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BOOK OF ABSTRACTS THE 3rd INTERNATIONAL CONFERENCE ON SUSTAINABLE GLOBAL AGRICULTURE AND FOOD

09 - 10 November 2018, Ho Chi Minh City, Vietnam

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MICROENCAPSULATION AND FREEZE DRYING OF *CLINACANTHUS NUTANS* EXTRACT WITH THE ADDITION OF ARABIC GUM

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ABSTRACT

Clinacanthus nutans is the native plant in Indonesia that is traditionally used to reduce blood sugar levels. It contains alkaloids, flavonoids and terpenoids. Those antioxidant compounds might regulate the blood glucose by improving insulin production system in pancreas. *Clinacanthus nutans* extract is freeze dried to prolong its storage shelf life. Microencapsulation process is conducted to maintain the activities of bioactive compounds during drying and to give the stability of dried product. This research aimed to produce the microencapsulated *Clinacanthus nutans* extract with the addition of Arabic gum and to analyze its physicochemical properties. Arabic gums were added in three concentrations, which were 20 grams, 30 grams and 40 grams. Firstly, the bioactive compounds of *Clinacanthus nutans* were extracted with ethanol and then freeze dried. Physicochemical analysis were conducted for measuring moisture content, water activity, water absorption, solubility, wetting ability and antioxidant activity of microencapsulated *Clinacanthus nutans extract.* Addition of 40 grams of Arabic gum produced the lowest moisture content (2.792 %), the lowest water activity (0,149), the highest water absorption (26.133%), and the highest solubility (19.852%), the longest wetting time (434.163 seconds) and the highest antioxidant activity (79,128%).

Keywords: *Clinacanthus nutans*, microencapsulation, freeze drying, Arabic gum

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Microencapsulation and freeze drying of *Clinacanthus nutans* extract with the Addition of Arabic Gum

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Abstract

Clinacanthus nutans is the native plant in Indonesia that is traditionally used to reduce blood sugar levels. It contains alkaloids, flavonoids and terpenoids. Those antioxidant compounds might regulate the blood glucose by improving insulin production system in pancreas. *Clinacanthus nutans* extract is freeze dried to prolong its storage shelf life. Microencapsulation process is conducted to maintain the degradation of bioactive compounds during drying and to give the stability of dried product. This research is aimed to produce the microencapsulated *Clinacanthus nutans* extract with the addition of Arabic gum and to analyze its physicochemical properties. Arabic gums were added in three concentrations, which were 20 grams, 30 grams and 40 grams. Firstly, the bioactive compounds of *Clinacanthus nutans* were extracted with ethanol and then freeze dried. Physicochemical analysis were conducted for measuring moisture content, water activity, water absorption, solubility, wetting ability and antioxidant activity of microencapsulated *Clinacanthus nutans extract.* Addition of 40 grams of Arabic gum produced the lowest moisture content (2.792 %), the lowest water activity (0,149), the highest water absorption (26.133%), and the highest solubility (19.852%), the longest wetting time (434.163 seconds) and the highest antioxidant activity (79,128%).

Keywords : *Clinacanthus nutans*, microencapsulation, freeze drying, Arabic gum

INTRODUCTION

Indonesia is rich in natural resources that are used as traditional medicine. The knowledge about the use of medicinal plants in Indonesia is only based on experiences inherited from time to time and has not been scientifically tested (Silviana, 2017). Medicines that are trusted by the community can cure diseases, namely dandang gendis plants. Dandang gendis is a plant in the Acanthaceae family and includes shrub plants that grow in Southeast Asia (Silviana, 2017). Dandang gendis (Clinacanthus nutans) is a shrub plant that is often used as a hedgerow and is known by the community as diabetes, difficulty urinating, and dysentery. According to Arullappan et al. (2014) that dandang gendis leaf plants can be used as antidiabetic drugs, antidotes, anti-inflammatories, pain relievers and as antioxidants.

This research carried out an extraction process which was followed by encapsulation. This study carried out both methods because the researcher wanted to increase the economic value of the gandang gendis plant, extend the shelf life and keep the content contained in the gendis cucumber plants undamaged. In this study an extraction process was carried out to physically or chemically separate using a solvent. The extraction method carried out in this study is maceration. The maceration method was chosen because it was able to easily extract chemical components that could be damaged by heat. Encapsulation technique is a technology that aims to protect active compounds with coating materials (Martins et al. 2010). In this study the encapsulation method used was the freeze drying method. According to Nurulita (2008) and Peng et al. (2014), that in gendis cucumber plants there are active compounds including flavonoids, alkaloids and saponins. These compounds are a class of antioxidant compounds. Antioxidant compounds themselves are easily damaged by heat and oxidized by oxygen so the encapsulation method used is the freeze drying method. This research is aimed to produce the microencapsulated *Clinacanthus nutans* extract with the addition of Arabic gum and to analyze its physicochemical properties.

MATERIALS AND METHODS

Materials

The materials used in this study are *dandang gendis* leaves obtained through farmers in Keji area, ethanol 96%, 99% methanol, DPPH (2,2-Diphenyl -1-picyrlhydrazil) solution, aquades and arabic gum.

Equipments

The equipments used in this study are freeze dryer, filter cloth, blender, analytical balance with Ohous brand, rotary evaporator with Buchi brand, fortex under the Thermolyne brand, centrifuge with Hettich brand, hot plate, glass jar, porcelain cup, chomameter with Konica Minolta brand, spectrophotometer with Shimadzu brand, aw meter with Rotronic brand and glass tools commonly used in laboratories such as beaker glass, test tubes, centrifuge tubes, and erlenmeyer.

Leaf Extraction of Dandang Gendis

The leaf sample of the *dandang gendis* is dried using a *demumidifier* for 72 at a temperature of 400C. Then smooth using a blender then weigh as much as 200 grams. Ethanol 96% solvent was poured in a jar which had been filled with *dandang gendis* leaf powder then the maceration extraction process was carried out for 5 days (sample ratio: ethanol = 1:10). Then the macerated solution is filtered until the filtrate is obtained. The filtrate was evaporated until extracts were obtained from the leaves of *dandang gendis*.

Encapsulation of Dandang Gendis Leaf Extract

Powder making is done using the freeze dryer method. Arab encapsulates gum weighed as much as 20 grams, 30 grams and 40 grams. Then the ethanol extract of dandang gendis leaves was weighed as much as 4 grams and dissolved with aquadest to 100 ml. After that the

extract was frozen in the freezer for 24 hours, then dried using a freeze dryer for 72 hours at -1010C. The dried samples were smoothed after testing chemical and physical characteristics.

Testing of Chemical Characteristics

Analysis of Antioxidant Actives

A sample of 0.5 grams was dissolved using 5 ml of distilled water and then allowed to stand until the sample dissolved. After that, 0.1 mL of filtrate was taken and added 3.9 ml of DPPH (2,2-Diphenyl -1-picyrlhydrazil). Let stand for 30 minutes in a dark room. After the solution was measured the absorbance was using a spectrophotometer with a wavelength of 515 nm. In making blanks, take 0.1 mL of distilled water and add 3.9 ml of DPPH (2,2-Diphenyl -1-picyrlhydrazil). This test is carried out 4 times for each sample. The absorbance results obtained were measured by% antioxidant activity using the formula:

$$\% \text{ Antioxidant activity} = 1 - [\frac{\text{Sample Absorbance}}{\text{Blank Absorbance}}] \times 100$$

.

Determination of Moisture Content

The porcelain cup is dried using a temperature of 1050C for 1 hour. The dried porcelain cup was then put into the desiccator and then weighed the empty cup weight. The 2 g dandang gendis leaf powder was put into a porcelain dish and heated in a 1050C oven for 16 hours. After that, the porcelain cup is cooled in a desiccator and weighed until the weight is constant. (AOAC, 1995). The percent moisture content of the sample can be calculated using the formula:

$$\% \text{ water content} = \frac{w - (w1 - w2)}{\text{w}} \text{ x } 100$$

Information :

w = initial sample weight

w1 = sample weight + dried cup

w2 = empty cup weight

Analysis of *Water activity* (A_w)

The measurement of A_w *dandang gendis* leaf powder using A_w meter. A_w meter is set for 15 minutes of operation, then a sample of 5 grams is inserted into a transparent cylinder container. Then the container is inserted and closed with a detector plate for 15 minutes then the measurement results can be seen.

Physical Characteristics Testing

Higroxicity Analysis of Dandang Gendis Leaf Ethanol Extract Powder

An empty porcelain cup was dried using an oven at a temperature of 1000C for 60 minutes, then cooled with a desiccator and then weighed to a constant weight. A sample of 2 grams was weighed and then put into a porcelain dish. Weigh the sample and cup then put into a desiccator that has been set RH 75% for 1 week. After 1 week the sample is weighed then calculated% Hygroscopy uses the formula:

$$\% \text{ Hygroscopy} = \frac{\text{M} + \text{M1}}{\text{M}} \; x \; 100\%$$

Information :
M = initial mass (gram)
M1 = the amount of free water in the sample (gram)

Color Intensity Analysis

Color analysis was measured using a *chromameter* CR 400 with the brand Konica Minolta L* a * b. Measurements are made at three different points. The data obtained are L * values, namely brightness value, a * which is a mixture of red and green chromatic color parameters, then b * as a mixture of yellow and blue chromatic color parameters. Testing is done with 3 replications.

Percent Analysis of Powder Yields of Ethanol Extract from Dandang Gendis Leaf

The yield of dandang gendis leaves ethanol extract powder was determined based on the ratio between the weight of the powder and the weight of the solids used. Then percent yield is calculated using the formula:

$$\text{Rendement (\%)} = \frac{\text{powder weight}}{\text{solid weight}} \; x \; 100$$

Water Absorption Analysis

A total of 1 gram of sample was dissolved into 10 mL aquadest, then vortexed for 2 minutes. Then let stand for 15 minutes. Then centrifuged at 3000 rpm for 25 minutes. Separate the supernatant with the filtrate, after that, the sample contained in the centrifuge tube is weighed. The difference between the weight of the sample after absorbing water and the dry sample shows the amount of water absorbed by the powder.

Solubility Analysis

The sample was weighed and then put into a cup containing 20 mL of water. Samples were stirred using a *magnetic stirrer* with the help of *hotplate* whose temperature was set to 0ºC. After that, 5000 rpm centrifugation was carried out for 5 minutes. Supernatant was taken as much as 5 mL, then dried in an oven for 24 hours at 100ºC. Solubility is calculated using the formula:

$$\% \text{ Solubility} = \frac{\text{Dry mass after oven x 20}}{\text{Initial mass x 5}} \text{ x } 100\%$$

Wetting Ability Analysis

Wetting analysis in this study was conducted to determine how long it took the ethanol extract powder to be wetted by water as well as to determine the effect of adding arabic encapsulate gum to the results of wetting analysis. First, 1 gram of sample was poured in 150

mL of *aquadest* prepared in a 500 ml *beaker glass*. Then the time when pouring all samples to the wettest sample is recorded. This time is recorded as the time of wetting.

Data Analysis

Data analysis was performed with SPSS version 16.0 using the ANOVA (Analysis of Variance) method to determine the effect of arabic gum. If there is a real difference from the results, then further testing will be carried out using the Duncan test.

RESULTS AND DISCUSSION

In this study, 987.5 grams of wet dandang gendis leaves were used which were then dried for 72 hours at 400C. After drying, it was found that there were 237 grams of dried dandang gendis leaves. Dandang gendis dried leaf powder was added 96% ethanol as much as 2000 mL or 2 liters which was then extracted maceration for 5 days and then filtered on the extract solution and obtained the results of 1440 mL or 1.44 liters. The ratio of solvents to dandang gendis leaf powder is 1:10. Ethanol 96% solvent was chosen because of its cheap and volatile price. Arifin et al. (2006) said that the selection of ethanol 96% as a solvent was due to its ability to dissolve polar, semi-polar and non-polar compounds. In the results, the evaporation process was carried out so that the ethanol extract of the dandang gendis leaf was free of ethanol. The results of the evaporation process obtained 29.521 grams of dandang gendis leaf ethanol extract.

Rendemen Percentage

This study also used the aid of encapsulates in the form of Arabic gum with the drying process freeze drying. According to Nugroho et al. (2006) arab gum has properties that can protect or bind chemical compounds contained in a material. In addition Glicksman (1983) added that arabic gum has a very high level of solubility against water solvents. In this study the ethanol extract of dandang gendis leaves obtained through a rotary evaporator was mixed with arabic encapsulate gum and added with water. Then dried using a freeze dryer. According to Hariadi, (2013) the mechanism of the drying technique using the freeze dryer tool is different from the other drying, this is because the freeze drying technique uses a sublimation mechanism. Before the material is dried using a freeze dryer, it is frozen for 24 hours. According to Hariadi (2013) the purpose of this freezing is that during the drying process using a freeze dryer the sublimation process can run smoothly. Samples that had been frozen for 24 hours were then dried using a freeze dryer for 72 hours at -1010C. On the yield percent yield it can be seen that the most powdered ethanol extract of dandang gendis leaves was obtained by adding 40 grams of arabic gum which is 89.543% and the lowest sample with the addition of 20 gram arabic gum, 88.696%.

Solubility and Absorption

Table 1. Physical Analysis Based on Arab Gum Concentration

Concentration	Solubility (%)	Absorption(%)
A	18,795±1,773[a]	19,550±1,849[a]
B	19,088±1,853[a]	22,450±2,597[a]
C	19,852±2,733[a]	26,133±3,477[b]

Notes :
A = Arabic gum 20 grams + Ethanol Extract of Dandang Gendis Leaves 4 grams
B = Arabic gum 30 grams + Ethanol Extract of Dandang Gendis Leaf 4 grams
C = Arabic gum 40 grams + Ethanol Extract of Dandang Gendis Leaf 4 grams

Based on Table 1, the highest solubility rate was found in the ethanol extract powder of dandang gendis leaves with the addition of 40 gram arabic gum, which was 19.852 ± 2.733. The lowest solubility results in the addition of 20 gram arabic gum, which is 18,795 ± 1,773. As for the addition of 30 gum as much as 30 grams the resulting solubility level is 19,088 ± 1,853. After statistical testing, the addition of 20 grams of gum, 30 and 40 grams is not significantly different. In the opinion of Bambang (2017) on powder drink products containing sucrose, this product will easily dissolve in water, but if there are components that are not easily soluble in water, it will affect the yield of solubility. However, based on the results of the study it was shown that the highest solubility rate was the addition of 40 grams of gum arabic. This is because if the amount of encapsulated gum arabyang is added more and more, it will also produce a higher level of solubility (Nugroho et al., 2010). Based on the results of the study, the addition of 40 grams of arab gum is the best powder product for the solubility level.

Analysis of water absorption in the powder of ethanolic extract of dandang gendis leaves, showed that the addition of 20 grams of arabic gum produced the lowest water absorption value of 19,550 ± 1,849. While for the addition of gum, as much as 40 grams produced the highest water absorbency value of 26.133 ± 3.477. In the results of this study it can be seen that the addition of 40 gram arabic gum produces the highest water absorption value. Based on the results of statistical tests, the addition of arabic gum 20 grams and 30 grams was not significantly different but the addition of 40 grams of arabic gum was significantly different from the addition of 20 grams of arabic gum and 30 grams. In arabic encapsulates gum has 2 types of protein groups, including galactan arabino protein and glycos protein (Hakim, 2013). According to Soeparno (2005), protein will affect the ability of water absorption. So if the protein content added is higher in a food ingredient, then the ability to absorb water in these food products will be even higher. According to Chatrine et al. (2013) if the absorption of water in powder type products is getting higher, the powder product has better quality.

Wetting and Hygroscopic

Wetting of a food product according to Selomulya et al. (2013) is the ability of a powder to prevent a tension between the solid phase and look for calculated based on the time needed for the powder to be wetted evenly by water. Whereas according to Hartono and Widiatmoko (1993), the purpose of doing an analysis of the ability of wetting on a food product is to know the nature of the food, whether the food is hydrophilic or hydrophobic.

Table 3. Results of physical analysis of wetting and hygroscopicity

Concentration	Wetting (seconds)	Hygroscopyitasitas(%)
A	14.125±2.283[a]	1,991±0.002[a]
B	25.288±3.289[b]	1,996±0.001[b]
C	34.163±2.619[c]	1,999±0.001[c]

Notes :
A = Arabic gum 20 grams + Ethanol Extract of Dandang Gendis Leaves 4 grams
B = Arabic gum 30 grams + Ethanol Extract of Dandang Gendis Leaf 4 grams
C = Arabic gum 40 grams + Ethanol Extract of Dandang Gendis Leaf 4 grams

In Table 3, the powder of ethanol extract from the leaves of Dandang Gendis with the addition of 40 grams of gum arabic, the ability for wetting is the longest. This is because the results of the test analysis of the water content of the powder of the ethanol extract of the leaves of Dandang Gendis by adding gumar as much as 40 grams produce the lowest moisture content. In the opinion of Gustavo & Barbosa (1999), arab gum is a product of drying agents that can affect the hydration of water molecules in a food material through a drying process so that with the addition of arabic gum the higher the water content in a food material the smaller also. Hygroscopicity is the ability of the material to absorb water vapor that is contained in the environment. The high level of hygroscopicity in a food substance is easily damaged because it can easily absorb moisture (Mohamad et al. 2015). In the results of the study it can be seen that the addition of 40 grams of arab gum is the concentration which results in the highest hygroscopicity value. According to Hector et al. (2004), the nature of hygroscopicity can be influenced by the level of dryness of a food. If the food has a low water content, the food has a high hygroscopicity ability (Yuwono and Susanto, 2001). In this study the value of hygroscopicity increased with the decreasing water content. At the addition of gumar as much as 40 grams has the results of a low water content analysis value, this is also followed by the results of the analysis of the higher level of hygroscopicity.

Color Analysis

Table 4. Results of Color Analysis

Consentration	Color L*	Color a*	Color b*
A	48,185±0,743[a]	-7,392±0,179[a]	11,285±0,459[a]
B	60,047±1,433[b]	-6,528±0,241[b]	12,173±0,229[b]
C	61,858±1,191[c]	-5,972±0,115[c]	13,297±0,336[c]

Notes:
A = *Arabic gum* 20 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram
B = *Arabic gum* 30 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram
C = *Arabic gum* 40 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram

In Table 4, it can be seen that the color of the ethanol extract of the leaves of dandang gendis leaves with the addition of 40 grams of arabic gum has powder with a color level of the L * parameter that is 61,858 ± 1,191. In the powder of ethanol extract of dandang gendis leaves with the addition of 20 grams of gum arabic produced L * color of 48.185 ± 0.743. Based on the results of the study, the higher the addition of the Arabic gumy encapsulate used in making micorkapsules of dandang gendis leaf ethanol extract will produce a brighter color. This is supported by the theory of Warsiki (2010) who says, if the rate of addition of gumar is higher, it will affect the level of brightness. So that in the doran gendis leaf ethanol extract powder, this different brightness level is due to the arab gum has the ability to cover the leaves of dandang gendis leaves during the drying process. So that after statistical tests, the color parameters L * produce significantly different for all concentrations of addition of arabic gum.

In the a * color parameter, the powder of ethanol extract with the addition of gum as much as 40 grams produces a * color of -5,972 ± 0,115. Whereas in the powder of ethanol extract of dandang gendis leaves by adding gumar, a total of 20 grams produced a * color of -7,392 ± 0.179. Based on the opinion of Glicksman (1983), that the addition of gum can be used as a protector of the components of compounds that make up color. So that the color constituent found in the leaves of the gendis is protected. Therefore, with the addition of gum encapsulates on the powder of the ethanol extract of the leaves of Dandang Gendis, the more it will affect the final color produced on the powder. The color of the powder extract of the leaves of Dandang Gendis ethanol can be seen in Figure 8.

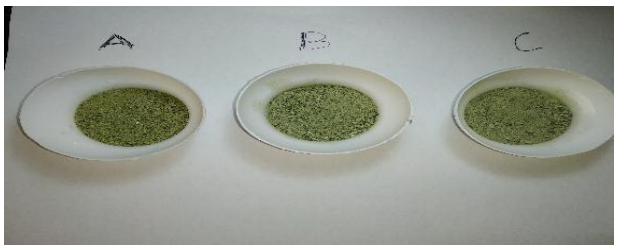

Figure 1. Powder of Ethanol Extract from the Leaf of Dandang Gendis with the Addition of Arabic Gum

Notes :
A. Powder of ethanol extract of dandang gendis leaves with the addition of 20 grams of gum.
B. Powder of ethanol extract of dandang gendis leaves with the addition of 30 grams of gum
C. Powder of ethanol extract of dandang gendis leaves with the addition of 40 grams of gum

Chemical Analysis (Antioxidants, Water Content, Aw)

Table 5. Chemical analysis of powder of ethanol extract from dandang gendis leaves based on 3 concentrations of gum with different additions.

Consentration	Antioxidants (%)	Water content (%)	*Water Activity*
A	71,647±3,321[a]	4,175±0,460[a]	0,212±0,006[a]
B	77,872±1,430[b]	3,342±0,204[b]	0,161±0,009[b]
C	79,128±1,703[c]	2,792±0,410[c]	0,149±0,004[c]

Notes :
A = *Arabic gum* 20 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram
B = *Arabic gum* 30 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram
C = *Arabic gum* 40 gram + Ekstrak Etanol Daun Dandang Gendis 4 gram

In this study the antioxidant activity in the powder of ethanol extract from the leaves of Dandang Gendis was analyzed using the DPPH method. Powder samples of ethanol extract from dandang gendis leaves were reacted with DPPH and then incubated in a dark room for 30 minutes. In Table 5, it shows that the treatment of addition of arabic gum increases the higher the antioxidant activity. In the results of the study, the highest antioxidant activity was found at a concentration of 40 grams of the use of arabic gum which was 79.128% ± 1.703 (79.128%) and the lowest activity was found in the use of 20 gram gum with antioxidant activity of 71.674% ± 3.231 (71.674%). According to Nugroho et al. (2010), Gum arabic is able to protect or bind to an active compound contained in a food ingredient. In this study antioxidant compounds bind with arabic gum so that the antioxidant compounds in the leaves of the dandang gendis leaf can be protected by arabic gum. So the results of the study show that, if the higher use of arabic gum, the antioxidant activity is also higher.

Water content in the form of food powder is an important parameter that makes whether the food has a long shelf life or not. According to Frakye et al. (2001) that if a powder has a low water content, the powder will be resistant to damage caused by microorganisms. In Table 5, it can be seen the results of the water content of the leaves of dandang gendis which have been dried for 72 hours at a temperature of 400C below 10%. In the results of the study, the value of water content in the powder of ethanol extract of dandang gendis leaves with the addition of 40 grams of arabic gum has a low water content compared to the addition of 20 grams of arabic gum and 30 grams. According to Denanda (2014) if the higher the amount of gum arabic added to the powder product, the total solids in the product will be higher. Increasing total solids will cause the amount of water present in the product to decrease. Srihari et al. (2010) added that the water content in a product will affect shelf life, product appearance and solubility level in water.

On the results of research on powder water activity, the ethanol extract of leaves of dandang gendis was below 0.6. According to Beuchat (1981) water activity from a powder-based product is required to have water activities of less than 0.6. This is because products that have water activities below 0.6 can inhibit damage caused by microorganisms. \\ In the results of the study the higher the amount of addition of arabic gum in the powder products of dandang gendis leaves ethanol extract, the smaller the water activity in the product. In the opinion of Denanda (2014), the higher the amount of addition of total solids in the powder products will be more so that the ability of products to bind water will be smaller. So that the free water contained in the product will be smaller. So the fine powder product of dandang gendis leaves ethanol extract seen from water activity is by adding 40 grams of gum, which is 0.149.

Conclusion

Increasing the addition of arabic gum contributed to the increase of colour intensity L*, b*,a* values, solubility, water absorption, wetting ability, hygroscopicity and antioxidant activity. Addition of 40 grams of Arabic gum produced the lowest moisture content (2.792 %), the lowest water activity (0,149), the highest water absorption (26.133%), and the highest solubility (19.852%), the longest wetting time (434.163 seconds) and the highest antioxidant activity (79,128%).

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