

CHAPTER 4 – RESULTS AND DISCUSSIONS

4.1 Material Preparation

Material preparation is a crucial step to ensure constant quality and efficient extraction of bioactive compounds. In this study, torch ginger stems were prepared through a drying process followed by grinding into powder form. These steps aim to reduce water content, prevent microbial growth, and increase the surface area for solvent interaction during extraction. After drying, the torch ginger stem's initial moisture content of 79.02% reduced to 6.21%. From 5kg of fresh material, 1.134kg of dried powder was obtained, resulting in a drying yield of 22.68%. This finding meets the quality requirement for dry simplicia, which requires moisture content below 10%, as reported by Effendi et al. (2019), who dried torch ginger stem and leaves and found moisture contents ranging from 5.557% to 9.928%.

4.2 Enzymatic Treatment

The glucose concentration profiles over time for different cellulase enzyme concentrations (0.5%, 0.7%, and 0.9% v/w) were analyzed during the Enzyme-Assisted Extraction (EAE). To evaluate the enzymatic activity, an enzyme reaction kinetics approach was applied by monitoring glucose release as a function of time. A second-order polynomial regression was used to model the kinetic behaviour of the enzymatic hydrolysis, and the optimum reaction time was identified as the peak of the polynomial curve, representing the point at which the maximum glucose concentration was achieved.

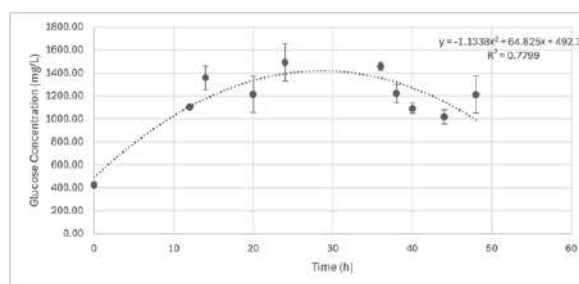


Figure 6. Glucose Concentration (mg/L) vs Time (h) at Enzyme Concentration 0.5% (v/w)

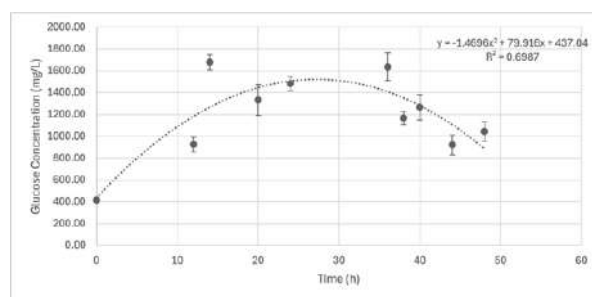


Figure 7. Glucose Concentration (mg/L) vs Time (h) at Enzyme Concentration 0.7% (v/w)

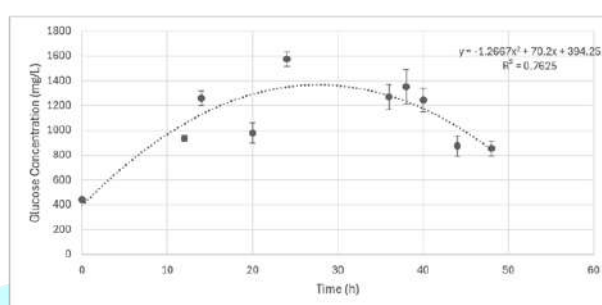


Figure 8. Glucose Concentration (mg/L) vs Time (h) at Enzyme Concentration 0.9% (v/w)

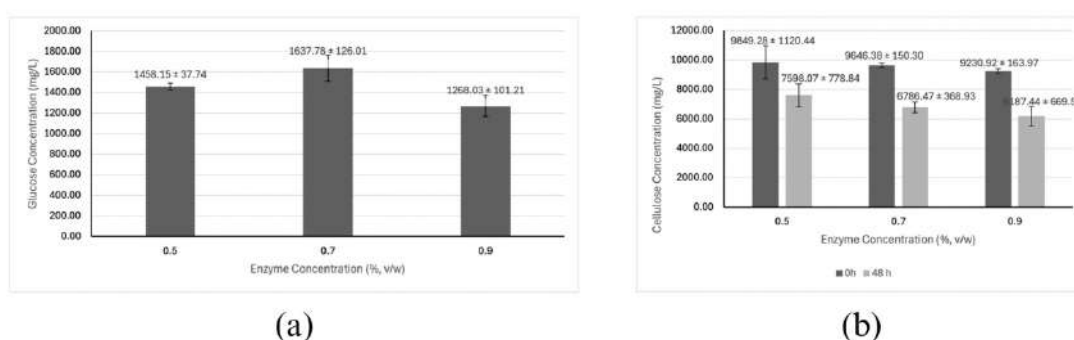


Figure 9. (A) Glucose Concentration (mg/L) vs Enzyme Concentration (% v/w) at 36 hours, (B) Cellulose Concentration (mg/L) vs Enzyme Concentration (% v/w) at 0 hour and 48 hours

For the 0.5% enzyme concentration, as shown in Figure 6, the glucose concentration gradually increased and reached its peak at 28.59 hours, after which it began to decline. At 0.7% enzyme concentration (Figure 7), the glucose concentration followed a similar parabolic trend but reached its maximum earlier, at 27.19 hours. Meanwhile, at 0.9% enzyme concentration (Figure 8), the optimum time was found to be 27.70 hours. These

findings indicate that the 0.7% enzyme concentration enabled the fastest enzymatic hydrolysis among all enzyme concentrations.

In addition to the optimum time, the glucose concentration at 36 hours was also considered to evaluate the overall effectiveness of each treatment, as shown in Figure 9 (A). The 0.7% enzyme concentration resulted in a significantly higher glucose concentration at 36 hours compared to both 0.5% and 0.9%. Although the 0.9% enzyme concentration had an optimum time close to that of 0.7%, its final glucose yield was lower. This may be due to less efficient reaction conditions at higher enzyme concentrations or possible reactions between glucose and other compounds in torch ginger stem, leading to undetectable glucose.

Based on these results, the 0.7% enzyme concentration was identified as the most effective condition for enzymatic treatment, as it provided both the fastest optimum time and the highest glucose concentration.

To further support evaluation, cellulose concentration before (0 h) and after (48 h) enzymatic hydrolysis was also measured as shown in Figure 9 (B). The reduction in cellulose concentration was calculated as an indicator of enzyme activity. The 0.5% enzyme concentration showed a decrease of (2251.21 ± 341.60) mg/L, while the 0.7% and 0.9% enzyme concentration showed reductions of (2859.90 ± 218.62) mg/L and (3043.48 ± 505.56) mg/L, respectively.

Table 2. Cellulose Concentration Reduction (mg/L) vs Variation

Variation	Cellulose Concentration (mg/L)
0.5% Enzyme Concentration (v/w)	2251.21 ± 341.60^b
0.7 % Enzyme Concentration (v/w)	2859.90 ± 218.62^a
0.9 % Enzyme Concentration (v/w)	3043.48 ± 505.56^a

^{a-b} : For each row, values with different letters indicate significant statistical difference ($p < 0.05$), t-test: paired two sample for means (appendix 4)

Although the 0.9% enzyme concentration showed the greatest numerical reduction, a statistical comparison using a paired t-test showed no significant difference between the 0.7% and 0.9% enzyme concentrations ($p\text{-val} > 0.05$) as shown in Table 2. This result suggests that increasing enzyme concentration from 0.7% to 0.9% did not produce a meaningful enhancement in cellulose degradation. Therefore, considering the shortest optimum time, the highest glucose concentration at 36 hours, and cellulose breakdown performance, the 0.7% enzyme concentration was selected as the most effective condition for enzymatic treatment.

4.3 Comparison of Maceration and Enzyme Assisted Extraction (EAE)

The purpose of this study was to evaluate the effect of enzymatic treatment on the extraction efficiency of total saponins from torch ginger stem. Enzyme-Assisted Extraction (EAE) was compared to conventional maceration using 70% ethanol to determine which method yields higher Total Saponin Content (TSC). The comparison involved three treatment variations with different approaches to extraction.

The Enzyme-Assisted Extraction (EAE) process modifies the structure of the torch ginger stem before the solvent extraction step. During enzymatic treatment step, cellulase enzymes break down cellulose, the primary component of the rigid plant cell wall. This cell wall acts as a natural barrier, limiting the solvent's access to the saponins stored within the cell.

The hydrolysis of cellulose by the cellulase enzyme breaks down this complex polysaccharide structure into simpler glucose units. This process not only disrupts the integrity of the cell wall but also significantly lowers the viscosity of the torch ginger powder and buffer solution slurry. The plant powder, which initially forms a relatively thick suspension, becomes more fluid. This reduction in viscosity is crucial as it enhances the efficiency of mass transfer during the subsequent maceration stage. With lower viscosity, the solvent can penetrate more easily and deeply into the plant tissue whose cell walls have been compromised. This allows for better contact between the solvent and the intracellular saponins, making the dissolution and diffusion of saponin into the solvent faster and more efficient.

Conversely, in the conventional maceration process without enzymatic treatment, the solvent must work harder to penetrate the intact cell walls. Consequently, the diffusion process is slower and less efficient, resulting in a lower yield of extracted saponins. Therefore, EAE offers a dual advantage, it breaks down the cell wall while also lowering viscosity for a superior extraction (Łubek-Nguyen et al., 2022; Pratiwi, 2021).

Based on the kinetic analysis in Figure 10, Figure 11, and Figure 12, the optimal extraction time was identified as 42 hours, corresponding to the peak saponin yield. This fixed duration was therefore used for all subsequent comparisons to consistently evaluate the effect of the different variation.

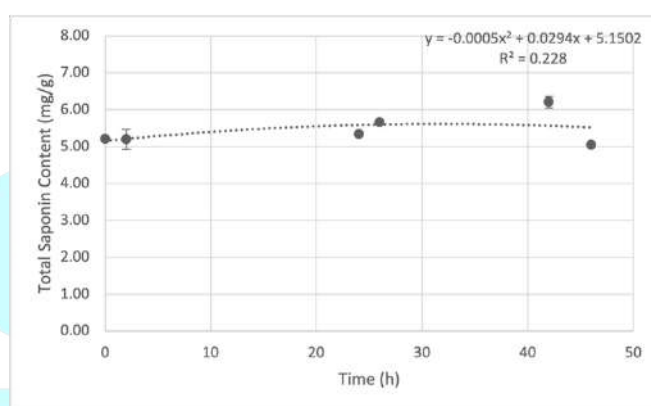


Figure 10. TSC (mg/g) vs Time (h) During Maceration After 0.5% Enzymatic Treatment

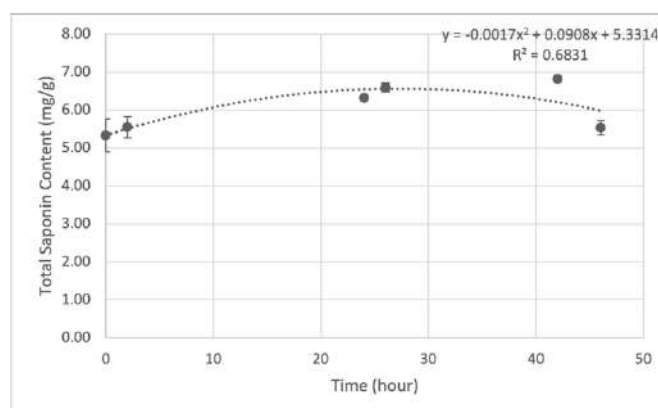


Figure 11. TSC (mg/g) vs Time (h) During Maceration After 0.7% Enzymatic Treatment

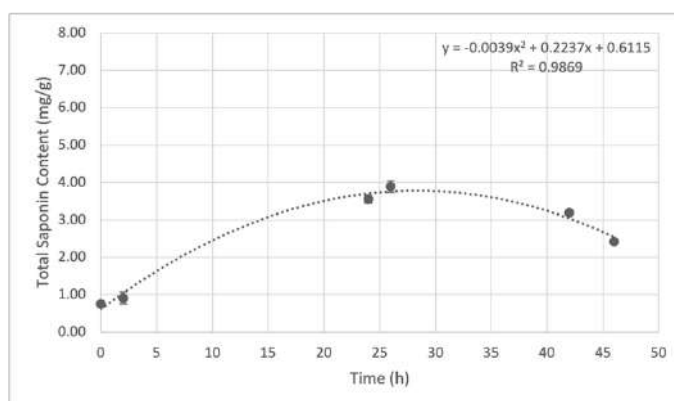


Figure 12. TSC (mg/g) vs Time (h) During Maceration without Enzymatic Treatment

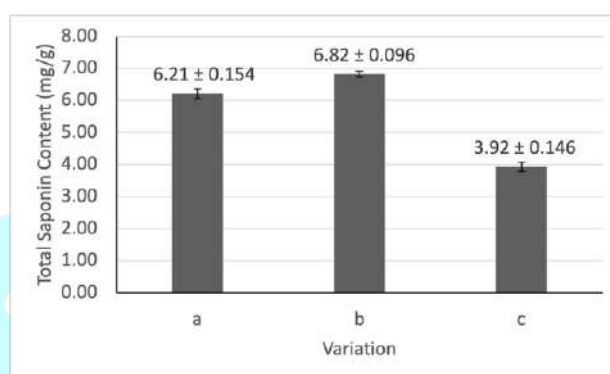


Figure 13. TSC (mg/g) at 42 hours for Different Variation:

- (A) EAE (0.5% enzyme v/w) + maceration,
- (B) EAE (0.7% enzyme v/w) + maceration,
- (C) maceration only (control)

The total saponin content (TSC) in the enzyme-assisted extraction samples (variation A and B) was nearly twice as high as in the non-enzyme-treated control (variation C), as shown in Figure 13, showing a clear advantage of enzymatic treatment. Variation A involved treatment with 0.5% enzyme followed by maceration produced the maximum TSC at 6.21 ± 0.154 mg/g. Variation B used a higher enzyme concentration of 0.7% under the same treatment conditions produced the highest TSC at 6.82 ± 0.096 mg/g. The control (variation C) showed the lowest TSC at 3.92 ± 0.146 mg/g.

The enzymatic treatment process may have caused this double increase in saponin yield. The rapid release of saponins was probably assisted by the enzymatic treatment, which

was carried out in a buffer solution with pH 5. Although the buffer is aqueous and not the same as aquadest, its aqueous property still allows for partial solubilization of saponins during the buffer treatment phase. As a result, some saponins were already extracted before ethanol maceration began. The overall effect resulted in significantly increased total yields when ethanol extraction was performed after the enzymatic treatment. This shows the efficiency of EAE in improving saponin recovery compared to maceration alone.

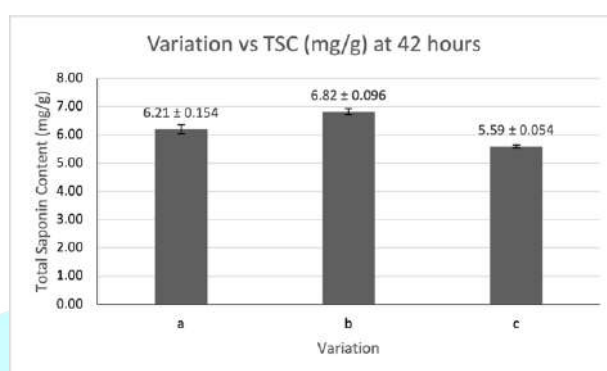


Figure 14. TSC (mg/g) at 42 hours for Different Variation:

- (A) EAE (0.5% enzyme) + maceration, (B) EAE (0.7% enzyme) + maceration,
(C) buffer treatment (no enzyme) + maceration

To ensure a fair comparison between EAE and maceration, variation C was also performed a treatment process using buffer only (without enzyme) prior to ethanol maceration. This adjustment allowed for a more direct comparison by isolating the effect of the enzyme itself. Despite receiving the same treatment conditions in terms of time and buffer exposure, variation C still produced significantly lower total saponin content ($5.59 \pm 0.054\text{mg/g}$) compared to both enzymes treated samples variation A ($6.21 \pm 0.154\text{mg/g}$) and variation B ($6.82 \pm 0.096\text{mg/g}$) as presented in Figure 14.

This indicates that the enzyme's presence is essential for increasing saponin yield. The enzyme helps break down the components of plant cell walls, especially cellulose, into simpler carbohydrates like glucose. This breakdown disrupts the cell wall structure, which increases the accessibility and extractability of intracellular saponins in the maceration (Łubek-Nguyen et al., 2022).

Table 3. Total Saponin Content (mg/g) vs Variation

Variation	Total Saponin Content (mg/g)
EAE (0.5% enzyme) + maceration	6.21 ± 0.154^b
EAE (0.7% enzyme) + maceration	6.82 ± 0.101^a
Buffer Treatment + maceration	5.59 ± 0.054^c

^{a-c}: For each row, values with different letters indicate significant statistical difference ($p < 0.05$), t-test: paired two sample for means (appendix 5)

Statistical analysis using paired t-tests confirmed that the differences in total saponin content among all three variations were significant ($p\text{-val} < 0.05$) as presented in Table 3. These results highlight the efficacy of enzyme-assisted treatment in improving the extraction efficiency of saponins from torch ginger stem.

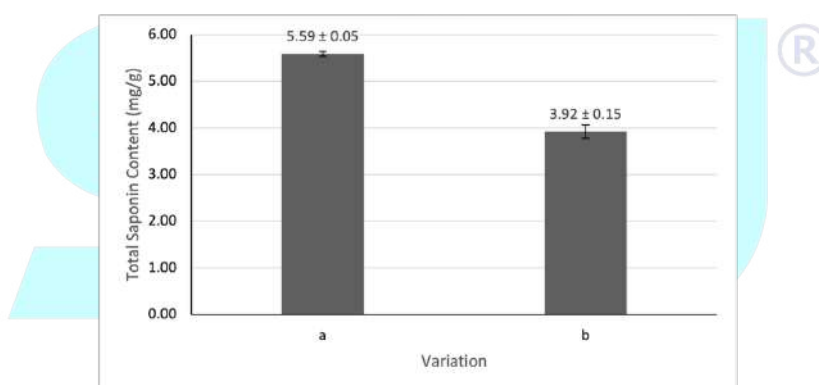


Figure 15. TSC (mg/g) at 42 hours for Different Variation:

(A) buffer treatment (no enzyme) + maceration, (B) maceration only

When comparing the saponin yield between maceration without any enzymatic treatment (B) with maceration followed by buffer treatment (A) as presented in Figure 15, it is evident that the addition of buffer treatment enhances the extraction outcome. Although no enzyme was involved, Variation A yielded a higher total saponin content ($5.59 \pm 0.054\text{mg/g}$) than Variation B ($3.92 \pm 0.146\text{mg/g}$).

This improvement can be caused by the effect of the aqueous buffer treatment. Firstly, the buffer enhances the solubility of the saponin molecules. Due to their amphiphilic

structure, the aqueous buffer interacts with the hydrophilic glycone chains, and for saponins containing acidic moieties, its pH promotes deprotonation. This ionization makes saponin more polar and easier to dissolve (Guclu-Ustundag & Mazza, 2007). Secondly, this pre-soaking process induces osmotic swelling of the plant cells, leading to the softening and rupturing of cell walls. This structural weakening then improves the mass transfer of saponins and ethanol penetration during the extraction process (Zhao et al., 2014).

Even without enzymes, buffer treatment improved extraction by increasing tissue permeability and allowing more saponin to dissolve. This highlights the benefit of adding enzyme-assisted extraction, which further enhances extraction by breaking down cell wall components.

To further evaluate the extraction process, the total saponin productivity is calculated. This gives a measure of how much saponin was extracted per gram of sample each hour (mg/g.h), which helps show how fast and effective each method is.

Table 4. Productivity of Different Extraction Method

Extraction Method	Extraction Duration (hours)	Productivity (mg/g.h)
EAE (0.5% enzyme) + maceration	76	0.064
EAE (0.7% enzyme) + maceration	76	0.074
Buffer Treatment (no enzyme) + maceration	76	0.056
Maceration Only	48	0.038

As shown in Table 4, the enzyme-assisted extraction (EAE) with 0.7% enzyme gave the highest productivity at 0.074mg/g.h, followed by the 0.5% enzyme at 0.064mg/g.h, both conducted over a total extraction duration of 76 hours. In comparison, maceration with buffer treatment (without enzyme) resulted in slightly lower productivity at 0.056mg/g.h. In contrast the conventional maceration method without any treatment, done in 48 hours, had the lowest productivity at 0.038mg/g.h. This indicates that the

productivity of EAE with 0.7% enzyme was 32.14% higher than buffer treatment + maceration, and 94.74% higher than conventional maceration.

These results show that using enzymes makes the extraction process not only more effective in terms of total yield, but also more efficient over time. The enzymes help break down cell wall components, making it easier for saponins to be released. While buffer treatment alone improved productivity compared to regular maceration, adding enzymes made the process even more efficient.

4.4 Qualitative Analysis

4.4.1 Saponin Analysis

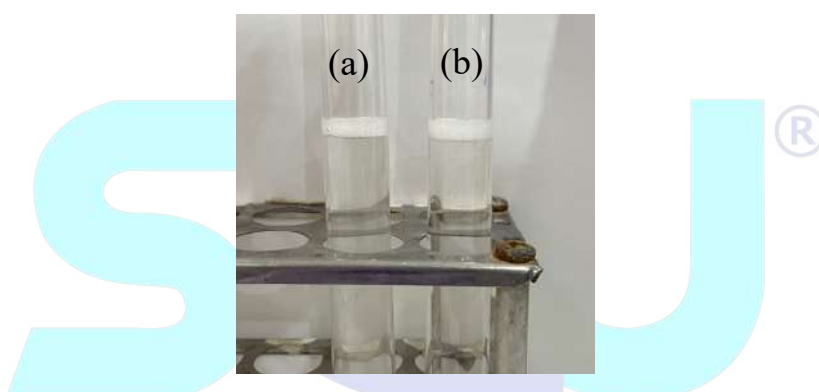


Figure 16. Saponin Analysis (a) Maceration (b) Enzyme Assisted Extraction

The foam test on torch ginger stem extract obtained through maceration and enzyme assisted extraction (EAE), Figure 16 showed foam heights of 1.5 and 2cm, respectively. Even after adding 1% HCl, the foam stayed steady at the same height after being left undisturbed for ten minutes. These results confirm the presence of saponins, based on the literature that stable foam that lasts for at least ten minutes after the addition of 1% HCl signifies the presence of saponin compound (Nur Azizah et al., 2022; Suryani et al., 2019).

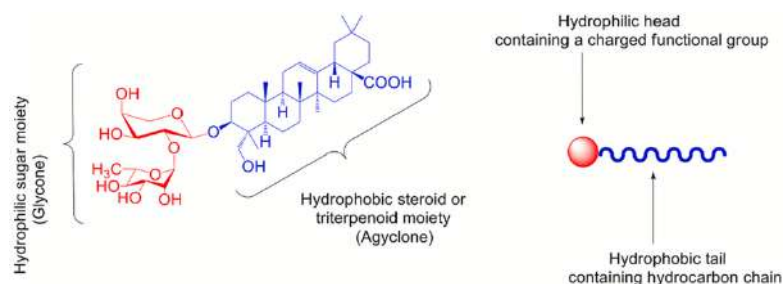


Figure 17. Saponin Structure (Rai et al., 2023)

Foam can be formed because saponins have the ability to lower water's surface tension. Similar to soap, saponins are big molecules containing both hydrophilic and hydrophobic groups as shown in Figure 17. When saponins dissolve in water, they have a tendency to align with the hydrophobic part away from the water, reducing surface tension and producing foam (Timilsena et al., 2023).

4.4.2 Type of Saponin

The type of saponin present in the torch ginger stem extract was identified through further identification after the presence of saponin was confirmed through qualitative analysis. Saponins are generally classified into two main types: triterpenoid and steroidal saponins, which differ in their aglycone (sapogenin) structures. In this study, the Liebermann-Burchard method was used to classify the saponin type present in the extract.

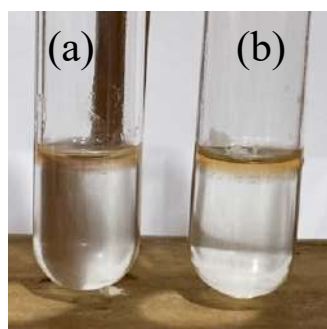


Figure 18. Type of Saponin Analysis

(a) Maceration (b) Enzyme Assisted Extraction

As illustrated in Figure 18, it can be observed that triterpenoid saponins are present in torch ginger stem extracts obtained from both maceration and enzyme assisted extraction. According to the literature, the presence of triterpenoid aglycone in saponin structures is indicated by the production of a brownish or violet ring (Indrayani et al., 2006; Nur Azizah et al., 2022; Suryani et al., 2019). The findings from this study do not align with those reported by Dakwan et al. (2024), who identified steroid saponins in the flower of *Etilingera elatior*, whereas this study found triterpenoid saponin in the stem of *E. elatior*.

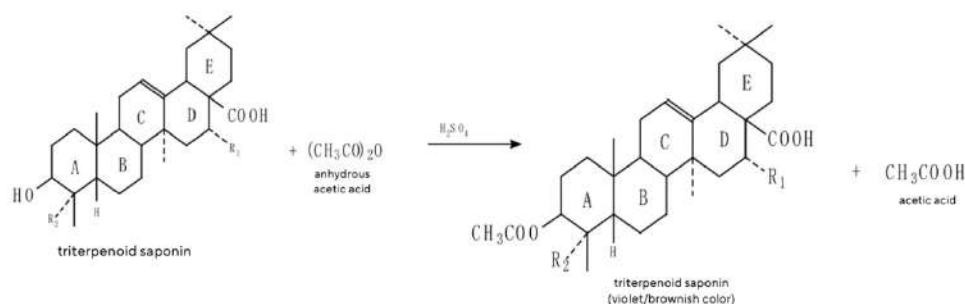


Figure 19. Triterpenoid Saponin Reaction with Liebermann-Burchard Reagent (Amananti et al., 2017)

The Liebermann-Burchard reagent is a mixture of acetic anhydride and concentrated sulfuric acid. When sulfuric acid is added to the extract containing acetic anhydride, a chemical reaction occurs that produces a highly reactive carbocation from the acetic anhydride. This carbocation then reacts with the oxygen atom in the hydroxyl (-OH) group found in triterpenoid compounds. The hydroxyl group from the triterpenoid and the acetic anhydride combine to produce an ester compound, which is known as an esterification reaction. Besides forming esters, this reaction also creates a conjugated system, which causes a colour change into violet or brownish (Setyawaty et al., 2020).

Saponins are amphiphilic compounds consisting of hydrophilic sugar chains and lipophilic aglycone groups. Because of this property, saponins dissolve better in polar solvents due to the more dominant of the sugar part. Since water is a pure

polar solvent, highly polar saponins, which are produced by the high density of hydroxyl groups on the sugar units, can dissolve in it. However, a combination of ethanol and water is frequently used to increase extraction efficiency (Rai et al., 2023).

Previous research also mentioned that triterpenoid saponin are commonly used in cleansing product such as natural soap, herbal shampoo, and detergents (Sochacki & Vogt, 2022).

