Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Rifda Naufalin
Assignment title:	Prof Rifda Naufalin
Submission title:	Naufalin_2021_IOP_Conf._Ser.__Earth_Environ._Sci._746_0120...
File name:	21_IOP_Conf._Ser.__Earth_Environ._Sci._746_012008_Prof_Rifd...
File size:	952.02K
Page count:	10
Word count:	3,969
Character count:	21,452
Submission date:	27-May-2021 10:11AM (UTC+0700)
Submission ID:	1594992977

IOP Conference Series: Earth and Environmental Science

PAPER · OPEN ACCESS

Extraction time optimization of antibacterial activities of kecombrang flower extract with microwave assisted extraction (MAE) method

To cite this article: R Naufalin *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **746** 012008

View the article online for updates and enhancements.

Naufalin_2021_IOP_Conf._Ser.__Earth_Environ._Sci._746_012008_Prof Rifda

by Rifda Naufalin

Submission date: 27-May-2021 10:11AM (UTC+0700)
Submission ID: 1594992977
File name: 21_IOP_Conf._Ser.__Earth_Environ._Sci._746_012008_Prof_Rifda.pdf (952.02K)
Word count: 3969
Character count: 21452

Extraction time optimization of antibacterial activities of kecombrang flower extract with microwave assisted extraction (MAE) method

Extraction time optimization of antibacterial activities of kecombrang flower extract with microwave assisted extraction (MAE) method

R Naufalin*, Erminawati, N Herliya, and N Latifasari

Food Technology Study Program, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto 53123

*Email: rifda.naufalin@unsoed.ac.id

Abstract. Kecombrang belongs to the *Zingiberaceae* group and is a type of spice plant that has long been used as a cooking ingredient. This research was conducted to determine the effect of optimal power and extraction time on the antibacterial activity of kecombrang flower extract using the microwave assisted extraction (MAE) method. This research is an experimental research type with the experimental design used is Response Surface Methodology (RSM) with Central Composite Design (CCD) using the Design Expert application ver 10. The factors studied were the extraction power with 3 levels, namely 200, 250, 300 watts and extraction time consisting of 3 levels, namely 3, 5, 7 minutes. Based on these 2 factors, there are 13 optimization formulas. The variables observed consisted of qualitative analysis, namely phytochemicals (alkaloids, flavonoids, saponins, steroids and triterpenoids, tannins, phenol hydroquinone, and glycosides), antibacterial activity, and extract characteristics (pH, color). The results showed the optimum conditions for inhibitory zone of *Escherichia coli* 8.256 mm and *Staphylococcus aureus* 7.987 mm. The qualitative test results showed that the kecombrang flower positively contained alkaloid compounds, flavonoids, saponins, triterpenoids, tannins, phenol hydroquinone, and glycosides.

1. Introduction

Kecombrang (*Etlingera elatior*) is a well-known spice that has various benefits when consumed by humans. One part that contains many benefits is kecombrang flowers. Kecombrang contains alkaloid compounds, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, vitamins, minerals, and glycosides which act as antibacterial and antioxidant [1]. Kecombrang flowers have a higher antioxidant content than other parts of the kecombrang plant, amount 61.61 - 83.17% [2].

The method commonly used in kecombrang flower extraction is the conventional method, namely maceration [3]. The maceration method has the disadvantage that it takes a long time to work and the extraction is less than perfect. Conventional methods are also generally time consuming and involve thermal processes that can destroy antioxidant compounds. In addition, other conventional methods also often use organic solvents and can produce toxic residues, chemical changes in extract compounds, and waste that is difficult to degrade [4]. Therefore, the need for well methods as well as

the use of safe solvents. A more environmentally friendly extraction method such as Microwave Assisted Extraction (MAE) is expected to be a solution.

MAE has several advantages including being able to work fast and efficiently. This is due to electromagnetic waves that can penetrate the material and evenly excite the molecules of the material. In addition, microwaves contained in microwaves can be absorbed by glass, ceramic, and some types of plastics, using less solvent, higher yield and lower cost [4]. Some of the factors that influence extraction with MAE technique are power and extraction time. The power is chosen precisely to avoid the degradative temperature of the target compound and overpressure in the extraction process. Meanwhile, the difference in extraction time using MAE affects the extraction yield value and the quality of the extract is indicated by the longer the extraction, the higher the total yield and the remaining solvent content decreases the longer the extraction is carried out. In previous studies, a way to extract kecombrang flowers has been found, but there has been no optimization for the power or strength and duration of extraction of the resulting antibacterial and antioxidant activity [5]. Therefore, this research was conducted, namely to optimize the power and extraction time using the Response Surface Methodology (RSM) method. Based on these descriptions, the objectives of this study was to determine the effect of power and extraction time on the antibacterial activity of kecombrang flower extracts using the MAE method.

2. Research Methodology

2.1. Experimental design

Optimization of power and duration of extraction of kecombrang flowers using MAE method using Response Surface Methodology (RSM) with a central composite design (CCD) which consists of 2 factors, namely as follows: Extraction power (X1) consists of three levels, namely 200, 250, 300 (watts) and the extraction time (X2) consists of three levels, namely 3, 5, 7 (minutes). These two factors were entered into the Design Expert Ver.10 application and resulted in 13 combinations as shown in Table 1.

Table 1. Combinations of treatments in the study

No	Variables	
	X_1 (strength)	X_2 (duration)
1	250	5
2	300	7
3	250	7,83843
4	179,289	5
5	250	5
6	250	5
7	320,711	5
8	200	3
9	250	2,17157
10	300	3
11	200	7
12	250	5
13	250	5

2.2. Kecombrang Flower Powder Production

Making kecombrang flower powder begins with flower sorting. The criteria for kecombrang flowers used are fresh red, have a distinctive kecombrang aroma, are not defective or have black stains and do not wither. The sorted flowers are then taken by the flower petals and then washed thoroughly using running water. After the water is drained, reduce the size using a knife so that the size of the petals is smaller so that drying is more optimal. The cut kecombrang flowers are then spread out on a

baking sheet. Kecombrang flower drying was done using a cabinet dryer with a temperature of 50°C for 4 hours. The dried kecombrang flower simpilisia is ground using a disc mill to form kecombrang flower powder [5].

2.3. Kecombrang Flower Extract Production

The homogeneous kecombrang flower powder was dissolved using distilled water with a ratio of ingredients and solvent of 1:10. The solution is homogenized until blended. Furthermore, the extraction was carried out using a microwave in accordance with the combination of power treatment and extraction time that had been determined. When finished, it is filtered using a filter cloth. The results of the filter are evaporated using a vacuum rotary evaporator at 60°C for 20 minutes [5].

2.4. Qualitative test

Qualitative variables include the tests for alkaloids, flavonoids, saponins, steroids and triterpenoids, tannins, phenol hydroquinones, and glycosides.

2.5. Antibacterial Activity

Disc paper soaked in 2 ml of kecombrang flower extract for 30 minutes. 100 μL of bacterial culture was taken and dissolved in 9 ml of physiological NaCl. A total of 100 μL of culture was placed in sterile petri dishes then mixed with sodium agar. The sodium should be allowed to solidify and then the disc paper is dried and then planted in NA media. Incubation was carried out for 24 hours at 37°C and then measured the diameter of the resulting clear zone.

2.6. Data Analysis

Data analysis was performed with the Response Surface Methodology (RSM) to find the optimum conditions of the factors that affect the response and the t test to validate the optimization model.

3. Result and Discussion

3.1. Qualitative Test

3.1.1. Alkaloid. Alkaloids are organic compounds found in plants that are alkaline and are mainly sourced from dicots. Alkaloid testing begins with the addition of HCl to extract alkaloid compounds that are alkaline [6]. The results of the qualitative analysis in Table 2 show that all positive treatments contain alkaloid compounds which are indicated by the presence of red-orange deposits in the kecombrang flower extract. The deposition is caused by a change of ligands in which nitrogen, which has a lone pair on the alkaloid, forms a coordinate covalent bond with the K + ion from potassium tetraiodobismutate, resulting in the alkaloid potassium complex which precipitates [7]. The results of the analysis are in accordance with the literature which states that kecombrang extract contains alkaloid compounds [8].

3.1.2. Flavonoid. The flavonoid compounds in kecombrang flowers are anthocyanins which are red pigments in flowers. The results of the qualitative analysis in Table 2 show that all positive treatments contain flavonoid compounds which are indicated by the formation of red, yellow, or orange colors in the kecombrang flower extract. The results of the analysis are in accordance with the literature which states that kecombrang extract contains flavonoid compounds [8]. The detection of flavonoids was caused by the sensitivity of flavonoids to treatment with alkaline compounds. The addition of base, in this experiment Magnesium powder, causes a spontaneous color change through a reduction reaction that occurs in flavonoids [6]. The content of flavonoids has great potential as antioxidants because of the molecular structure and position of the hydroxyl groups [9]. The flavonoid content in kecombrang flowers was identified as kaemferol and quartzetin [2].

Table 2. Qualitative test results of kecombrang flower extract.

Treatments	Alkaloid	Flavonoid	Saponin	Triterpenoid	Tanin	Fenol	Glikosida
250 W, 5 minutes	+	++	++	+++	+	+++	+
300 W, 7 minutes	++	++	+	+++	+	+++	+
250 W, 7,82 minutes	+	++	+	+++	+	+++	+
179,28 W, 5 minutes	++	++	+	++	+	+	+
250 W, 5 minutes	++	++	+	++	+	+++	+
250 W, 5 minutes	+	++	+	++	-	+++	+
320,71 W, 5 minutes	+++	+++	-	+++	-	++	+
200 W, 3 minutes	++	++	-	++	-	++	+
250 W, 2,17 minutes	++	++	-	++	-	+++	+
300 W, 3 minutes	+	++	+	++	-	+++	+
200 W, 7 minutes	++	+++	+	+++	-	+++	+
250 W, 5 minutes	++	+++	+	++	-	+++	+
250 W, 5 minutes	+++	+++	+	+++	+	+++	+

3.1.3. Saponin. The results of the qualitative analysis in Table 2 show that as many as 3 negative treatments contained saponin compounds and 10 positive treatments contained saponin compounds which were characterized by the formation of a stable foam on the surface of the kecombrang flower extract. The formation of foam is because saponins contain glycosyl groups that act as polar groups as well as steroid and triterpenoid groups as nonpolar groups that are surface active so that when shaken with water saponins can form micelles, where the polar structure faces outward while the nonpolar groups face inward [10]. A total of 3 negative treatments contained saponins because the foam produced after shaking only lasted a few seconds, did not last long.

3.1.4. Triterpenoid. The results of the qualitative analysis in Table 2 show that all positive treatments contain triterpenoid compounds which are indicated by the formation of red or purple colors in the kecombrang flower extract. The color change to red or purple is based on the ability of triterpenoid compounds to form color by H_2SO_4 in anhydrous acetic acid solvent [7]. The results of this analysis are in accordance with the literature which states that kecombrang extract contains triterpenoid compounds [8]. Triterpenoid compounds can also be bound to sugar groups so that they will be attracted by semi-polar and even polar solvents [4].

3.1.5. Tannin. Tannins are a group of active plant compounds that are phenolic, have a cool feeling and have the ability to tan the skin [4]. The results of the qualitative analysis in Table 2 show that as many as 7 negative treatments contained tannin compounds and 6 positive treatments contained tannin

compounds which were marked by the formation of a green-blue color in the kecombrang flower extract. Tannin testing was carried out by adding $FeCl_3$. The function of $FeCl_3$ is to hydrolyze the tannin group so that it will produce a blackish blue color change and condensed tannins that produce a green-black color [10].

3.1.6. Phenol. Phenol has potential as an antimicrobial and causes cell death by several mechanisms, including denaturation of proteins, inactivating bacterial enzymes, and destroying cell membranes [6]. The results of the qualitative analysis in table 2 show that all positive treatments contain phenolic compounds which are indicated by the formation of a green-blue-black color in the kecombrang flower extract. The green-blue-black discoloration is due to $FeCl_3$ reacting with the aromatic -OH group [6].

3.1.7. Glycoside. The results of qualitative analysis in table 2 show that all positive treatments contain glycoside compounds which are indicated by the formation of a blue or green color in the kecombrang flower extract. The results of the analysis are in accordance with the literature which states that kecombrang extract contains glycoside compounds [8]. The formation of a green color is thought to be due to the glycoside compounds contained in the kecombrang flower extract are non-sugar glycosides. Glycoside testing using anhydrous acetic acid and concentrated sulfuric acid can only test for non-sugar glycosides and cannot detect the presence of sugar glycosides. The content of chemical compounds glycosides has the potential to be antibacterial by penetrating into the cell wall, causing damage to the cell wall [7].

3.2. Optimization of Kecombrang Flower Extract

This study used a central composite experimental design (Central Composite Design) which consisted of 2 factors, namely power and extraction time. These two factors were entered into the Ver.10 expert design application and a response was obtained from the central composite design (CCD) which is presented in Table 3.

Table 3. Response data from the central composite design (CCD).

No	Variables		Respond	
	X_1	X_2	Antibacterial Activity *Escherichia coli* (mm)	Antibacterial Activity *Staphylococcus aureus* (mm)
1	250	5	9,83333	8,5
2	300	7	9,66667	6,5
3	250	7,83843	10	6,33333
4	179,289	5	7	6,83333
5	250	5	7,33333	9
6	250	5	9,83333	9,33333
7	320,711	5	9	8,33333
8	200	3	6,33333	10,3333
9	250	2,17157	6,83333	8,5
10	300	3	8	8,66667
11	200	7	8,83333	6,33333
12	250	5	7	8
13	250	5	7,66667	7,16667

The expert design program will provide a regression model that matches the response data. The responses analyzed were antioxidant activity, antibacterial activity of *Escherichia coli* and *Staphylococcus aureus*.

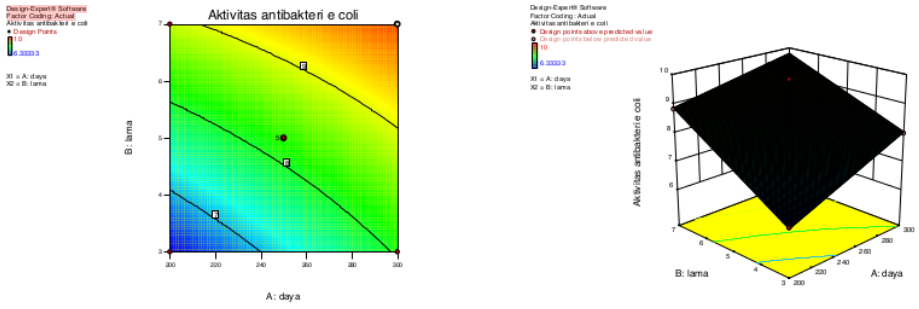
3.2.1. Antibacterial Activity. Testing for antibacterial activity was carried out by the disc diffusion method, in which paper discs containing the test compound were placed on the surface so that previously inoculated with the test microorganism. The test compound diffuses into the agar medium to cause inhibition of the growth of microorganisms. Petri dishes were placed at room temperature before incubation, then the inhibition zone was measured [8].

The type of regression selected using ANOVA analysis in Design Expert ver 10 for the analysis of *Escherichia coli* antibacterial is the 2FI model obtained a significant value, amounting to 0.0272 (p-value <0.05) with a coefficient of determination (R2) of *Escherichia coli* antibacterial activity of 0, 6211. Based on the lack of fit tests, the result is 0.9994 which shows insignificant results. The regression equation model is obtained as follows:

Y = 8.25641 + 0.666053 (A) + 1.08063 (B) + -0.208333 (AB)

Description:
Y = Antibacterial Activity respond of *Escherichia coli*
A = strength (Watt)
B = Duration (Minutes)

According to the regression model obtained by RSM, power treatment and extraction time of kecombrang flowers did not significantly affect the antibacterial activity of *Escherichia coli*. The antibacterial activity value of *Escherichia coli* is presented in Figure 1.



(a) (b)

Figure 1. Two-dimensional (a) and three-dimensional (b) contour graph of *Escherichia coli* antibacterial activity of kecombrang flower extract

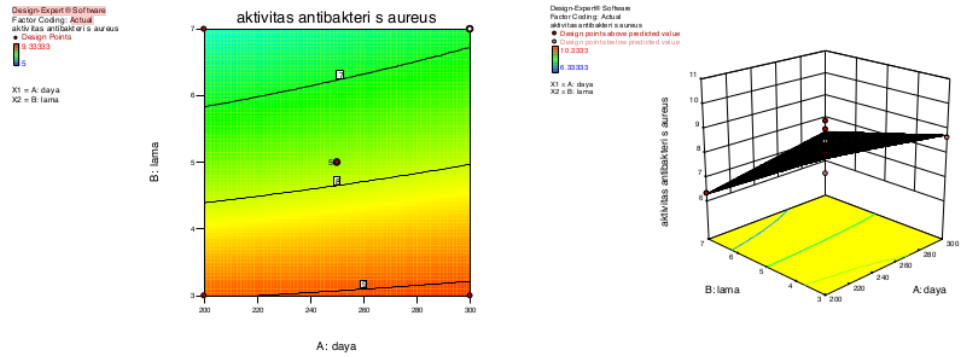
The type of regression selected using ANOVA analysis on Expert Design ver 10 for antibacterial analysis of *Staphylococcus aureus* is a 2FI model obtained a significant value, amounting to 0.0332 (p-value <0.05) with a coefficient of determination (R2) of antioxidant activity of 0.6030. Based on the lack of fit tests, the result was 0.385 which showed insignificant results. The regression equation model is obtained as follows:

Y = 7.98718 + 0.0776651 (A) + -1.15385 (B) + 0.458333 (AB)

Description:
Y = Anatibacterial activity respond of *Staphylococcus aureus*
A = Strength (Watt)

B = Duration (Minutes)

In accordance with the regression model obtained by RSM, power treatment and duration of extraction of kecombrang flowers significantly affected the antibacterial activity of *Staphylococcus aureus*. The antibacterial activity value of *Staphylococcus aureus* is presented in Figure 2.



(a) (b)

Figure 2. Contour diagram of *Staphylococcus aureus* antibacterial activity from kecombrang flower extract

The test results above show that kecombrang flower extract can inhibit both types of bacterial activity, both gram-negative (*Escherichia coli*) and gram-positive (*Staphylococcus aureus*). The ability of kecombrang flower extract to inhibit bacteria is due to the presence of terpenoids, flavonoids and alkaloids. The polarity of kecombrang flower extract greatly affects the antibacterial activity [10]. A compound that has optimum polarity will have maximum antimicrobial activity, because for the interaction of an antibacterial compound with bacterial cells, hydrophilic-lipophilic balance (HLB) is required. To ensure that the antimicrobial compound dissolves in the water phase which is the place for microbes to live, it is necessary to know its hydrophilic properties [8]. The polar compounds in kecombrang flower extracts easily penetrate the gram-positive walls so that the *S. aureus* inhibition zone results are greater than *E. coli*. It is also written based on the peptidoglycan structure and lipid content that gram-negative bacterial cells (*S. aureus*) are more easily damaged by active compounds of kecombrang flower extract than *E. coli* [9,11].

3.3. Verification of Optimization Results

After testing the antioxidant and antibacterial activity of 13 response data treatments, the optimum formula recommendation was obtained according to the specified criteria. In the optimization of kecombrang flower extract, the criteria for antioxidant and antibacterial activity are inrange. Based on these criteria, RSM recommends one formulation, namely 250 W power and 5 minutes extraction time with desirability of 1.

Table 4. RSM recommendation formula for kecombrang flower extract

strength (Watt)	duration (Menit)	Antibacterial Activity *Escherichia coli* (mm)	Antibacterial activity *Staphylococcus aureus* (mm)	Desirability
250	5	8,256	7,987	1

The desirability value is the value of the optimization objective function which shows the program's ability to fulfill the desires based on the criteria set in the final product. The value ranges from 0 to 1.0. Furthermore, verification is carried out in accordance with the results of the RSM recommendation to find out whether the actual value of the response is within the Prediction Interval (PI) and is close to the predicted value given by RSM. The results of the verification of kecombrang flower extract are presented in Table 5.

Table 5. Verification results of kecombrang flower extract

Respond	Verification value	Prediction value	PI 95% Low	PI 95% High
Antibacterial activity				
Escherichia coli (mm)	6,67	8,256	6,05	10,47
Staphylococcus aureus (mm)	7,67	7,987	7,67	10,14

The verification values for the antioxidant, antibacterial activity of *E. coli* and *S. aureus* were 435.386 ppm, 6.67 mm and 7.67 mm, respectively. The verification value falls into the predicted interval (PI) range with a significant level of 95%. A good response value is the value in the PI range. So, it can be concluded that the optimum formula is in accordance with what the application recommends.

4. Conclusion

Based on the RSM calculation model, the power factor and extraction time had a significant effect on the quality of the extract in terms of antibacterial activity of *Staphylococcus aureus* and *Escherichia coli*. The optimum condition for the antioxidant and antibacterial activity of kecombrang flower extract is at a power of 250 W and an extraction time of 5 minutes. The optimum conditions resulted in a predictive value of antibacterial activity of *Escherichia coli* of 8.256 mm and antibacterial activity of *Staphylococcus aureus* of 7.0987 mm and also contained bioactive compounds.

Acknowledgements

Authors wish to thanks to Institute for Research and Community Service of Jenderal Soedirman University which has provided research funding through Fasilitasi Guru Besar Scheme 2020..

References

[1] Naufalin R 2019 Natural preservation opportunities and challenges in improving food safety *AIP Conf. Proc.* **2094**
[2] Chan E W C, Lim Y Y and Omar M 2007 Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia *Food Chem.* **104** 1586–93
[3] Naufalin R and Rukmini H S 2013 Microcapsule Application of Kecombrang Flower Extract : Effects of Concentration, Types of Fraction, pH of Medium, and NaCl on Microbiological Properties of Minced Beef *Anim. Prod.* **15** 8–14
[4] Harborne J B 1984 *Metode Fitokimia : Penuntun Cara Modern Menganalisis Tumbuhan* (Bandung: ITB Bandung)
[5] Naufalin R and Rukmini H S 2018 Antibacterial activity of kecombrang flower extract (Nicolaia speciosa) microencapsulation with food additive materials formulation *IOP Conf. Ser. Earth Environ. Sci.* **102**
[6] Haryati N, Saleh C and - E 2015 Uji Toksisitas Dan Aktivitas Antibakteri Ekstrak Daun Merah Tanaman Pucuk Merah (Syzygium Myrtifolium Walp.) Terhadap Bakteri Staphylococcus Aureus Dan Escherichia Coli *J. Kim. Mulawarman* **13** 35–40
[7] Jannah R, Husni M A and Nursanty R 2017 Inhibittion Test of Methanol Extract from Soursop

Leaf (Annona muricata Linn.) Against Streptococcus mutans Bacteria *J. Nat.* **17** 23

[8] Niswah L 2014 Uji Aktivitas Antibakteri dari Ekstrak Buah Parijoto (Medinilla speciosa Blume) Menggunakan Metode Difusi Cakram *Progr. Stud. Farm. Fak. Kedokt. dan Ilmu Kesehat. Univ. Islam Negeri Syarif Hidayatullah Jakarta*

[9] Hudaya A 2011 Uji antioksidan dan antibakteri ekstrak air bunga kecombrang (edigera elatior) sebagai pangan fungsional terhadap staphylococcus aureus dan escherichia coli *Skripsi*

[10] Naufalin R, Jenie B S L, Kusnandar F, Sudarwamto M and Rukmini H 2005 Antibacterial Activity of Kecombrang Flower Extract Toward Pathogenic and Food Spoilage Bacteria *J. Teknol. dan Ind. Pangan* **16** 119–25

[11] Putri F A, Naufalin R and Wicaksono R 2019 Antimicrobial edible coating application of Kecombrang flower concentrate to reduce microbial growth on gourami fish sausage *IOP Conf. Ser. Earth Environ. Sci.* **250**

Naufalin_2021_IOP_Conf._Ser.__Earth_Environ._Sci._746_012...
Rifda

ORIGINALITY REPORT

18%	3%	18%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Farah Fatimah, Natalia Christin Larasati, Rumpoko Wicaksono, Rifda Naufalin. " Optimization of temperature and time of extraction of kecombrang stem and leaf based on the quality of product bioactive components ", IOP Conference Series: Earth and Environmental Science, 2019 Publication	10%
2	R Naufalin, Erminawati, R Wicaksono, A T Febryani, N Latifasari. "Antioxidant activity of kecombrang preserving powder using foam mat drying method", IOP Conference Series: Earth and Environmental Science, 2021 Publication	6%
3	Khairunnisa Nisrina Effendi, Nurahmahdiani Fauziah, Rumpoko Wicaksono, Erminawati, Poppy Arsil, Rifda Naufalin. " ANALYSIS OF BIOACTIVE COMPONENTS AND PHYTOCHEMICAL OF POWDERS STEM AND LEAVES OF KECOMBRANG ", IOP Conference Series: Earth and Environmental Science, 2019	3%

Publication

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	On		