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Preliminary Study of Gaultherin Extraction from Gandapura Through Uv-Photo Extraction

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Abstract. Gaultherin in gandapura can be extracted using hot water and calcium carbonate, followed by solvent extraction using acetic ester hydrate at a temperature of 100 ºC. However, the process yields only 4 g/kg of fresh leaves. A technique of simultaneously gaultherase enzyme inactivation and gaultherin extraction with ethanol solvent should be studied. Preliminary studies of Gaultherin extraction with ethanol has been done and it is able to obtain gaultherin up to 14.46% at pH 8. This study will examine the development of the process photo-extraction-UV and osmosis dehydration simultaneously. The advantages of this process are (i) the gaultherase enzyme experiences unfolding, resulting in reduced hydrolysis reaction, (ii) increase in gaultherin yield, (iii) needs less energy, and (iv) purification of the product would be lighter. Study of the effect of solvent concentration and extraction time on gaultherin recovery was carried out for 2 months in the Laboratory of Chemical Engineering Operations at UNDIP Vocational School. Variables include dependent and independent variables. Dependent variables include the solvent-feed ratio of 5:1, pH 4.8, 75 rpm of mixer speed, chopper blades speed of 125 rpm, a drying temperature of 80 °C. Independent Variables include ethanol concentration at 80, 85, 90, and 95%. Extraction time for 10, 20, 30, 40 and 50 minutes. The result shows that gandapura extraction using ethanol had dual function, inactivate gaultherase enzymes and extract the active compounds gaultherin. Gaultherin yields was increased by increasing the duration of extraction. Whereas increasing the concentration of the solvent, gaultherin extracted was decreased.

1. Introduction

Gandapura is an essential oil-producing plant that in the list of commodities fostered of Directorate General of Plantation. Product diversification from gandapura can be a form of gaultherin production, which has a higher economic value (US $ 690 per 10 mg) compared with gandapura oil (US $ 22 per 15 ml). Theoretical analysis shows that gaultherin productivity 12 times higher than gandapura oil production (Yulianto et al., 2013). Gaultherin has properties that make it as a natural aspirin and anti-cancer (Ribnicky et al., 2003; Hoffman, 2007). As estimated, the aspirin consumption reaches 20-50 million pounds per year (West, 1998). The global pharmaceutical industry's demand for gaultherin is expected to increase in the coming years.

At this time, taking gaultherin from gandapura plant has not been effective, because during the extraction process gaultherin hydrolyzed into methyl salicylate components and disaccharides. The hydrolysis process is to be catalyzed by the enzyme gaultherase contained in the plant itself (Waters et al., 1931). Extracting method of Gaultherin plant when conditions gaultherase minimal activity or inactive need to be sought so that the hydrolysis reaction gaultherin be methyl salicylate and the disaccharide is not going to happen.

In 1928, gaultherin on Gaultheria procumbens extracted using hot water and the addition of calcium carbonate, and followed by solvent extraction using acetic ester hydrate at a temperature of 100ºC. The process only produces the final yield of 4 g / kg of fresh leaves (Bridel and Grillon, 1928), Small yield caused by gaultherin contained has been hydrolyzed by gaultherase. It is believed that alcohol, methylene chloride, acetonitrile can inhibit gaultherase activity (US Patent No. 2002/0031562 A1; Yulianto et al., 2008; Kusumo et al., 2013; US Patent No. 7033618).

For gaultherin production of gandapura, enzyme inactivation techniques and extraction gaultherin gaultherase simultaneously with ethanol should be examined. Ethanol solvent will have dual function, i.e simultaneously extract gaultherin enzyme inactivation. Preliminary studies of recovery gaultherin through the extraction process have been carried out (Yuniastuti et al., 2008, Faizah et al., 2011, Kusumo et al., 2013). The results show that inactivation of the enzyme using ethanol gaultherase potential and prospective, because it can increase the active compound gaultherin acquisition of up to 14.46% at pH 8. However, gaultherin recovered can not be produced optimally. It is caused by: (i) the diffusion of ethanol into the cellular cytoplasm of cells retained, so that not all the enzymes gaultherase through a process of unfolding, and (ii) the hydrolysis reaction gaultherin be methyl salicylate and disaccharide still occur. Thus, it is need photo-extraction-UV process development and drying agent simultaneously, which is able to summarize gaultherase inactivation treatment, the extraction process, and the process of osmotic dehydration in a single stage. Ethanol solvent functions as inactivation of the gaultherase enzyme while extracting gaultherin. The UV light-based extractor functions as the inactivation of the enzyme gaultherase and cellular cell destruction, so that the gaultherin can optimally recovered. While the *drying agent* serves to minimize the reaction of hydrolysis gaultherin to methyl salicylate. UV Oxidizing properties makes UV as a disinfectant in drinking water treatment (US Patent No. 5.78086 million) and the degradation of the organic components of wastewater (US Patent No. 2006/0163168 A1 and US Patent No. 7279092), as well as purification and sterilization of food production (US Patent No.7.391.041 and US Patent No. 2007/0114465 A1). The use of ultraviolet light was also developed to inactivate the enzyme and simultaneously extract the bioactive compounds in the extractor (Rizki et al., 2018).

Scheme process photo of extraction-UV and dehydration osmosis simultaneously is expected to provide benefits i.e. (i) the enzyme gaultherase experienced unfolding as penetrated ethanol, resulting hydrolysis reaction gaultherin into methyl salicylate catalyzed by the enzyme gaultherase reduced, (ii) yield gaultherin increased, due to the degradation mobile cell by UV light, (iii) less energy requirements for enzyme inactivation and gaultherin extracting, (iv) lighter product purification load, and (v) less amount of waste.

For this reason, the research activities aimed at: (i) design and fabrication of a 5 liter/day capacity UV photo-extractor prototype for inactivating the enzyme gaultherase, reducing water (inhibiting the hydrolysis reaction) and extracting the active compound gaultherin, (ii) studies of the effect of adding *drying* magnesium *agent* sulfate, sodium sulfate, and calcium chloride to increase gaultherin productivity, (iii) the optimization of process parameters on productivity gaultherin, and (iv) techno-economic evaluation study for feasibility of investment. Therefore, production gaultherin can be one of the diversification options for the gandapura plants. Hopefully, by producing gaultherin may increase the economic value gandapura industry.

2. Method

Research on the inactivation of the enzyme gaultherase and the extraction of gaultherin simultaneously through UV-photobio extraction technology has been investigated experimentally. This UV-photo extractor is a multi-function, which is able to summarize three stages in one device, namely the inactivation treatment, the extraction process gaultherin and dehydration process of osmosis. Fotoektstraktor-UVberupa stirred tank equipped with blade/knife enumerators as in a blender and is also equipped with UV light. The series of UV-stage photo-extractor and osmotic dehydration devices used for recovery of active compounds of gaultherin are presented in Figure 1. This series of devices consists of UV-based enzymatic inactivation extractor equipped with a stirring motor, a chopper *blade,* temperature control and stirrer rotation**.**

3. Materials and Devices Research

Raw materials to be used in this study are the leaves and flowers of plants gandapura obtained from KT Sikunang, Wonosobo. The raw material is frozen in order to inhibit the enzyme activity gaultherase. Another necessary ingredient is a chemical of PT. Bratachem Semarang is used as a solvent such as ethanol, pH buffer, drying agent (gelatin, calcium chloride and sodium sulfate) and the standard material for the purposes of analysis gaultherin levels, levels of salicylic acid and methyl salicylate levels using HPLC-MS analysis.

The main equipment used in this research is UV-photo-extractor and one-stage osmotic dehydration which is used to inactivate enzymes, reducing water and extract gaultherin. Supporting tools needed include: filtration equipment, oven, centrifuge and heater to assist in determining the levels of salicylic acid and methyl salicylate levels. Identification and analysis gaultherin performed using a spectrophotometer and HPLC-MS.

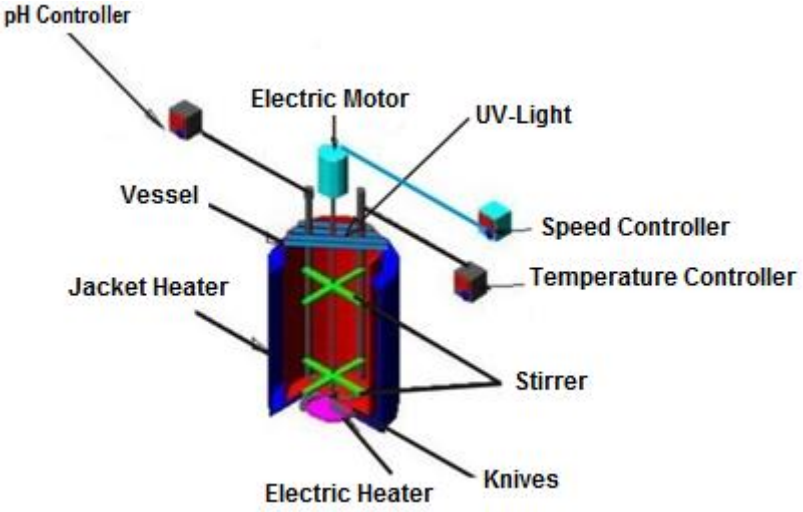


Figure 1. UV-photobio Extractor

4. Study Effect of Concentration and Time Solvent Extraction

Study of the effect of solvent concentration and extraction time on gaultherin recovery was carried out for 2 months in the Laboratory of Chemical Engineering Vocational School Operations UNDIP. Efforts to improve productivity including the effect of the addition of drying agent (sodium sulfate, calcium chloride, and calcium sulfate) to UV-photobio extraction. In general, the level of productivity gaultherin rather than with the addition of the drying agent. The use of *drying agent is* useful to bind, reduce or eliminate the water content in solution or in a mixture of water so that no hydrolysis reaction occurs by the enzyme gaultherase. It is expected that with the addition of the drying agent, the yield obtained in the production of gaultherin will be higher when compared to the production process without the addition of a *drying agent*.

5. Variable Experiment

Variables include the experiment remains variable and independent variables. Fixed variables include the solvent-feed ratio of 5: 1, pH 4.8, 75 rpm rotational speed mixer, chopper blades rotating speed of 125 rpm, a drying temperature of 80°C product. Free Variable includes concentration ethanol at 80, 85, 90, and 95%. Extraction time for 10, 20, 30, 40 and 50 minutes. Type drying agent sodium sulfate, calcium chloride, and calcium sulfate concentration of drying agent 5; 7.5; 10; 12.5% (w / w).

6. Experimental procedure

In this experiment, the leaves and flowers of the plant gandapura ice or frozen using liquid nitrogen. Extraction was carried out in a UV- enzyme photobio extraction enzyme with ethanol as a solvent. UV-photobio extraction is equipped with a *blade* or chopper knife at the bottom and UV light at the top. The ratio of solvent: feed, 5: 1. Ethanol is added at a certain concentration. The pH was maintained at pH 4.8 using a pH buffer. Speed of stirrer rotary and rotational chopper blades, respectively 75 and 125 rpm. Drying agent added according to experimental variables. Extraction was held for a certain time. The solids are then separated from the extract using a filter or centrifugation.

The extract was separated from the solids then added with chemicals or heated to remove the solvent. To get gaultherin in the form of a solution, extract that has been heated can be resuspended using a buffer or water. The extraction and then analyzed the content of gaultherin, methyl salicylate, salicylic acid using HPLC-MS analysis. HPLC-MS used were HPLC Alliance 2695 (Waters) with Photodiode Array Detector 2996 (Waters), column Symmetry C18 5 um, 4.6 mm x 150 mm (Waters).

7. Result and Discussion

Gandapura plant extraction using polar compound in the form of ethanol has a dual function, which inactivates enzymes gaultherase and extract the active compounds gaultherin. Diffusion of ethanol into gandapura leaves (Figure 2) intended to make the enzyme gaultherase are located in the cytoplasm penetrated with solvent, causing the enzyme activity to be inhibited. The statement also expressed by Poulev et al. (2003) that gaultherase activity inhibited by the addition of polar compounds. The next mechanism that the ethanol solvent will infiltrate the walls of the tonoplast membrane and phase contact occurs with the active compound gaultherin. The polar solvent will diffuse out of the leaf cell by carrying gaultherin. This is caused by differences in solubility.

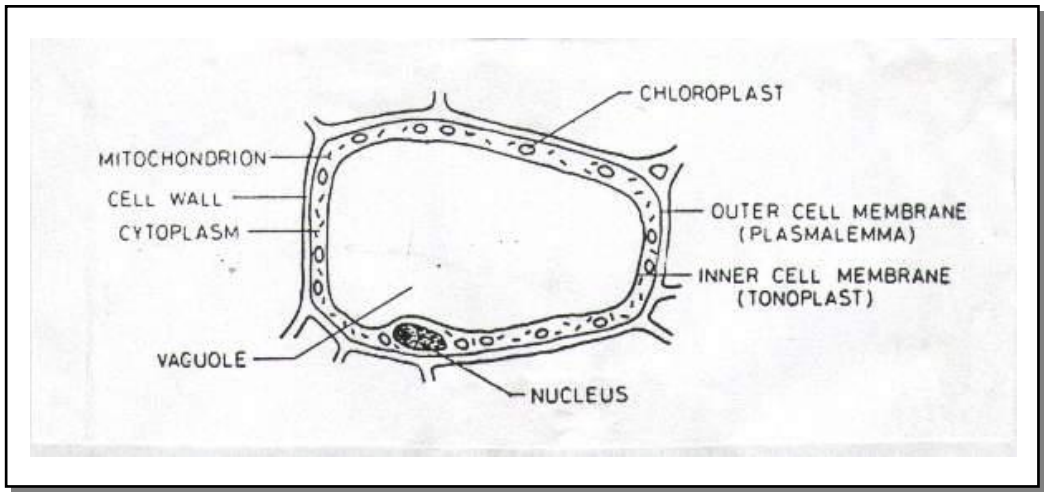


Figure 2. Gandapura leaf cell

Enzymes are giant molecules with a molecular weight that varies between 5000 Da-5 million Da. The enzyme belongs to a group that is larger macromolecular protein and consists of a series of linear chains of specific amino acids. In optimum conditions, the enzyme will through a folding process (Figure 3). The process of forming the composition of the enzyme folding is a spontaneous process that occurs in seconds (Bugg, 2004; Yang et al., 2004). Therefore, if the enzyme gaultherias is in a *folding* state, and damage to the tonoplast membrane also occurs, the enzyme catalyzes the reaction of the hydrolysis of the gaultherin compound to methyl salicylate. This cause acquisition of the active compound gaultherin is relatively low.

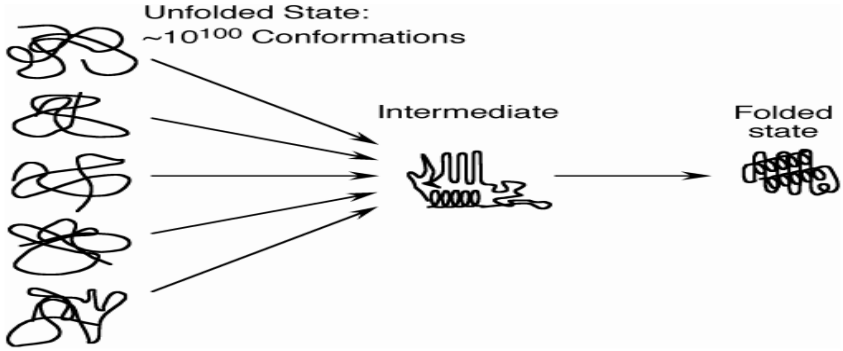


Figure 3. The Folding Process

The amino acid sequence of the enzyme will form a three-dimensional arrangement of certain, specific to each type of enzyme (tertiary structure). Part of the tertiary structure of the enzyme that is responsible for the catalytic activity of the enzyme called the active side. The number of active sides of an enzyme reaches 10-20% of the total volume of the enzyme (Bugg, 2004; Yang et al., 2004). The active side of an enzyme usually a hydrophilic gap consisting of a series of amino acid chains that binds the substrate (Figure 4.a) or bind a cofactor (Figure 4.b) and catalyzes the reaction.

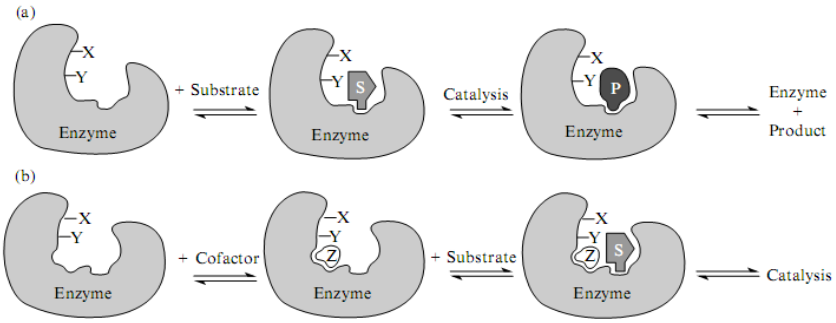


Figure 4. The active side of the enzymes

The *folding* process in an enzyme is a process that involves the inclusion of amino acid chains that are hydrophobic to the inner side of the enzymes and process out or shifting the amino acid chain that is hydrophilic to the exterior of the three-dimensional arrangement of the enzyme.

7.1. Effect of Extraction Time

Figure 5 presents the relationship gaultherin recovery to extraction time. The longer the extraction time, the gaultherin obtained increases, but after 10 minutes, the extraction approaches equilibrium. The longer the contact time between the phase dispersion phase (gaultherin) with the continuous phase (solvent phase), causing many dispersion phases to drag and cross the continuous phase based on differences in solubility. This is accordance with the statement Yuniastuti et al., (2017), the longer the time, the yield gaultherin has increased.

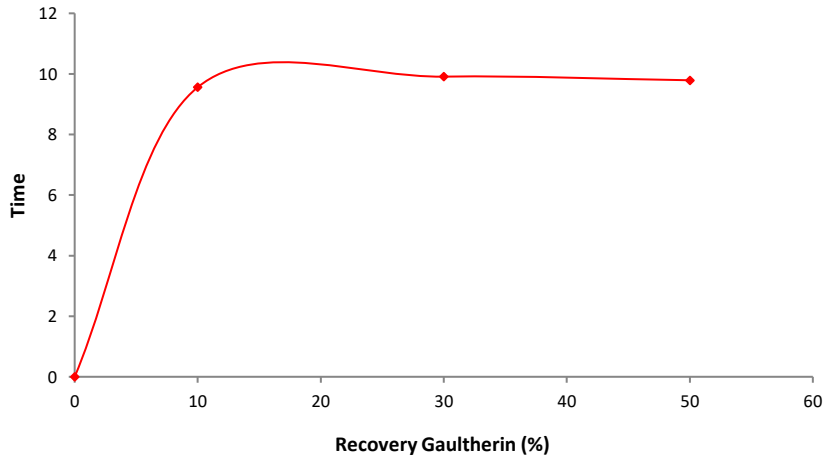


Figure 5 Gaultherin acquisition against extraction time graph

7.2. Effect of Solvent Concentration

Figure 6 presents a graph of the relationship between the ethanol concentration gaultherin levels. The greater the concentration of the solvent, gaultherin extracted decreases. It is possible, increasing concentrations of ethanol above 85%, causing some diluents such as methyl salicylate shipped to the continuous phase due to the increased solubility (figure 7). This is in contrast with the statement Yuniastuti et al., (2017), by increasing the concentration of solvent means enlarging the continuous phase, resulting in the volume fraction of the dispersed liquid phase and the smaller the particle diameter is also smaller. With decreasing particle diameter, it will expand inter-phase contact brought about by increased solute was dragged into the solvent phase.

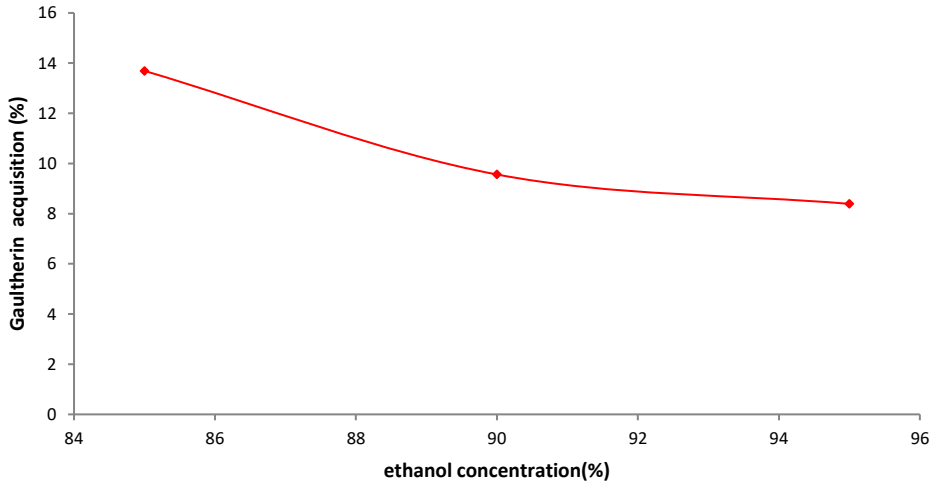
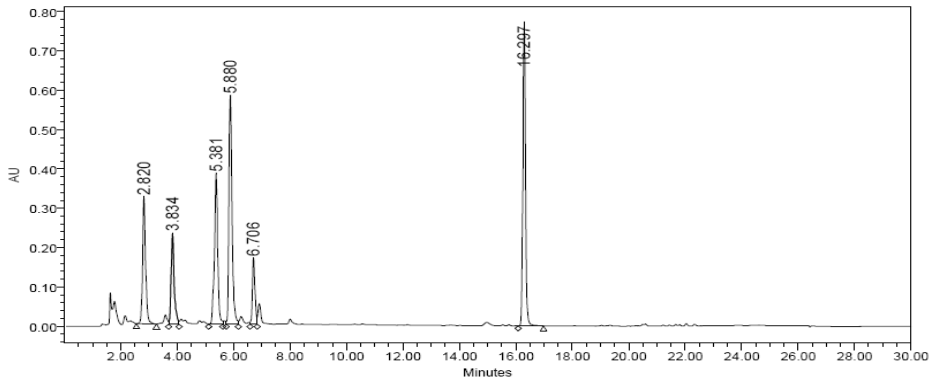


Figure 6. Gaultherin acquisition of ethanol concentration

It is inexplicable that gaultherase a type hydrolase enzyme, which has optimum activity around pH weak acid solution. Therefore, on the condition of a weak base bio-extraction condition cause enzyme gaultherase is unfolding, consequently it will reduce the hydrolysis reaction of gaultherin to methyl salicylate which is catalyzed by the enzyme gaultherase.



	RT	Area	% Area	Height
1	2.820	2441581	13.69	323410
2	3.834	1734364	9.72	230749
3	5.381	3073729	17.23	382806
4	5.880	4444864	24.92	582563
5	6.706	1095720	6.14	170395
6	16.297	5044594	28.29	769152

Figure 7. HPLC-MS chromatogram for gaultherin components and methyl salicylate in a 10-minute extraction using ethanol 85%.

8. Conclusion

Gandapura extraction using ethanol to dual function, which activates enzymes gaultherase and extracts the active compounds gaultherin. The longer the extraction time, the gaultherin obtained is increasing. The greater the concentration of the solvent, gaultherin extracted decreases.

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