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Research Article

Extraction of oxalic acid from porang tuber by mechanical separation method

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Abstract. Porang tuber is a source of food that has many benefits. It also has the disadvantage of increasing the risk of health problems because of its high oxalate content. One of the methods for capturing oxalic acid from porang tuber flour is the Mechanical Separation method. Porang flour was added to ethanol-water solvent with a ratio of the solvent mixture-porang flour and an ethanol-water composition according to the variable. The mixing of porang flour and the solvent mixture were heated and added with HCl. The filtrate obtained was taken and diluted. The diluted filtrate was taken and titrated using KMnO4. This study aims to examine the effect of material size (between 60 - 80 mesh, between 80 - 100 mesh, and >100 mesh), the composition of the solvent mixture (60:40 v/v, 78:22 v/v, 96:4 v/ v), and the ratio of solvent with porang tuber flour (10:1, 15:1, 20:1) to oxalic acid yield in porang tuber flour. The results showed that the optimum conditions obtained from this RSM method are flour size of 100 mesh, composition of ethanol: water = 96:4, and the ratio of solvent to flour 20:1. Oxalic acid yield obtained in this variable is 30.2469%.

Keywords: Mechanical Separation, Oxalic Acid, Porang Tuber, Yield

1. Introduction

Plants are one of the resources that are closely related to human life. Plants are the supplier of their needs (Mutaqin et. al., 2020). Porang is one of the plants that is used in Indonesia as a food source. Porang is a type of bulbous plant that generally grows in Indonesian forests. The porang plant (Amorphophallus oncophyllus) is a member of the Araceae family and is known as the corpse flower because of its unpleasant odor. Porang plants have long been used as food and exported as industrial raw materials as plants that produce carbohydrates, fats, proteins, minerals, vitamins, and dietary fiber (Mutaqin et. al., 2020).

Porang tubers still cannot be directly consumed because they contain oxalic acid, even though they contain many benefits in porang tubers (Bahlawan et. al., 2021). The content of calcium oxalate can cause itching if not well treated. Several methods have been developed to extract and purify the glucomannan content in porang, either by mechanical (dry processing) or chemical (wet processing) methods (Bahlawan et. al., 2021).

The glucomannan purification methods can be treated with several extraction methods. That is the extraction method using MAE (Microwave Assisted Extraction), the extraction method using a Reflux Condenser, and the extraction method using modified maceration with a stirrer. This maceration modification has more advantages in relatively lower operational costs, and the process saves more solvents than other extraction methods (Bahlawan et. al., 2021). The glucomannan purification using a modified maceration technique can be treated using ethanol as a solvent (Nurlela et. al., 2019). The experiment resulted in the highest glucomannan content of 66.56% obtained by the Multi Concentration method.

At food extraction, water is preferable as a solvent than the other. That is because it can save research costs, has low health risks when mixed, suitability for food applications, and compatibility with the human body (Lajoie et. al., 2022). However, previous literature also reported that ethanol as a solvent could increase the yield of bioactive ingredients and glucomannan. It also can reduce harmful substances, such as oxalate (Hikmawanti et. al., 2021).

Faridah et. al. (2013) conducted a study on optimizing the production of calcium oxalate levels on porang chips mechanically using the response surface method. study showed that the response surface methodology could be used to optimize the experimental variables. The variables in this study are extraction time, stirring speed, and flour solvent ratio. These variables are important factors that affect the mechanical extraction process. The experiment showed that the extraction time was 4 hours 6 minutes 18 seconds, the stirring speed was 443.45 rpm, and the ratio of solvent to flour was 8.92 ml/g, which increased the glucomannan content and decreased the calcium oxalate content in the flour. This study optimizes the extraction of oxalic acid from porang flour using the RSM (Box-Behnken Design) method with various variables, namely flour size, ethanol-water solvent mixture, and the ratio of the solvent mixture to porang flour.

2. Material and methods

2.1. Materials

Porang flour is made from porang tubers in Tembalang area, Semarang, which is taken directly by porang farmers. This porang flour is made using a grinder. The porang chips are put into the grinding machine to grind and get porang flour. Porang flour is then sieved using a vibrating screen to get flour of between 60 - 80 mesh, 80 - 100 mesh, and >100 mesh. Ethanol (60%, 78%, and 96%), Hydrochloric Acid (HCl), Potassium permanganate, Sulfuric acid (H2SO4), and aquadest was used by Merck Ltd.

2.2. Extraction Method

Porang flour of each mesh (between 60 - 80 mesh, between 80 - 100 mesh, and > 100 mesh) be pondered as much as 5 grams, then added ethanol-water solvent with a ratio of the solvent mixture and porang flour 10:1, 15:1, and 20:1 with an ethanol-water composition of 60:40 v/v, 78:22 v/v, and 96:4 v/v. The mixing of porang flour and the solvent mixture was heated at 60oC for 15 minutes. Then 10 ml of 6 M HCl was added and reheated at 60oC for 15 minutes. The filtrate and precipitate were separated using a centrifuge for 15 minutes at 3000 rpm.

The filtrate obtained from the separation process was taken as much as 1 ml and diluted to 100 ml. The diluted filtrate was taken as much as 50 ml and added with 10 ml of 20% sulfuric acid (H_2SO_4), then titrated the solution using 0.05 M KMnO4 at 70^{o}C. The titration process is complete when the solution changes color to pink, which does not disappear for 30 seconds. The required titrant is recorded, and then the concentration and yield of oxalic acid are calculated using the following formula:

$$Oxalic\ Acid\ Level = \frac{5 \times M_{KMnO_4} \times V_{titrant} \times MW_{H_2C_2O_4}}{2 \times 1000 \times V_{solution}} \times D_F \times 100\% \quad (1)$$

$$Oxalic\ Acid\ Yield = \frac{m_{oxalic\ acid\ obtained}}{m_{sample}} \times 100\% \quad (2)$$

$$m_{oxalic\ acid\ obtained} = m_{total\ oxalic\ acid} \times Oxalic\ Acid\ Level \quad (3)$$

$$m_{total\ solution} = \rho_{sample} \times V_{solution} \quad (4)$$

2.3. Sample Analysis Method

Fourier Transform Infrared (FTIR) analysis is used to identify the functional groups of the samples. FTIR spectroscopy is used to characterize organic and inorganic compounds. The main advantage of FTIR spectroscopy is its ability to identify the molecular structure of both simple and complex functional groups.

2.4. Experimental Design

The research design was made using Response Surface Methodology (RSM). The Box-Behnken Design (BBD) is chosen as a design with three factors and three levels. Program Design Expert 13.0 with Response Surface Methodology (RSM) Box-Behnken Design is used to research and select process conditions from a combination of factor levels that produce an optimal response. The independent variables or factors used in the extraction process are the size of the material (X1 = between 60 - 80 mesh, between 80 - 100 mesh, and >100 mesh), a mixture of two solvents (ethanol-water) (X2 = 60:40 v/v, 78:22 v/v, 96:4 v/v), and the ratio of the solvent mixture and porang flour (X3 = 10:1, 15:1, 20:1). The dependent variable or the observed response was oxalic acid levels. Table 1 shows the independent variables and levels used for Box-Behnken Design. Table 2 shows the research design with Response Surface Methodology.

Table 1. Independent variables and levels used for BBD

Independent *v*ariables	Levels		
	-1	0	+1
Size of the material (X_1)	60 mesh	80 mesh	100 mesh
Ethanol : Water (X_2)	60:40	78:22	96:4
Solvent mixture : flour (X_3)	10:1	15:1	20:1

Table 2. Research design with Response Surface Methodology

Std	Run	Factors		
		X_1	X_2	X_3
15	1	1	-1	0
1	2	0	0	0
6	3	0	0	0
11	4	-1	0	1
8	5	-1	1	0
13	6	-1	0	-1
10	7	1	0	1
2	8	0	1	-1
4	9	0	0	0
14	10	0	1	1
12	11	1	0	-1
9	12	0	-1	-1
7	13	1	1	0
5	14	-1	-1	0
3	15	0	-1	1

3. RESULTS AND DISCUSSION

3.1. Model Response Surface Methods

The response surface method carries out for determining the suggested model to predict the response of oxalic acid levels. The model analysis is used to define the appropriate model in RSM and to predict the response of the independent variables in the study (Faridah and Widjanarko, 2013). The models evaluated include linear, 2FI, quadratic, or cubic. The model selection was selected based on the P-value, lack of fit test, and model summary statistics (R^2, Adjusted R^2, and standard deviation) (Faridah and Widjanarko, 2013). The following is an Analysis of Variance (ANOVA) test for various models.

The model is chosen based on the sum of squares sequential model, so that can show in table 4 that the significant and recommended model is 2FI. P-value smaller than 0.05 indicates that the variable is significant. P-value smaller than 0.001 indicates that the variable is very significant. It can be interpreted that the variable has a greater influence than other variables (Faridah and Widjanarko, 2013). The 2FI model is recommended because the p-value generated is 0.0109. This value means the p-value < 0.05. It indicates that this 2FI model has a significant effect on the response.

Table 4. Analysis of Variance (ANOVA) test for various models on the Mechanical Separation method

Model	Sequen-tial p-value	Lack of Fit p-value	Std. Dev.	R^2
Linear	0.142	0.419	3.91	0.378
2FI	**0.011**	**0.812**	**2.37**	**0.835**
Quadratic	0.378	0.878	2.25	0.907
Cubic	0.878		3.09	0.929
Model	*Adjust-ed* R^2	*Pre-dicted* R^2		
Linear	0.209	-0.21		
2FI	**0.711**	**0.63**	**Suggested**	
Quadratic	0.738	0.47		
Cubic	0.506		Aliased	

The 2FI model has a standard deviation that is smaller than other models, where the resulting R^2 is 0.8349 and the Adjusted R^2 is 0.7111 to increase the yield of oxalic acid in porang flour by the Mechanical Separation method. The value of R^2 indicates that the variable, such as the size of flour (mesh), the composition of ethanol: water (v/v), and the ratio of solvent and flours, are the variables that are significant on the response of 71.11%. The remaining 28.89% is affected by other factors that are not variables. Differences in flour size (Rehman et. al., 2020), ethanol-water composition, and the ratio of solvent and flour mixtures are the variables that can affect the yield of oxalic acid (Faridah and Widjanarko, 2013).

3.2. Response Surface and Effect of Variables on Oxalic Acid Yield in Porang Flour

The 2FI equation uses to predict the response of several levels. The following is a 2FI equation obtained.

$$Y = 19{,}22 + 3{,}20X_1 + 1{,}60X_2 - 0{,}083X_3 + 3{,}87X_1X_2 + 3{,}51X_1X_3 + 1{,}90X_2X_3 \quad (5)$$

This study uses three different variables as factors in the RSM method. These factors are the size of the flour (X_1), the composition of ethanol: water (X_2), and the ratio of solvent to flour (X_3). The response generated in this study is the yield of oxalic acid (Y). The effect of flour size, ethanol: water composition, and the ratio of solvent to flour processed in a 2FI model on the oxalic acid yield response is shown in the figure below.

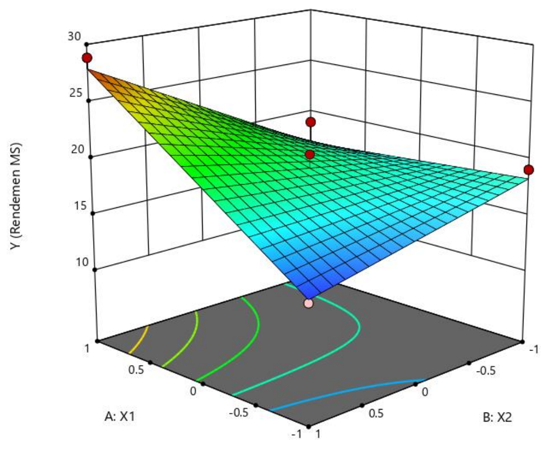

Fig. 1. The surface plot of oxalic acid yield in the Mechanical Separation method is influenced by flour size (X_1) and ethanol:water composition (X_2)

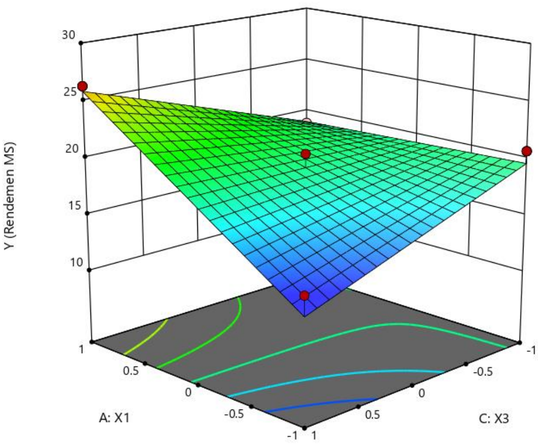

Fig. 2. The surface plot of oxalic acid yield in the Mechanical Separation method is influenced by the size of flour (X_1) and the ratio of solvent and flour (X_3)

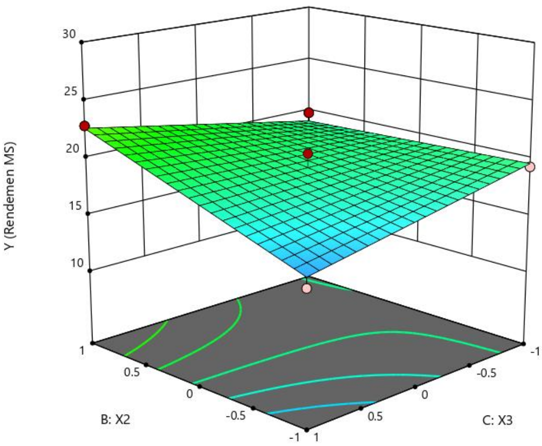

Fig. 3. The surface plot of oxalic acid yield in the Mechanical Separation method is influenced by the composition of ethanol: water (X_2) and the ratio of solvent and flour (X_3)

3.3. The Effect of Flour Size on Oxalic Acid Yield in Porang Flour

The oxalate levels in porang flour have a high concentration (Nurlela et. al., 2019). The content of oxalic acid in this porang flour will cause irritation and itching of the skin (Nurlela et. al., 2019). The oxalic acid in porang flour causes the low selling price of porang flour or porang tubers (Nurlela et. al., 2019). Oxalic acid consumption in adults ranges from 0.6 grams to 1.25 grams per day for six consecutive weeks (Nurlela et. al., 2019).

Extraction by the Mechanical Separation method is one method to reduce the levels of oxalic acid in porang flour. This method uses porang flour of various sizes as samples that contain oxalic acid. There are three sizes to be tested in this study, namely 60-80 mesh, 80-100 mesh, and >100 mesh. The difference in flour size in this study was used as a variable that affects the yield of oxalic acid because it is an important variable that affects extraction efficiency (Rehman et. al., 2020).

The smaller the particle size of porang flour can make more impure particles, such as starch and oxalate (Hermanto et. al., 2019). Other components, namely impurities such as oxalic acid in porang flours are smaller and easily broken into fine particles, while glucomannan is a component that is larger and harder (Nurlela et. al., 2019). This explains that the flour size of >100 mesh has more oxalic acid content than the flour size of 60-80 mesh and 80-100 mesh.

3.4. The Effect of the Ethanol-Water with Various Compositions on Oxalic Acid Yield

The extraction process with the Mechanical Separation method is carried out using a mixture of the solvent mixture ethanol-water in various compositions. This extraction process was carried out with 3 ethanol-water (v/v) compositions, namely 60:40 (60% ethanol), 78:22 (78%), and 96:4 (96%). This different composition of ethanol-water allows a particular component to dissolve in this solvent based on its polarity. If ethanol concentration is higher, that impact lowers the polarity level, allowing non-polar components to dissolve (Faridah and Widjanarko, 2013).

Oxalic acid in plants divides into two groups, soluble in water oxalate and insoluble in water oxalate (Nurlela et. al., 2019). Washing porang flour using ethanol can separate non-glucomannan compounds (Faridah and Widjanarko, 2013). That phenomenon happens because ethanol has a high polarity and can dissolve several compounds, such as resins, fats, oils, fatty acids, carbohydrates, and other organic compounds. In addition, ethanol solvent can also prevent glucomannan granules from expanding and dissolving into solution (Faridah and Widjanarko, 2013).

The composition of ethanol-water (v/v) 60:40 used as a solvent in the extraction process by the Mechanical Separation method will dissolve polar impurities, such as protein and sugar (Xu et. al., 2014). This phenomenon can happen because there is lots of water composition in the solvent. At ethanol-water (v/v) 78:22, it will cause dissolved starch (Nurlela et. al., 2019). The ethanol-water (v/v) 96:4 is the solvent with the lowest polarity (Faridah and Widjanarko, 2013). The ethanol-water (v/v) 96:4 will cause dissolved fat, crude fiber, and oxalate, including calcium oxalate (Nurlela et. al., 2019). The following statement explains that the ethanol-water composition (v/v) of 96:4 has a higher yield of oxalic acid than the composition of ethanol-water (v/v) of 60:40 and 78:22.

3.5. The Effect of the Ratio of the Solvent Mixture and Porang Flour on Oxalic Acid Yield

The ratio of the solvent mixture and porang flour is the variable used in the extraction process using the Mechanical Separation method. The ratio variable ratio of the solvent mixture and porang flour (v/w) has three variations, namely 10:1, 15:1, and 20:1. The fixed ratio (1) is the ratio for flour, and the changing ratio (10, 15, or 20) is the ratio for the ethanol-water solvent mixture. The ratio of the solvent and flour mixture in the extraction process is one of the things that affects the efficiency of the extraction process (Faridah and Widjanarko, 2013).

The volume of solvent that is more than the number of samples extracted will dissolve the impurity compounds more effectively than the amount of flour (Faridah and Widjanarko, 2013). The dissolved solids in the solvent will increase when the amount of solvent is added. This can occur due to particle dispersion that will increase mass transfer between particles into the solution (Faridah and Widjanarko, 2013).

A higher solvent ratio will produce porang flour and contains high glucomannan (Faridah and Widjanarko, 2013). The yield of oxalic acid in the solution will increase along with the amount of ethanol water used in the extraction process (Faridah and Widjanarko, 2013). The following statement explains that the ratio of ethanol-water and porang flour of 20:1 has a higher yield of oxalic acid.

3.6. Response Optimal Point and Verification

Based on the predicted value of the Response Surface Method for optimizing the Mechanical Separation method using ethanol-water solvent, verification was performed by conducting experiments using optimum conditions prediction. The prediction of optimum conditions obtained from this RSM method are flour size of 100 mesh, composition of ethanol: water = 96:4, and the ratio of solvent to flour 20:1. The following is a verification of the analysis value obtained from the research and the predicted response value from the design expert application.

Table 5. Optimum conditions for the application of the Mechanical Separation method expert design

	X_1	X_2	X_3	Oxalic Acid Yield (%)
Prediction	100	96	20	33.22
Actual	100	96	20	30.25
		Accuracy level (%)		91.04
		Difference value (%)		8.96

Verification is complete by comparing the value of the response analysis from the research to the response value from the design expert application (Table 5). The level of accuracy of oxalic acid yield is 91.04%, while the difference value is 8.96%. A significant difference in predictive value ($p<0.05$) was obtained from the experiment. It is shown in the validation of the RSM model. The analysis results confirm that the response model is sufficient to reflect the expected optimization and the model is satisfactory and accurate.

3.7. FTIR Test Results on Porang Flour Samples

Fourier Transform Infrared (FTIR) spectroscopy used to characterize organic and inorganic compounds (Baltacıoğlu et. al., 2021). FTIR allows for direct quantification of all components in a sample. The main advantage of FTIR spectroscopy is its ability to identify the molecular structure of both simple and complex functional groups and the interactions between two molecules by monitoring the molecular vibrations of the functional groups involved (Nolte et. al., 2022). The results of the analysis of the specific functional groups of oxalic acid in porang tubers that have been made into flour using FTIR are shown in Figure 4.

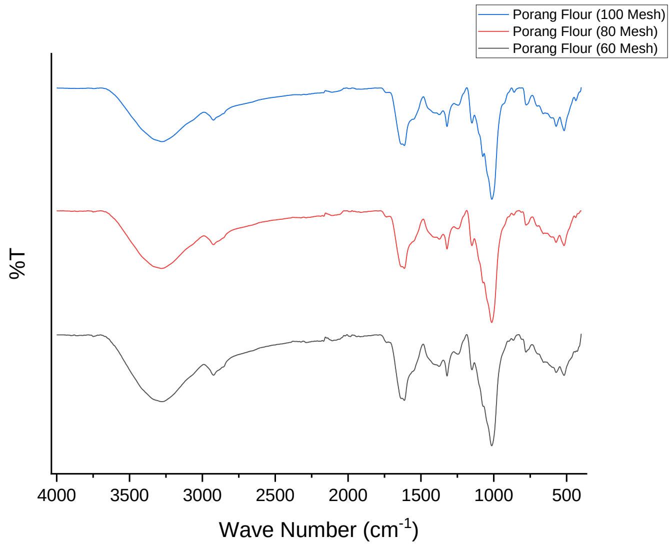

Fig. 4. FTIR Spectrum Results

Based on the FTIR spectrum above, the wavelengths representing oxalic acid in flour are 60 mesh, 80 mesh, and 100 mesh. In 60-80 mesh flour, the wavelength is 3278.19 cm^{-1} – 517.96 cm^{-1}. In 80-100 mesh flour, the wavelength is 3281.17 cm^{-1} – 518.20 cm^{-1}. In >100 mesh flour obtained a wavelength of 3278.65 cm^{-1} – 436.85 cm^{-1}. The wavelength of oxalic acid in various size of flour is visible in the groups obtained at the FTIR peak. Based on Figure 4, it is found that the functional group that is a constituent group of oxalic acid is the O – H group, which is a hydroxyl group with wave numbers of 3281.17 cm^{-1} dan 2922.90 cm^{-1}. An O-H group (the water bond) find at a wavelength of 3400 cm^{-1} (Azevedo et. al., 2006). There is also a C – C group, an alkaline group with a wave number is 2112.69 cm^{-1} – 2108.02 cm^{-1}. The C-C group is found only in oxalate crystals (Muthuselvi et. al., 2016). There is also a C = O group which is a carboxylate group with a wave number of 1614.15 cm^{-1} – 1613.91 cm^{-1}, and a C - O group, which is an ether or carboxylate group with a wave number of 1375.71 cm^{-1} – 777.44 cm^{-1}. The C = O carboxylate group is usually strong and appears at wavelengths between 1725 cm^{-1}- 1600 cm^{-1} and carboxylic acid group finds at a wavelength of 1260 cm^{-1} (Muthuselvi et. al., 2016).

4. Conclusions

The optimum conditions obtained from this RSM method are flour size of 100 mesh, composition of ethanol: water = 96:4, and the ratio of solvent to flour 20:1. A significant difference in predictive value ($p<0.05$) was obtained from the experiment. It is shown in the validation of the RSM model and the analysis results confirm that the response model is sufficient to reflect the expected optimization and the model is satisfactory and accurate. The impurity particles, such as oxalic acid, are found in porang flour, which has a smaller particle size. This variable compositional difference of ethanol-water allows a particular component to dissolve in this solvent based on its polarity. The composition of ethanol-water (v/v) 96:4 will cause dissolved fat, crude fiber, and oxalate, including oxalic acid. A higher solvent ratio will produce porang flour that contains high glucomannan. The yield of oxalic acid in the solution will increase along with the amount of ethanol water used in the extraction process. In the FTIR analysis, the groups contained in oxalic acid are O – H (hydroxyl group), C – C (alkaline group), C = O (carboxylate group), and C – O (ether or carboxylate group)

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References

Azevedo, W. M. D., Oliviera, G. B., Silva Jr, E. F., Jesus, E. F. O., 2006. "Highly Sensitive Thermoluminescent Carbon Doped Nanoporous Aluminium Oxide Detectors." *Radiation Protection Dosimetry*, 119(4).

Bahlawan, Z. A. S., Damayanti, A., Megawati. Cahyari, K., Andriani, N., Hapsari, R. A., 2021. "Study of Glucomannan Extraction with Hydrichloric Acid Catalyst and Alcohol Solvent Based on Porang Tuber Flour (Amorphophallus Oncophyllus)." *IOP Conf. Series: Earth and Enviromental Sciences*, 700 (2021).

Baltacıoğlu, H., Baltacıoğlu, C., Okur, I., Tanrıvermis, A., & Yalıç, M., 2021. "Optimization of Microwave-Assisted Extraction of Phenolic Compounds from Tomato: Characterization by FTIR And HPLC And Comparison with Conventional Solvent Extraction." *Vibrational Spectroscopy*, 113, 103-204.

Faridah, A & Widjanarko, S. B., 2013. "Optimization of Multilevel Ethanol Leaching Process of Porang Flour (Amorphophallus muelleri) Using Response Surface Methodology." *International Journal on Advanced Science Engineering Information Technology*, 3(2).

Hikmawanti, N. P. E., Fatmawati, S., & Asri, A. W. 2021. "The Effect of Ethanol Concentrations as The Extraction Solvent on Antioxidant Activity of Katuk (Sauropus androgynus (L.) Merr.) Leaves Extracts". *IOP Conf. Series: Earth and Environmental Science*, 755 (2021), 012060

Hermanto, M. B., Widjanarko, S. B., Suprapto, W., & Suryanto, A., 2019. "The Design and Performance of Continuous Porang (Amorphophallus muelleri Blume) Flour Mills." *International Journal on Advanced Science Engineering Information Technology*, 9 (6).

Lajoie, L., Sylvie, A., Tixier, F., and Chemat, F. 2022. "Water as Green Solvent: Methods of Solubilisation and Extraction of Natural Products—Past, Present and Future Solutions". *Pharmaceuticals*, 15 (12), 1507.

Mutaqin, A. Z., Kurniadie, D., Iskandar, J., Nurzaman, M., & Partasasmita, B., 2020. "Ethnobotany of Suweg (Amorphophallus Paeoniifolius): Folk Classification, Habitat, and Traditional Conservation in Cisoka Village, Majalengka District. Cimanuk Watershed Region, Indonesia." *Biodiversitas*, 21(2).

Muthuselvi, C. Arunkumar, A. & Rajaperumal, G., 2016. "Growth and Characterization of Oxalic Acid Doped with Tryptophan Crystal for Antimicrobial Activity." *Pelagia Research Library*, 7(4), 55-62.

Nurlela, Ariesta, N., Laksono, D. S., Santosa, E., & Muhandri, T., 2019. "Effect of harvest timing and length of storage time on glucomannan content in porang tubers". *IOP Conf. Series: Earth and Environmental Science,* 299 (2019), 012012.

Nolte, L., Nowaczyk, M., & Brandenbusch, C., 2022. "Monitoring and Investigating Reactive Extraction Of (Di–) Carboxylic Acids Using Online FTIR – Part I: Characterization of the Complex Formed Between Itaconic Acid and Tri-N-Octylamine." *Journal of Molecular Liquids*, 352.

Rehman, M. U., Abdullah, Khan, F., & Niaz, K., 2020. "Chapter 1 – Introduction to Natural Products Analysis." *Elsevier*, 3-15.