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Total Phenols and Bioactive Compounds in Karamunting Leaves Based On Temperature, Drying Time, and Different Leave Levels

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ABSTRACT

Karamunting leaves (*Rhodomyrtus tumentosa*,(W.Ait),Myrtaceae) are very potential leaves in Kalimantan. Karamunting leaves contain bioactive compounds of flavonoids, alkaloids, steroids and saponins. Flavonoids are known to play a role in capturing free radicals or function as natural antioxidants. In lowering blood sugar, flavonoids have mechanisms of action, including inhibiting α-glucosidase enzyme activity, inhibiting fatty acid oxidation, and capturing free radicals. Karamunting leaves are a very abundant source of flavonoids. The use of karamunting leaf flavonoids as a medicine for diabetes mellitus is obtained by extraction, this also has a patent certificate. The treatment that will be used in this study is drying by providing variations in temperature and drying time, and based on the level of leaf location. This research aims to: Determining the optimal temperature and drying time and protecting the total phenol and bioactive compounds in karamunting leaves, determine the level of leaves that have optimal total phenol and bioactive compounds. This study used Response Surface Method/RSM Analysis with a Central Composite Design consisting of 3 factors. The response variable (Y) is water content and the amount of phenol resulting from drying, while the independent variable (X) consists of 3 factors, namely drying time (X_1) consisting of 3 levels (120, 150 and 180 minutes), drying temperature (X_2) consisting of 3 levels (50, 60, and 70 ºC), and the karamunting leaf stage (X_3) consists of 3 levels (1-5 from shoots, 6-10 from shoots, and 11-15 from shoots). Parameters observed were: amount of phenol, water content, and components of bioactive compounds. The results showed that optimization of the drying treatment was obtained when the drying time reached 180 minutes, the drying temperature was 70 ºC, and the 3rd leaf level, would give the best results, namely total phenol 61.038 mg GAE/g and water content 3.504%.

Keywords:

Drying karamunting, leaf, shoots, temperature, time

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INTRODUCTION

Karamunting leaves (Rhodomyrtus tumentosa,(W.Ait),Myrtaceae) are very potential leaves in Kalimantan. Karamunting plants are very easy to grow and difficult to control, this is shown by the density of karamunting plants of 2.18 trees/m2, with a wet leaf production range of 2.5 – 3 tonnes/ha [1]. Karamunting leaves contain bioactive compounds of flavonoids, alkaloids, steroids and saponins. Based on research results, all organs of the karamunting plant contain bioactive compounds such as: flavonoids, alkaloids, sapaonins, and anthocyanins [2]: [3]: [4] and [5]. Flavonoids are known to play a role in capturing free radicals or function as natural antioxidants [6].

In lowering blood sugar, flavonoids have a mechanism of action, including inhibiting α-glucosidase enzyme activity, inhibiting fatty acid oxidation, and capturing free radicals [7]. In addition, saponins in karmunting leaves have the ability to lower blood sugar, precipitate intestinal mucous membrane proteins and form a layer that protects the intestines, thus inhibiting glucose intake and the rate of increase in blood glucose [8]. Therefore, karamunting plants have great potential to be developed into phytopharmaca, especially as a medicine for diabetes mellitus.

Diabetes Mellitus is a condition in which the level of sugar in the blood is higher than usual or normal, due to a lack of insulin in the body. This disease is chronic, and if it is left uncontrolled or the patient is not aware of the disease, various chronic complications will arise which are fatal [9]. In general, people still think that diabetes is a parental disease or a disease that only arises due to hereditary factors. In fact, everyone can have diabetes, both young and old. According to data from the International Diabetes Federation (IDF), in 2020 the number of people with diabetes is 415 million and is expected to continue to increase in 2040 to around 642 million people or 55% of the total world population. There are an estimated 10 million people with diabetes in Indonesia, and Indonesia is ranked 7th out of 10 countries with the largest diabetes sufferers worldwide. The number of sufferers increases potentially every year [10]. If this is allowed to continue, it will have a negative impact on society. On this basis it is necessary to look for a solution to the treatment.

So far, diabetes mellitus sufferers have always relied on treatment using chemical ingredients that are easy to obtain and practical, without thinking about the risks that exist when consumed regularly for a long time. So it is necessary to find alternative medicines for people with diabetes mellitus that do not cause negative effects. Alternative treatment besides using chemical drugs, is treatment using traditional medicine by utilizing flavonoid compounds found in karamunting plants as a medicine for diabetes mellitus. Based on the amount of flavonoid compounds in plant organs, the largest content is found in leaves, namely 5.625%, so this is a consideration for researchers to use leaves as a medicine for diabetes mellitus [11].

The use of karamunting leaves as a medicine for diabetes mellitus has been studied in 2008 with the results that karamunting leaves have the

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ability to lower blood glucose levels in rats affected by diabetes mellitus such as glibenclamide (a diabetes drug). The results showed that karamunting leaf extract has the potential to be used as medicine for people with diabetes mellitus. This is indicated by its ability to lower blood sugar levels in mice. Doses of karamunting leaf extract of 50 mg/kg to 150 mg/kg of karamunting leaf extract were able to reduce blood sugar levels in mice, both given once or twice a day with the highest ability at the treatment dose of 150 mg/kg given 2 times a day [1].

The use of karamunting leaves as a medicine for diabetes mellitus has a patent certificate. The raw material in the form of karamunting leaves used in this study were karamunting leaves of all ages and leaf locations, without sorting. Even though it does not rule out, from differences in age and level of location the leaves may contain different bioactive compounds. For example as in tea leaves, where the number of active compounds and antioxidants is different. Apart from the different ages and levels of leaf location, the conditions for processing karaunting leaves into diabetes mellitus drugs also need to be considered [12].

Considering that the extraction results are easily damaged, we think that to extend the shelf life of karamunting leaves, as well as facilitate their distribution to consumers, it is necessary to carry out further processing and packaging of the finished product later. The treatment to be used in this study is drying by providing variations in temperature and drying time. As we all know that giving different temperatures in the extraction and processing processes will definitely give different results, even for certain groups of plants, the higher the temperature given will result in damage to the active compounds in the processed material [13]. Likewise with the time given. The longer processing or drying time given will cause a longer heating effect on the processed or dried material [14].

The activity to be carried out is to determine the most effective raw materials in producing bioactive compounds by selecting the most appropriate leaf age and optimal drying process conditions. The aims of this research are: 1). determining the optimal temperature and drying time and protecting the total phenol and bioactive compounds in karamunting leaves, and 2). determine the level of leaves that have optimal total phenol and bioactive compounds.

METHOD

The research was conducted at the Agricultural Product Technology Laboratory, Faculty of Agricultural Technology, Borneo Tarakan University, from April 2022 to November 2022. The research used a quantitative method. The drying process in this study used the Response Surface Method/RSM Analysis [15]. The response variable (Y) is the amount of phenol resulting from drying, while the independent variable (X) consists of 3 factors, namely drying time (X1) (120 minutes, 150 minutes, 180 minutes), drying temperature (X2) (50ºC, 60ºC, 70ºC) , and grades of karamunting leaves (X3) (1-5 leaves from shoots (1), 6-10 from shoots (2),

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11-15 shoots (3)). This experiment uses a central composite design (Central Composite Design).

The mathematical model of this experiment is as follows:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_0 X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 \sum \sum_{i<j=1}^{2} \beta_{ii} X_i X_j + \in$$

……..[15]

Y is the response (result), β0 is a constant, βi, βii, βij are the coefficient of the independent variable (X), X is the independent variable with no code. X1 is the variable drying time (X1) (120 minutes, 150 minutes, 180 minutes; drying temperature (X2) (50ºC, 60ºC, 70ºC), X3 the level of caramunting leaves (1, 2 and 3) and ε is random errors. Parameters The total phenol, water content, and components of the bioactive compounds were measured using a spectrophotometer using the Folin-Ciocalteau reagent [16], the water content was tested using the gravimetric method [18], and the components of the bioactive compounds were measured using GC-MS [18].

RESULT AND DISCUSSION

The total phenolic test aims to determine the total phenolic compounds contained in the sample, so it is suspected that if the content of phenolic compounds in the sample is high, the antioxidant capacity will be high [19]. The total phenolic content in each leaf based on its grade and temperature treatment, as well as drying time is expressed as Gallic Acid Equivalent (GAE). Apart from phenol, the parameters observed were water content and the constituent compounds of dried kaarmunting leaves. The following is the total phenol and water content of caramunting leaves due to the treatment of drying time, drying temperature and leaf level.

Table 1. Total Phenol (mg GAE/g) and water content (%) of caramunting leaves due to variations in temperature (º C), drying time (minutes) and leaf age.

Treatment	Drying time (minutes)	drying temperature (˚C)	Age of leaves	Total Phenol (mg GAE/g)	Water content (%)
P1	120.00	50.00	1.00	46.83	2.08
P2	180.00	50.00	1.00	45.61	6.9
P3	120.00	70.00	1.00	45.37	1.65
P4	180.00	70.00	1.00	44.83	5.8
P5	120.00	50.00	3.00	63.64	5.18
P6	180.00	50.00	3.00	63.25	4.26
P7	120.00	70.00	3.00	65.23	2.4
P8	180.00	70.00	3.00	56.01	2.38
P9	99.55	60.00	2.00	40.23	6.33
P10	200.45	60.00	2.00	38.5	2.84
P11	150.00	43.18	2.00	39.92	7.08
P12	150.00	76.82	2.00	53.75	1.51
P13	150.00	60.00	0.32	53.09	2.75
P14	150.00	60.00	3.68	55.87	3.2
P15	150.00	60.00	2.00	54.46	2.25
P16	150.00	60.00	2.00	55.42	2.5
P17	150.00	60.00	2.00	56.72	3.08
P18	150.00	60.00	2.00	56.72	3.4
P19	150.00	60.00	2.00	55.05	3.32
P20	150.00	60.00	2.00	56.27	3.38

a. Predictive Model Analysis

The results of the analysis of the model to be used, based on the results of the sum of square test, show that the right model is Quadratic vs 2FI. This is indicated by the probability that the value of p > F is smaller than 0.05, which is 0.0379. Next is to look at the fit between the second order models, with the lack of fit test. A good model is a model that does not have a lack of fit or if the value of "p-value prop > F" ≥ 5%. There are two models that do not have a lack of fit, namely the quadratic and cubic models. This is because the p-value prob > F is greater than 5%, and the þ-value is the largest in the quadratic model, so the quadratic model is suitable.

The highest Predicted R-Square value and the lowest PRESS value are in the Quadratic model. So from this test, the quadratic model was chosen

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as the best model. The R-Square value in the quadratic model is 0.978 or 97.8%, which means that the quadratic model is able to explain the diversity contained in the data regarding the effect of extraction time and temperature on total phenol by 97.8%. While the remaining 12.2% is explained by errors and other factors not examined. From the response surface method, the second order model polynomial equation is obtained with the coded equation as shown below. The quadratic regression model obtained is as follows:

Total phenol = $55.688 - 4.842\ X_1 + 0.584\ X_2 - 1.086\ X_3 - 3.030\ X_1X_2 + 1.270\ X_1X_3 + 1.705\ X_2X_3 - 0.245\ X_1^2 + 0.240\ X_2^2 + 0.099\ X_3^2$ .....(1)

Where :

Y1 : Total Phenol Response Variable

X1 : Drying Time Factor

X2 : Drying Temperature Factor

X3 : Level of leaves

This equation shows that the total phenol response will increase in direct proportion to the increase in temperature, time and level of leaves as indicated by a positive constant value. The quadratic equation model (Equation 1), based on the results of the analysis of variance, explains that 97.8% of the total variance is included in the total phenol value, so the model can explain the actual conditions of temperature, time, and leaf age to total phenol.

b. Factor Influence

1. Drying Time

The results of the research on the value of analysis of variance show that drying time does not have a significant effect on the model. The total phenol due to the drying time treatment is presented in Figure 1 below

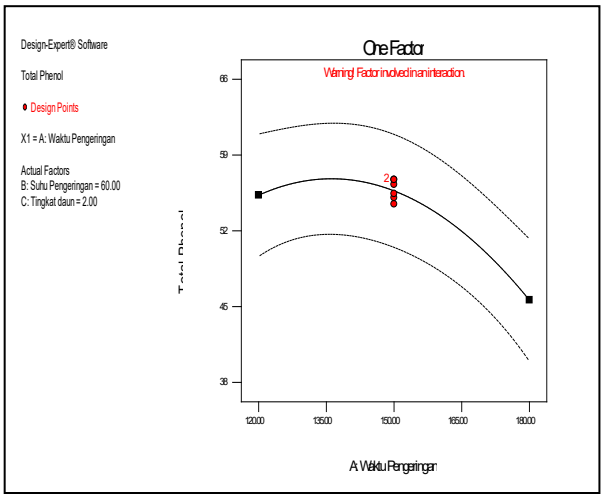

Figure 1. Profile of drying time to total phenol

In Figure 1. it can be seen that in the drying process, the total phenol will increase with the increase in the time given. But the total phenol will decrease with the use of time above 150 minutes.

2. Drying Temperature

The results of the analysis obtained the value of analysis of variance for the drying temperature treatment of total phenol shown in Figure 2.

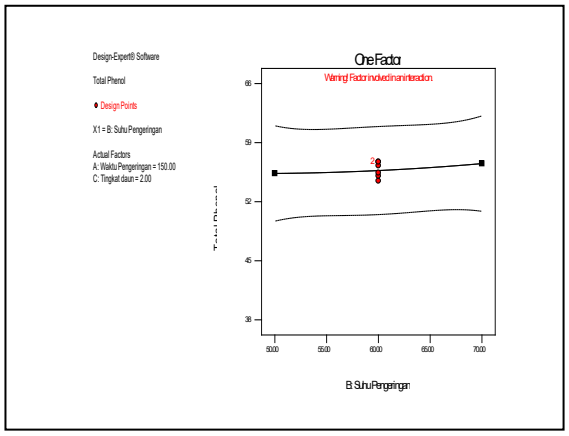

Figure 2. Profile of drying temperature to total phenol

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In Figure 2, it can be seen that the higher the drying temperature the total phenol increases up to a certain temperature limit. The higher the temperature and drying time, the higher the total phenol content in caramunting leaves, this is also in accordance with research conducted by [20] on the process of drying bamboo leaves into tea. It is suspected that the antioxidant compounds in the form of phenolic acids are resistant to the heat used.

According to [21] the phenolic acids can be active at temperatures of 50℃, 60℃ and 70℃ for 100 minutes, 160 minutes and 180 minutes. So if drying at 60° C for 3 hours is the optimal temperature to activate the phenolic compounds, however, drying temperatures that are too high at 100°C can cause a decrease in the phenol content in the material. This happens because heat that is too high can cause damage to the constituent components of leaf cell walls, namely carbohydrates (including cellulose fiber) and protein as insoluble components. This damage can facilitate the release of polyphenolic compounds from the leaves, this is because polyphenols are compounds that have a low molecular weight, making them easy to infuse into solvents.

3. Leaf Age Profile

Based on the results of the study, the value of the analysis of variance was obtained that the age level of the leaves did not have a significant effect on the model. The profile of leaf age level to total phenol is presented in Figure 3 below.

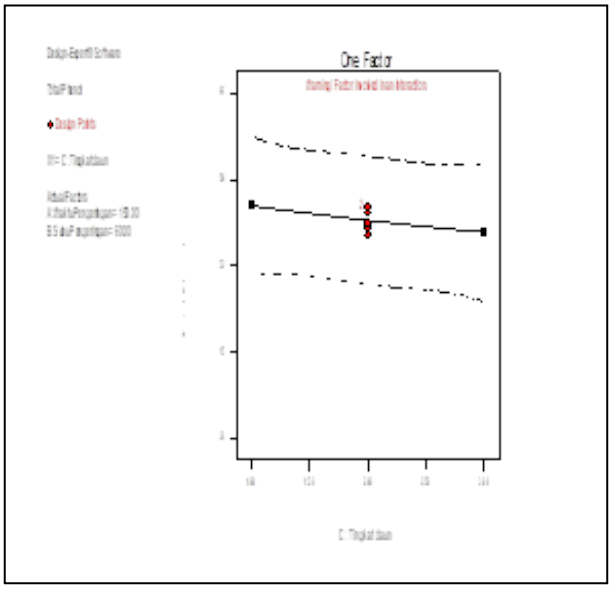

Figure 3. Leaf age level profile to total phenol

Based on the pictures above, it can be seen that the total phenol is directly proportional to the length of the drying process, the total phenol of karamunting fruit will be higher as the drying time increases, but the total phenol will decrease after the optimum time has passed. The graph shows that after 150 minutes of drying time, the total phenol will decrease. [22] stated that the longer the time, the longer the heating effect on the caramunting leaves and the greater the chance of contact with the material so that the result will increase to the saturation point.

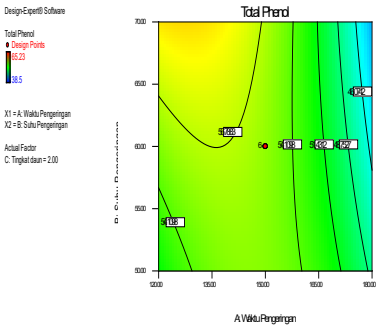

Figure 4. Response Surface Plot 3 Dimensional Contour Form

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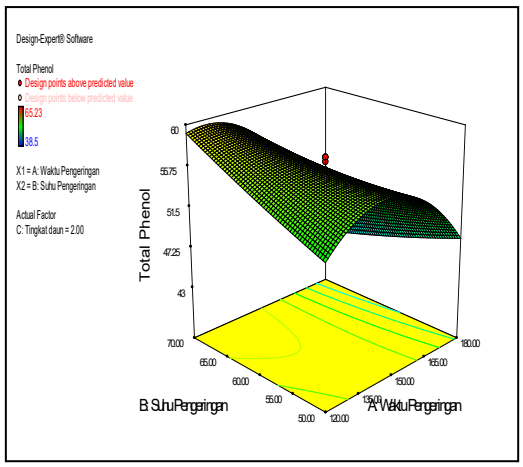

Figure 5. Effect of Variation of Temperature and Drying Time Treatments, and Leaf Age Levels on Total Phenol Response (mg GAE/g)

The figure represents the total phenol due to the influence of temperature variations and extraction time. The temperature and extraction time variable values were set to the optimum values on runing # 13 of the RSM (Response Surface Methodology) experimental design. In Figure 5 it can be seen that at various temperatures, the total phenol content in the extract of karamunting fruit obtained increased with increasing drying temperature, then stabilized and tended to decrease again. This is also the same as the effect of drying time. The use of high temperatures in the drying process will increase the solubility of cell walls or bound phenolic compounds, this is caused by damage to cell elements, causing more and more phenolic compounds to be extracted.

Figure 5. shows that the increasing the drying temperature and the longer the drying time the total phenol will increase up to a certain point. The higher the temperature, the easier it will be for the phenol to come out of the caramunting leaf cells. Heating during the drying process also serves to inactivate the polyphenol oxidase enzyme. The higher the temperature used in the drying process, the higher the inactivation of the polyphenol oxidase enzyme so that the enzyme activity will be lower, and the phenol damage will be smaller. However, the phenol content will also be disturbed by increasing drying temperature so that the total amount of phenol detected will reach a maximum peak then be constant and tend to decrease.

Higher phenol levels were found in the treatment of old caramunting leaves, namely at level 3 (ie 11-15 from shoots) had total phenol between 56.01 - 65.23 mg GAE/g body material, which were treated with variations in temperature and duration. different drying. This is in accordance with what was reported by [23] on Nypa fruticans leaves, namely old leaves have higher total phenol levels than young leaves. During the growth period, plants synthesize different amounts of secondary metabolites and bioactive compounds which are influenced by leaf morphology and age.

The processing method also greatly influences the total phenolic content of avocado leaf powder herbal tea. In general, the phenol content will decrease during drying with increasing temperature, phenolic acids will show a decrease with increasing temperature. In the presence of heat and oxygen, phenol compounds can be oxidized due to the activity of polyphenol oxidase enzymes to form orthosemiquinone radicals which are reactive and can react further with amino compounds to form brown products with

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high molecular weights. Therefore, karamunting leaves which were dried at a higher temperature for a longer time had lower total phenol levels.

4. Prediction of factor values to obtain optimal antioxidant capacity results

Optimum prediction of drying time, drying temperature and leaf age level, there are 16 computational solutions for optimizing factors sorted by desirability level**.**

Table 2. Computational Solutions for Prediction of Optimal Total Phenol Value and Moisture Content Using the Design-Expert DX 7.1.1 Program

Number	Drying Time	Drying Temperature	Leaf rate	Total Phenol	Water content	Desirability	
1	180.000	70.000	3.000	61.038	3.504	0.479	Selected
2	179.774	70.000	3.000	61.049	3.506	0.479	
3	179.999	70.000	2.991	61.055	3.501	0.479	
4	179.479	69.996	3.000	61.060	3.462	0.479	
5	180.000	69.894	3.000	60.955	3.494	0.479	
6	178.998	70.000	2.992	61.102	3.504	0.479	
7	178.661	70.000	3.000	61.104	3.420	0.478	
8	180.000	69.755	3.000	60.847	3.423	0.478	

Based on Table 2, it can be seen that the treatment solution to obtain the optimum yield (total phenol and water content) was 180 minutes drying time, 70 ºC drying temperature, with the 3rd leaf level.

5. Identification of Drying Result Compounds based on Total Phenol

There are 33 constituent compounds identified in dried caramunting leaves which are presented in Table 3.

Table 3. Compounds of Karamunting Dried Leaves

peak	compound	Compound class	% RA
1	p Cymene	aromatik	0,85149
2	β Pinene	aromatik	0,96152
3	Limonene	aromatik	0,96152
4	Myrcene	Flavonoid	1,48947
5	Myrtenal	Flavonoid	1,58175
6	Rosefuran	aromatik	1,05802
7	Caffeic acid	Phenolic acid	2,21473
8	β Ionone	karotenoid	0,94068
9	Ferulic acid	Phenolic acid	1,89295
10	β Caryophyllene	Aromatik	2,02761
11	β Sesquiphellandrene	Aromatik	1,41364
12	β Elemene	aromatik	0,93689
13	Kaempferol	Flavonoid	4,53045
14	Quercetin	Flavonoid	3,97659
15	Myricetin	Flavonoid	1,58164
16	Campesterol	Flavonoid	3,07878
17	Stigmasterol	Flavonoid	2,55941
18	β Sitosterol	Flavonoid	2,07134
19	β Amyrin	Triterpenoid	4,98656
20	Taraxerol	Komponen lain	3,17736
21	Lupeol	Terpenoid	4,03251
22	Friedelin	Terpenoid	6,66648
23	Rhodomyrtoxin	Phloroglucinol	2,84213
24	ψ Rhodomyrtoxin	Phloroglucinol	3,79533
25	Rhodomyrtoxin B	Phloroglucinol	6,15375
26	Rhodomyrtoxin C	Phloroglucinol	3,05528
27	β Amyrenonol	Triterpenoid	2,38998
28	Rhodomyrtosone A	Phloroglucinol	7,31293
29	Rhodomyrtosone B	Phloroglucinol	6,43381
30	Rhodomyrtone	Phloroglucinol	3,76308
31	Betulin	Terpenoid	5,17220
32	3 β 21 α 22(30) hopene 3,29 diol	Komponen lain	3,07757
33	Betulin monoacetate	Triterpenoid	2,84125

In Table 3 it can be seen that the results of the GC-MS of dried karamunting leaves produced 33 compounds, with 7 compounds belonging to the aromatic group, 8 compounds belonging to the flavonoid group, 2 compounds belonging to the phenolic acid group, 1 compound belonging to the carotenoid group, 3 compounds belonging to the triterpenoid group, 3 compounds belonging to the terpenoid class, 7 compounds phloroglucinol group, and 2 other components.

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CONCLUSION

The results showed that optimization of the drying treatment was obtained when the drying time reached 180 minutes, the drying temperature was 70 ºC, and the 3rd leaf level, would give the best results, namely total phenol 61.038 mg GAE/g and water content 3.504% with 33 bioactive compounds that make up dry caramunting leaves. .

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