* with P<0.01 if using hot water extraction. Temperature significantly influence concentration of phenol and tannin content, at 120 °C phenol and tannin extract more than 70 °C with P<0.01. Phenol extract significantly effected by pressure, 1 bar get phenol and tannin more than 2 bar with P<0.01. Treatment in this study have interaction, so generally concluded that to get phenol and tannin extract the highest used *Hevea brasiliensis* with temperature 120 °C and pressure 1 bar.

1. **Introduction**

Tannin is a plant defense mechanism from herbivores, parasites, fungi, insects, and environmental stress [1]. There is a lot of tannin in the roots, stems, skin, leaves and fruit of plants [2]. Tannin over addition reduce feed palatability and digestibility [3]. However the used of tannins precisely dosage has a beneficial effect on the ruminant metabolism [4]. Tannin can protect proteins from degradation process by rumen microbes [5,6], reduce methane from entheric fermentation [7,8], Decrease Biohydrogenation prosses and Increase CLA[9,10].

Tannin dosage depent on the type of tannin, each plant has different molecular form [11]. But in general tannin compounds are classified into two groups, namely condensed tannin (CT) and hydrolyzed tannin (HT). Compounds of CT and HT have different metabolic patterns in the rumen. CT has high stability so it is difficult to digest by enzymes, heat and acid, while hydrolyzed tannins have low stability so easily broken down into phenol acid and simple sugar. High stability allows CT to be a
good nutrient by-pass compound, whereas HT with a phenol group which is easily released will produce antimicrobial compounds [1].

Tannin is generally consumed by livestock along with feed ingredients that contain these compounds, so that the dosage of tannins cannot be measured. The practical use of extraction and addition technology is very important to determining doses. Tannins added by spraying soybean meal decreases degradation in the rumen and small intestine[12].

2. Materials and Methods
The bark sample is prepared by taking newly felled bark either in cutting wood or in the garden, then cut the bark with a uniform size of 0.5cm x 1cm. The bark was dried in an oven at 60 °C. The bark grinded with a sieve of 0.5mm and dissolved in 100% water that reach temperature 70 °C or 120 °C. The extraction process was continued by putting pressure 1 bar or 2 bar for 25 minutes. The liquid centrifuged at 4 °C for 10 minutes at a speed of 3000 rpm. The supernatan was measured for the total phenol and total tannin values, using a spectrophotometer with a wave of light 724nm. The bark extract was characterized by the number of CT and HT through the spectrophotometer method. Data were statistically analyzed by using the full factorial randomie disigne. Computation of the statistical analysis was performed by using SPSS software version 16.

3. Results and Discussion
The general test showed that the levels of phenol and tannin were significantly affected by the bark type and temperature treatment (70 °C and 120 °C) with P values<0.01, while the pressure of 1 bar and 2 bar had a significant effect on the amount of phenol content and tannin obtained P<0.01 (table 2). phenol and tannin levels were very significantly influenced by interactions between types and temperatures P<0.01 and significantly affected by interactions between types and pressure P<0.05. These results indicate to get the most extract content from the three types of bark requires a specific temperature and pressure, so that the next observation looks for the best temperature and pressure for the bark.

Table 1: Temperature and pressure treatment effect on phenol and tannin contain.

| Treatment | Phenol(%) | Tannin(%) |
|-----------|-----------|-----------|
| Temperature |  |  |
| 70 °C | 0.60** | 0.56** |
| 120 °C | 2.59** | 2.52** |
| Pressure |  |  |
| 1 bar | 2.08** | 2.02** |
| 2 bar | 1.11** | 1.06** |
| Bark |  |  |
| Karet | 2.47** | 2.37** |
| Durian | 0.74** | 0.70** |
| SEM | 0.05 | 0.04 |
| Pvalue Temperature | <0.01 | <0.01 |
| Pvalue Pressure | <0.01 | <0.01 |
| Pvalue Bark | <0.01 | <0.01 |
| Pvalue Temperature*Pressure | <0.01 | <0.01 |
| Pvalue Temperature*Bark | <0.01 | <0.01 |
| Pvalue Pressure*Bark | <0.01 | <0.01 |
| Pvalue Temperature*Pressure*Bark | <0.01 | <0.01 |
Table 2: Characteristic *Hevea brasiliensis* bark extract affected by temperature and pressure

| Parameter          | Treatment | Phenol | Tannin | T/F(%) | CT    | HT    | CT/T(%) | CT/HT |
|--------------------|-----------|--------|--------|--------|-------|-------|---------|-------|
| Temperature (°C)   | 70        | 1.03** | 0.95** | 92.55** | 0.15  | 0.80** | 84.68** | 0.18** |
|                    | 120       | 3.90** | 3.80** | 97.32** | 0.22  | 3.57** | 94.48** | 0.06** |
| Pressure (Bar)     | 1         | 3.33** | 3.23** | 95.84** | 0.27* | 2.96** | 88.28** | 0.14*  |
|                    | 2         | 1.58** | 1.51** | 94.03** | 0.10* | 1.41** | 90.88** | 0.10*  |
| SEM                | 0.06      | 0.06   | 0.65   | 0.01   | 0.04  | 4.47   | 0.00    |
| Pvalue Temperature | <0.001    | <0.001 | <0.001 | 0.26   | <0.001| <0.001 | <0.001  |
| Pvalue Pressure    | <0.001    | <0.001 | <0.001 | <0.05  | <0.001| 0.07   | 0.05    |
| Pvalue*Pressure    | <0.001    | <0.001 | 0.42   | 0.34   | <0.001| 0.48   | 0.35    |

Different superscripts in the same column are significantly different at P<0.05. T/F = proportion tannin/phenol, CT = condensed tannin, HT = Hydrolyzable Tannin, CT/T = Condensed tannin proportion in the total tannin, CT/HT = Condensed tannin per Hydrolyzable tannin. ; SEM, standard error of mean.

Table 3: Characteristic *Durio zibethinus* extract affected by temperature and pressure

| Parameter          | Treatment | Phenol | Tannin | T/F(%) | CT    | HT    | CT/T(%) | CT/HT |
|--------------------|-----------|--------|--------|--------|-------|-------|---------|-------|
| Temperature (°C)   | 70        | 0.79** | 0.75** | 92.18** | 0.04**| 0.71**| 12.23** | 0.15**|
|                    | 120       | 0.68** | 0.64** | 89.74** | 0.06**| 0.58**| 17.67** | 0.24**|
| Pressure (Bar)     | 1         | 0.19   | 0.16   | 85.56*  | 0.04**| 0.12* | 24.75** | 0.33**|
|                    | 2         | 1.28   | 1.23   | 96.36*  | 0.06**| 1.17* | 5.16**  | 0.06**|
| SEM                | 0.17      | 0.16   | 1.73   | 0.06   | 0.16  | 3.08   | 0.04    |
| Pvalue Temperature | <0.001    | <0.001 | <0.001 | <0.001 | <0.001| <0.001| <0.001  |
| Pvalue Pressure    | 0.06      | 0.06   | <0.05  | <0.05  | <0.05 | <0.05 | <0.05   |
| Pvalue*Pressure    | 0.34      | 0.34   | <0.05  | <0.05  | 0.16  | 0.83  | <0.05   |

Different superscripts in the same column are significantly different at P<0.05. T/F = proportion tannin/phenol, CT = Condensed tannin, HT = Hydrolyzable Tannin, CT/T = Condensed tannin proportion in the total tannin, CT/HT = Condensed tannin per Hydrolyzable tannin. ; SEM, standard error of mean.

Temperature treatment on HB bark of 120°C produces TF, TT, TT / TF in percent higher than the 70 °C with p < 0.01, but it is the opposite of the CT / TT value in percent, HT, CT / HT which is lower than the 70 °C temperature treatment with p < 0.01. Pressure method of 2 bars produce TF concentrations, TT, TT / TF (%), CT / TT (%) lower than the pressure of 1 bar with P < 0.01, a pressure treatment of 2 bar produce CF and HT higher than 1 bar pressure with P < 0.01. The temperature and pressure treatment has interaction with several parameters including TF, TT, HT, CT / TT and CT / HT. From this treatment to get the highest extract used temperature at 120 °C and 1 bar pressure (table 2).

The third extraction process was carried out on D skin, the temperature treatment of 120°C produced TT, TF, TF / TT (%), and HT higher than 70°C. But CT, CT / TT and CT / HT (%) is lower than 70°C with Pvalue < 0.01. The pressure treatment at 2 bar has a higher TT, TF, TF / TT, CT, HT compared of 1 bar, but the pressure of 2 bar has a CT / TT value (%) and lower CT / HT compared to
the pressure of 1 bar. The process of giving temperature and pressure have interactions on CT parameters Pvalue <0.01, CT / TT, CT / HT with Pvalue <0.05. The highest value for CT parameters is in the temperature treatment of 700 °C and 2 bar. Where the further test is not different from the temperature of 1200 °C and the pressure of 2 bar, then for CT / TT (%) and the highest CT / HT is at 1200 °C and the pressure is 2 bar (table 3).

4. Conclusion
The result for this study is Hevea brasiliensis has phenol and tannin content more than Durio zibethinus with P<0.01. Temperature significantly influence concentration of phenol and tannin content, at 1200C phenol and tannin extract more than 700C with p< 0.01. Phenol extract significantly effected by pressure, 1 bar get phenol and tannin more than 2 bar with p<0.01. Treatment in this study have interaction, so generally concluded that to get phenol and tannin extract the highest used Hevea brasiliensis with temperature 1200C and pressure 1 bar.

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