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Gaultherin production from gandapura *(Gandapura fragantissima)* using photo extractor-UV machine

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ABSTRACT

Gandapura extraction process using ethanol as a polar compound has two functions, namely: deactivating the gaultherase enzyme and extracting the active compound gaultherin. This study examines the effect of extraction using UV from the concentration of ethanol in the extraction process of gandapura leaves into gaultherin on the extraction time, with a ratio of solvent and feed of 5:1, the rotational speed of the stirrer and the rotational speed of the chopper blades are 75 and 125 rpm, respectively. Drying agent is added according to the experimental variables; the independent variables includes ethanol concentrations of 85, 90, and 96%, while the extraction times are 10, 30 and 50 min, respectively. Gaultherin concentration was obtained from HPLC Alliance 2695 (Waters) analysis with Photodiode Array Detector 2996 (Waters) at a wavelength of 254 nm. The effect of UV photo extraction on the acquisition of gaultherin compounds has succeeded in deactivating the gaultherase enzyme and opening when it is penetrated with ethanol. In this study, the peak of the dominant gaultherin compound was found at the retention time of 5.8 min, and the highest area was at 90% ethanol concentration variable with an extraction time of 50 min; the area was 22.783% of the total gaultherin area.

1. Introduction

Gandapura *(Gaultheria fragantissima)* is one of the essential oil-producing plants which can grow in the highlands around 1300–3300 m above sea level. Gandapura oil contains methyl salicylate between 93 and 98% [1]. However, gandapura oil produced by the Farmers Group in Indonesia only contains 82.23% of methyl salicylate [2]. The main obstacle for the gandapura oil refinery farmer group is that Indonesian gandapura oil is not yet competitive in the market since China is already able to produce synthetic gandapura oil [3].

Product diversification from Gandapura can basically be in the form of gaultherin production. Gaultherin products have a much higher economic value when compared to gandapura oil. In this case, the price of gandapura oil is 22 dollars per 15 mL, while 10 mg of gaultherin is 690 dollars. Theoritical analysis shows that the productivity of gaultherin is about 12 times higher than that of gandapura oil produced by farmer groups in Indonesia. However, gaultherin production from the gandapura plant in Indonesia has not yet been commercialized [4].

Gaultherin has properties that make it the best candidate for natural aspirin, anti-cancer, anti-inflammatory, and cardiopulmonary [1,4]. Empirically, plants from the gaultheria family have been used to treat cancer and leukemia. Plants derived from gaultheria are also reported to have anticarcinogenic properties. As a natural aspirin, gaultherin has the same healing power as synthetic aspirin but has minimal negative effects [5,6,7]. Currently, aspirin (Acetylsalicylic acid) is the most widely consumed drug by the world's population due to its anti-pyretic, anti-inflammatory, and analgesic properties [4,6,8,9]. According to estimates, world aspirin consumption reaches 20–50 million pounds per year. Therefore, it is estimated that the world pharmaceutical industry's demand for gaultherin will increase in the upcoming years [2,4].

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However, there has been no effective gaultherin extraction method from the gandapura plant until recently. The difficulty experienced in the process of gaultherin production is that during the extraction process; with tissue damage, gaultherin will immediately be hydrolyzed into its individual components, namely methyl salicylate and disaccharides. The hydrolysis process is believed to be catalyzed by an enzyme found in the plant itself, i.e., gaultherase [2,5,6,10]. Therefore, an appropriate method is needed to extract gaultherin from plants under conditions where the activity of the gaultherase enzyme is minimal or inactive, to minimize the hydrolysis reaction of gaultherin into methyl salicylate and disaccharides [3].

The extractor modification functions as inactivation of the gaultherase enzyme and media for destroying cellular cells, so that gaultherin is maximally recovered. The scheme of the UV-extraction photo process and simultaneous osmosis dehydration is expected to provide benefits for the opening of the gaultherase enzyme and can penetrate ethanol. Thus, the hydrolysis reaction of gaultherin into methyl salicylate catalyzed by the gaultherase enzyme decreases, while the yield of gaultherin increases due to degradation of cellular cells by UV light, requires less energy for enzyme inactivation and gaultherin extraction, and produces lighter product purification burden and less waste [3,13].

Therefore, the novelty of this research is the use of UV-based extraction which can reduce much higher economic value and optimize the inactivation of the gaultherase enzyme and the media for destroying cells, so that gaultherin can be recovered. This study is designed to increase the production of gaultherin from gandapura using the inactivation process of the gaultherase enzyme through UV photo extraction and to determine the effect of adding ethanol as a solvent to the extraction process to minimize the hydrolysis reaction of gaultherin conjugation, as well as analyzing the production process using HPLC analysis. The positive impact from gaultherin production is expected to improve the economy for farmer group businesses to the gandapura producing industry.

2. Material and method

2.1. Material

The raw material used in this research is gandapura leaves obtained from KT Sikunang, Wonosobo. The raw materials are frozen to inhibit the activity of the gaultherase enzyme. Ethanol, pH buffer solution and drying agent (calcium chloride) were purchased from Merck.

2.2. Method

2.2.1. Variable preparation

Fixed variables in this study include solvent-feed ratio of 5:1, pH of 4.8, mixer rotational speed of 75 rpm, and chopper blade rotational speed of 125 rpm. Independent variables include ethanol concentrations at 85, 90, and 96%; while the extraction times are 10, 30, and 50 min, respectively. This study uses calcium chloride drying agent.

2.2.2. Experimental procedure

The main equipments used in this research are UV-photo-extractor and one-stage osmotic dehydration which is used to inactivate enzymes, reduce water, and extract gaultherin in Fig. 1. Supporting tools needed include filtration equipment, oven, centrifuge, and heater. Extraction was carried out in UV-enzyme extraction with ethanol solvent. UV extraction is equipped with a chopper blade at the bottom and a UV lamp at the top. Solvent:feed ratio is 5:1. Ethanol was added at a predetermined concentration according to the variable. pH was maintained at pH 4.8 using a pH buffer. The stirrer rotational speed and chopper blade rotation are 75 and 125 rpm, respectively. Drying agent was added according to the experimental variables. Extraction was carried out for a certain time. The solids were then separated from the extract using filters or centrifugation. The extract was separated from the solid then chemical was added or heated to remove the solvent. To obtain gaultherin solution, the heated extract can be resuspended using a buffer solution or water.

2.2.3. HPLC-MS analysis

The chemical profile of Gaultherin extracts was defined using HPLC-MS. Analysis of the samples in this study includes analysis of the composition of the bioactive compounds of Gaultherin extract such as shogaol and Gaultherin ol using HPLC Alliance 2695 (Waters) with Photodiode Array Detector 2996 (Waters) and Symmetry C18 5 um, 4.6 mm × 150 mm (Waters). The analysis was performed by applying two types of effluents. The first one is water and 0.1% of formic acid, and the second one is Acetonitrile and 0.1% of formic acid. The injection of 10 μL of sample volume was conducted at a flow rate of 1 mL/ minute with a wavelength of 254 nm, and analytical data were compared using the patent standard gaultherin.

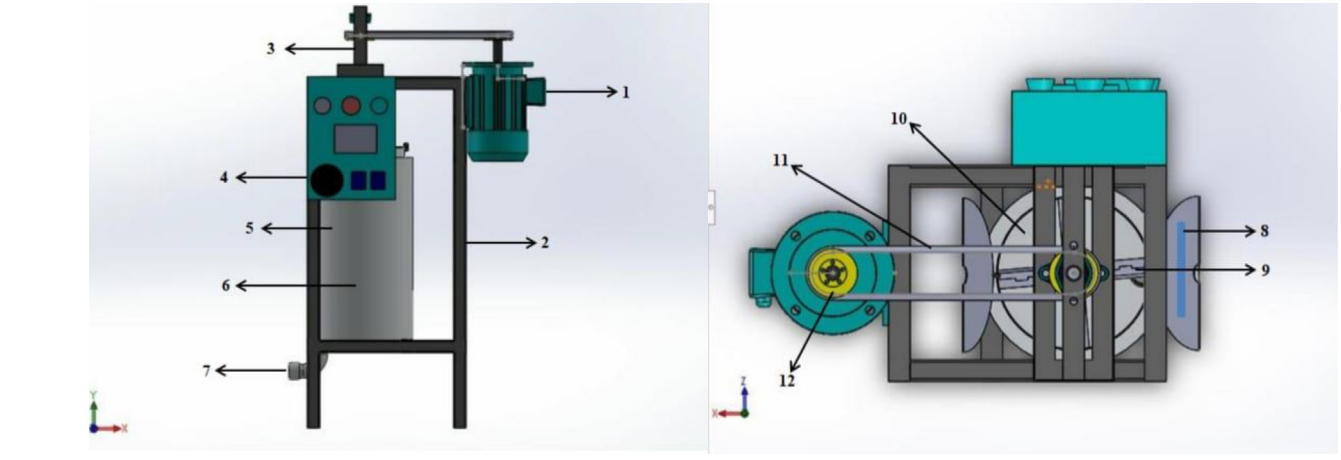
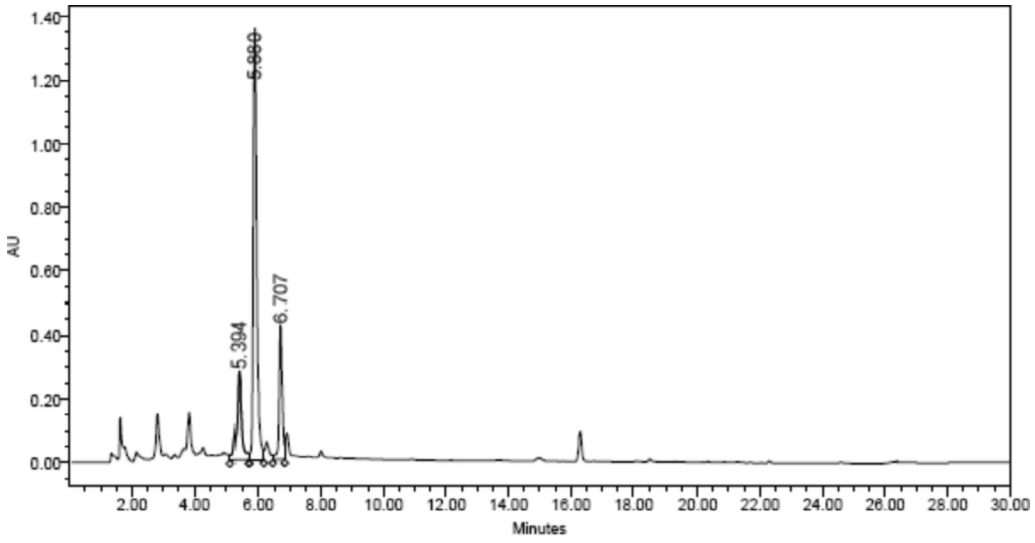


Fig. 1. Schematic UV-Photo Extraction. Where: 1. Motorbike, 2. Skeletons, 3. Shaft, 4. Panel buttons, 5. Tubes, 6. Heaters, 7. Outputs, 8. UV lamps, 9. Knives, 10. Inputs, 11. Beltsm, 12. Pulleys.

3. Result and discussion

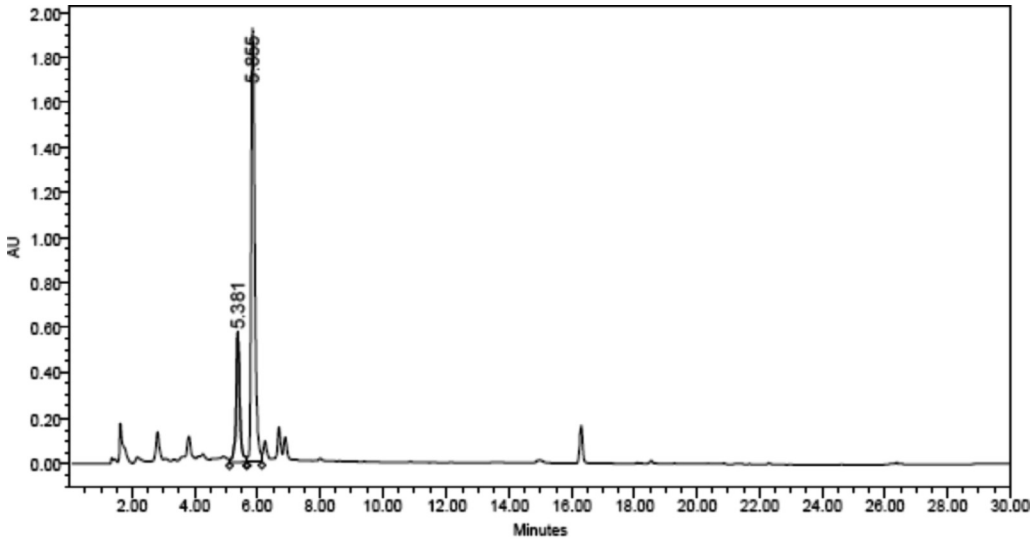
The gandapura extraction process using ethanol as a polar compound has two functions, namely deactivating the gaultherase enzyme and extracting the active compound gaultherin. In this case, the gaultherase activity can be stopped by polar compounds. The next mechanism is that the ethanol solvent enters through the tonoplasmic membrane wall so that phase contact occurs with the active compound gaultherin in leaf cells. The polar solvent diffusion that occurs will bring gaultherin out of the leaf cells, which is caused by differences in the solubility. The addition of drying agents, such as calcium chloride, can catalyze the reaction. Therefore, the production of gaultherin is higher and is useful for the process of extracting water from material by placing the material in a solvent with a high concentration where there is a semipermeable membrane between the two materials [3]. The mass transfer occurs from water to a higher concentration solvent. As a result, the probability of a hydrolysis reaction converting gaultherin to methyl salicylate is low [2].

The analysis to determine the levels of ghaultherin was carried out using HPLC-MS analysis and the curve obtained can be seen in Figs. 2 to 6 and compared using the standard on the patent [12].



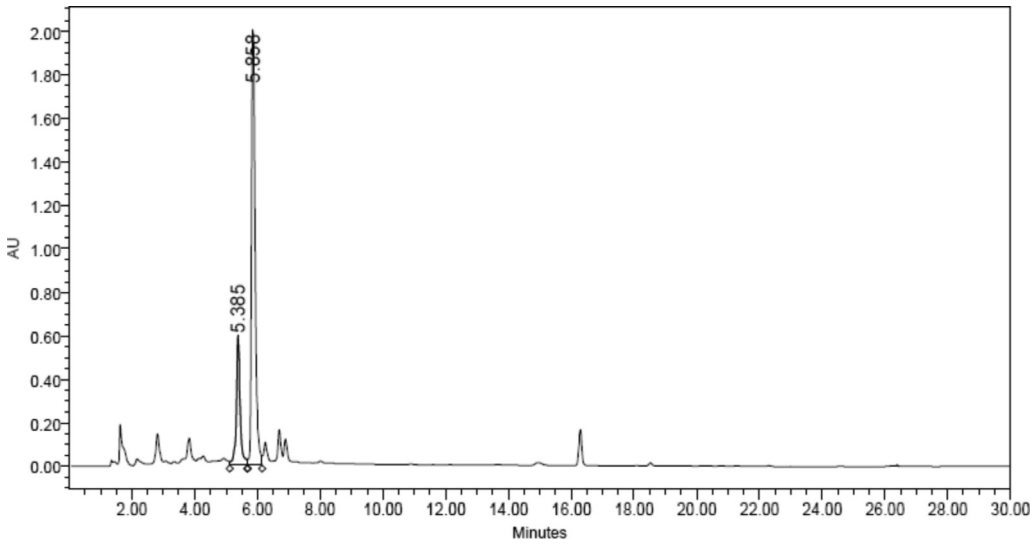
	RT	Area	% Area	Height
1	5.394	2732199	16.91	277974
2	5.880	10476457	64.86	1353584
3	6.707	2944785	18.23	424668

Fig. 2. Gaultherin HPLC-MS Analysis with Concentration Ethanol 85%-10 Minutes.



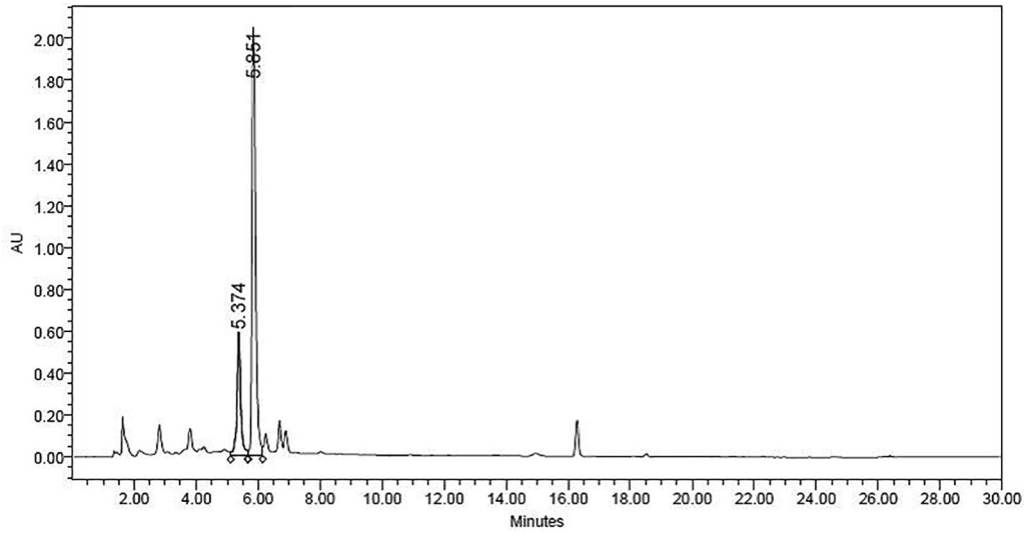
	RT	Area	% Area	Height
1	5.381	4676283	23.32	568331
2	5.855	15377226	76.68	1925661

Fig. 3. Gaultherin HPLC-MS Analysis with Concentration Ethanol 90%-10 Minutes.



	RT	Area	% Area	Height
1	5.385	5020103	23.78	597144
2	5.858	16094667	76.22	2023848

Fig. 4. Gaultherin HPLC-MS Analysis with Concentration Ethanol 90%-30 Minutes.



	RT	Area	% Area	Height
1	5.374	5041140	23.66	592922
2	5.851	16264585	76.34	2038768

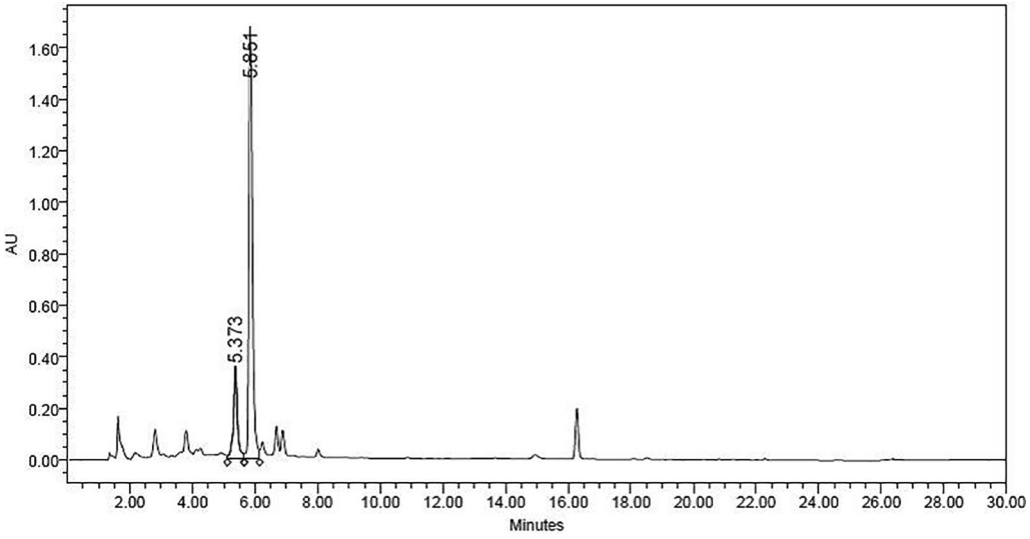
Fig. 5. Gaultherin HPLC-MS Analysis with Concentration Ethanol 90%-50 Minutes.

Referring to the patent, the gaultherin peak was detected at the retention time of 5.8 min. In Fig. 2, which is the result of analysis on the extraction of gandapura using an ethanol concentration of 85% and extraction time of 10 min, it has an area of 10,476,457 and has a height of 1.353 a.u.

In Fig. 3, which is the result of analysis on the extraction of gandapura using an ethanol concentration of 90% and extraction time of 10 min, gaultherin area is 15,377,226 and its height is 1.925 a.u. The area and altitude values from the analysis were higher than in gandapura extraction with an ethanol concentration of 85%-10 min. This means that the higher the ethanol concentration, the higher the gluttherin gain; which is in line with [1,11]. The research states that the addition of polar compounds can increase the yield of gaultherin.

In Fig. 4, which is the result of analysis on the extraction of gandapura using an ethanol concentration of 90% and extraction time of 10 min, gaultherin area is 16,094,667 and its height is 2.023 a.u. The higher the extraction time, the higher the yield of gaultherin; which is in accordance with research by [1] stating that the increase in extraction time can increase the yield of gaultherin.

In Fig. 5, which is the result of analysis on the extraction of gandapura using an ethanol concentration of 90% and extraction time



	RT	Area	% Area	Height
1	5.373	3230528	19.69	360165
2	5.851	13176977	80.31	1673426

Fig. 6. Gaultherin HPLC-MS Analysis with Concentration Ethanol 96%-10 Minutes.

Table 1
Comparison of gaultherin compounds obtained in each analysis variable.

Variable	Gaultherin Area	Area (%)
Gandapura with Ethanol 85%-10 min	10,476,457	14.67498
Gandapura with Ethanol 90%-10 min	15,377,226	21.53978
Gandapura with Ethanol 90%-30 min	16,094,667	22.54474
Gandapura with Ethanol 90%-50 min	16,264,585	22.78275
Gandapura with Ethanol 96%-10 min	13,176,977	18.45776
Total	71,389,912	100

of 50 min, gaultherin area is 16,264,585 and its height is 2.038 a.u. The higher the extraction time, the higher the yield of gaultherin; which is in accordance with research by [3] stating that the increase in extraction time can increase the yield of gaultherin.

In Fig. 6, which is the result of analysis on the extraction of gandapura using an ethanol concentration of 90% and extraction time of 10 min, gaultherin area is 13,176,977 and its height is 1.6734 a. u. The increase in the ethanol concentration up to 96% resulted in a decrease in gaultherin from the 90% ethanol concentration. This means that the 96% ethanol concentration is not optimum for use in the extraction process.

Table 1 is a comparison of the area of the gaultherin compound obtained at the 5.38 min retention. The result indicates that the higher the concentration of ethanol and the longer the extraction time used, the higher the gultherin compound obtained, when the ethanol concentration is increased to 96% of the total area in gaultherin compounds decreased. This is because the 96% ethanol concentration was not optimally used, and the best variable was the addition of ethanol with a concentration of 90% and extraction time of 50 min. From the results obtained, UV Photo extraction was proven to be able to properly remove gaultherin compounds from the gandapura plant.

4. Conclusion

The gandapura extraction process using ethanol as a polar compound has two functions, namely deactivating the gaultherase enzyme and extracting the active compound gaultherin. The effect of UV photo extraction on the acquisition of gaultherin compounds has succeeded in deactivating the gaultherase enzyme and opening when it is penetrated with ethanol. Therefore, the hydrolysis reaction of gaultherin into methyl salicylate catalyzed by the gaultherase enzyme decreases and the yield of gaultherin increases due to degradation of cellular cells by UV light. In this study, the peak of the dominant gaultherin compound was found at the retention time of 5.8 min, and the highest area was at 90% ethanol concentration variable with an extraction time of 50 min, and the area was 22.783% of the total gaultherin area. From the results obtained, UV Photo extraction was proven to be able to properly remove gaultherin compounds from the gandapura plant.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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