Optimization of temperature and time of extraction of kecombrang stem and leaf (Etlingera elatior) based on the quality of product bioactive components

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Abstract. Foodstuffs are perishable due to the nutritional component contained therein. Therefore it is necessary to prevent food damage, one of which is the addition of preservatives in food. One of the natural ingredients that has the potential as a natural preservative is kecombrang plant. Kecombrang (Etlingera elatior) is known to have bioactive compounds including polyphenols, alkaloids, and flavonoids. Extraction is influenced by several factors including temperature and time. These factors need to be controlled so that the extract produced has the best quality. This study aims to determine the optimum temperature and time extraction of kecombrang stems and leaves on the quality of the bioactive components of the product produced. This study uses the Response Surface Methodology (RSM) method with the Design Expert v10 program. The experimental design used is Central Composit Design (CCD). The factors that were tried in this study were temperature and liquid extraction time. The temperature consists of three levels, namely 40°C, 50°C, and 60°C, and time consists of three levels namely 3, 4 and 5 hours. The results showed that the optimum temperature and time in the extraction of kecombrang stem using a temperature of 40°C with a duration of 5 hours produced a pH value of 3.85, a total flavonoid of 239.92 mg QE / 100 g, a total phenol of 111.41 mg TAE / 100 g and optimum temperature and time in kecombrang leaf extraction using a temperature of 51°C with a duration of 3 hours produced a pH value of 4.35, a total phenol of 327.13 mg TAE / 100 g and a total flavonoid of 24.911 mg QE / 100 g. Qualitative test results indicate that positive liquid extracts contain alkaloids, flavonoids, glycosides, saponins, phenolics and triterpenoids.

Keywords: kecombrang, phenol, flavonoids, optimization, extraction

1. Introduction

The current development is demanding food and beverage products that are all practical, durable and have an attractive appearance. The addition of preservatives is a solution made by the food industry so that the final product has good quality with a longer shelf life. The use of synthetic preservatives and antioxidants at this time is not recommended because it is suspected to cause degenerative diseases and even cancer [1]. Therefore it is necessary to develop alternative preservatives sourced from natural ingredients, one of which is kecombrang.
Kecombrang is a Zingiberaceae plant group that has long been known and used by the community as a nutritious food ingredient as a food preservative. Kecombrang stem section itself is a part that is often used as food additives in the community. According to research [2,3] kecombrang stem contains a number of bioactive substances such as alkaloids, saponins, phenolics, flavonoids, triterpenoids, steroids and glycosides. Kecombrang stem section is more able to inhibit oxidation when compared to other leaves and rhizomes. This shows that kecombrang stem has the potential as a bioactive compound that functions as an antioxidant, antimicrobia, antimicrobial coating for food product [4, 5, 6, 7].

Extraction process of bioactive compounds in kecombrang plants needs to be carried out to test its activity. Extraction is the activity of withdrawing soluble chemicals so that they are separated from insoluble substances by liquid solvents [8]. Extraction is influenced by several factors. There are many factors that influence the extraction process including temperature and time. These factors need to be controlled so that the extract produced can have the best quality. If the extraction process uses temperatures that are too high for too long, it is feared that the bioactive component in the kecombrang will suffer damage and can reduce the antioxidant activity of the extract produced. Previous studies have found the best way to extract kecombrang plants, but there is no optimization for the temperature and extraction time [9]. Therefore this research was conducted, namely to optimize the temperature and extraction time with the Response Surface Methodology (RSM) method.

The objectives of this study are: 1) knowing the optimal combination of treatment between temperature and extraction time to produce liquid extracts of kecombrang stems and leaves with good quality; 2) determine the effect between temperature and extraction time on the quality of liquid extracts of kecombrang stem and leaves produced.

2. RESEARCH METHODOLOGY

2.1 Experimental design

All treatments consisted of 13 processes in which each process condition followed the central composite design (Table 1) obtained from the Design Expert application ver 10. Temperature and time extraction estimates in making kecombrang stem extract using Response Surface Methodology (RSM) with central composite design (CCD composite design). The factors tested in this study were temperature and duration of extraction with 3 levels, are:

X₁ = The extraction temperature consists of 40, 50 and 60 (°C).
X₂ = The length of time consists of 3, 4 and 5 (hours).

| No. | X₁ (Temperature) | X₂ (Time) | X₁ (Temperature) | X₂ (Time) |
|-----|------------------|-----------|------------------|-----------|
| 1   | 64.1421          | 4         | 50               | 5.41      |
| 2   | 40               | 5         | 50               | 4         |
| 3   | 50               | 2.58579   | 50               | 2.59      |
| 4   | 40               | 3         | 35.86            | 4         |
| 5   | 50               | 5.41421   | 40               | 5         |
| 6   | 50               | 4         | 60               | 3         |
| 7   | 50               | 4         | 60               | 5         |
| 8   | 50               | 4         | 50               | 4         |
| 9   | 60               | 5         | 50               | 4         |
| 10  | 35.8579          | 4         | 50               | 4         |
| 11  | 60               | 3         | 40               | 3         |
| 12  | 50               | 4         | 64.14            | 4         |
| 13  | 50               | 4         | 50               | 4         |
2.2 Kecombrang preparation and powder

Young kecombrang stems and leaf are sorted. After that the kecombrang stems are cut into small pieces which are then dried using a cabinet dryer for 6 hours at 60°C. The simplicial kecombrang stem is size reduction using a disc mill until a homogeneous kecombrang stem powder is obtained. In kecombrang leaves, young kecombrang leaves are sorted. After that kecombrang leaves are cut into small pieces which are then dried using a cabinet dryer for 2 hours at 60°C. The kecombrang leaves are size reduction using a disc mill until a homogeneous kecombrang leaf powder is obtained [7, 9].

2.3 Kecombrang stem and leaf extraction

The extraction method used is the maceration method. Homogeneous kecombrang powder is put into the extractor, then added distilled water with a ratio of 1:14 through the extractor closing pipe. The extraction process is carried out in accordance with the treatment produced by the Expert Design application ver.10. Kecombrang liquid extract is filtered using a filter cloth and a press to separate the extract from the pulp. Kecombrang liquid extracts are dried again using a cabinet dryer for approximately 2-3 hours with a temperature of 40-50°C to dry, then extracted again with the same water ratio. The resulting kecombrang liquid extract slurry is refined using a filter cloth and a press to get the second kecombrang stem extract. The first and second extraction results were mixed until homogeneous to obtain liquid extracts of kecombrang stem and leaves.

2.4 Qualitative test

The variables observed in the study consisted of qualitative and quantitative variables. Qualitative variables include phytochemical tests including alkaloids, phenols, flavonoids, glycosides, saponins, steroids and tannins [10].

2.5 Quantitative test of total phenol

The total phenol test uses the Singleton method with Folin-ciocalteu reagents with the standard used tannic acid. A sample of 400 µL was added with 1.5 mL of Folin-Ciocalteu then allowed to stand for 5 minutes at room temperature. After that added 1.5 mL of NaHCO3, 0.556 M then shaken and allowed to stand in a dark room for 90 minutes. After that the absorbance of the solution was measured using a spectrophotometer at a wavelength of 725 nm [11].

2.6 Quantitative test of total flavonoid

1000 µL samples were added with 1 mL of 2% AlCl 3 (2 grams of AlCl 3 in a 5% glacial acetic acid solution) and 1 mL of 120 Mm potassium acetate solution (1,176 grams of potassium acetate in 100 mL of distilled water). After that, it was incubated for 1 hour at room temperature and the absorbance was measured using a spectrophotometer at a wavelength of 435 nm [12].

2.7 Analysis of statistic

Data analysis was performed with Response Surface Methodology (RSM) which is a combination of statistical and mathematical techniques to find optimum conditions. Furthermore, the validation results are then tested independently to test whether there is a difference between the predicted results from the Design Expert application ver.10 and the results of the optimization treatment.

3. RESULT AND DISCUSSIONS

3.1 Qualitative test

The qualitative variables examined in this study include alkaloids, phenols, flavonoids, glycosides, saponins, steroids and tannins. The results of testing the qualitative variables of
kecombrang stem liquid extract are presented in Table 2 and the results of testing the qualitative variables of kecombrang leaf liquid extract are presented in Table 3.

Table 2. The results of the qualitative test were kecombrang stem liquid extract.

| Treatment       | Alkaloids | Phenol | Flavonoids | Glycosides | Saponin | Triterpenoid | Tannin |
|-----------------|-----------|--------|------------|------------|---------|--------------|--------|
| 64°C, 4 hours   | ++        | +++    | +          | +          | +       | -            | +++    |
| 40°C, 5 hours   | +         | ++++   | +          | +          | +       | +            | ++     |
| 50°C, 2.5 hours | ++        | +      | +          | +          | -       | +            | +      |
| 40°C, 3 hours   | ++        | ++++   | +          | -          | +       | +            | +      |
| 50°C, 5.5 hours | +++       | ++     | ++         | +          | +       | -            | ++     |
| 50°C, 4 hours   | +++       | ++     | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |
| 50°C, 4 hours   | +++       | +      | +          | +          | -       | +            | +      |

Note: ++++: strong; ++: father strong; +: a little strong; -: over load.

Table 3. The results of the qualitative test were kecombrang leaf extract.

| Treatment       | Alkaloids | Phenol | Flavonoids | Glycosides | Saponin | Steroids | Tannin |
|-----------------|-----------|--------|------------|------------|---------|----------|--------|
| 50°C, 5.4 hours | +++       | ++++   | ++++       | -          | ++      | +++      | +++    |
| 50°C, 4 hours   | ++++      | +++    | +++        | -          | ++++    | +++      | +++    |
| 50°C, 2.6 hours | +++       | ++++   | ++++       | -          | +++     | +++      | +++    |
| 36°C, 4 hours   | +++       | +++    | +++        | -          | +++     | +++      | +++    |
| 40°C, 5 hours   | +++       | ++++   | +++        | -          | +++     | +++      | +++    |
| 60°C, 3 hours   | ++        | ++++   | +++        | -          | +       | +        | +++    |
| 60°C, 5 hours   | ++        | ++++   | +++        | -          | +       | +        | +++    |
| 50°C, 4 hours   | +++       | +++    | +++        | -          | +       | +        | +++    |
| 50°C, 4 hours   | +++       | +++    | +++        | -          | +       | +        | +++    |
| 50°C, 4 hours   | +++       | +++    | +++        | -          | +       | +        | +++    |
| 40°C, 3 hours   | ++        | ++++   | +++        | -          | +       | +        | +++    |
| 64°C, 4 hours   | ++        | ++++   | ++++       | -          | +       | +        | +++    |
| 50°C, 4 hours   | +++       | +++    | +++        | -          | +       | +        | +++    |

Note: ++++++: very strong, ++++: strong; ++: rather strong; +: a little strong; -: over load.

3.1.1. Alkaloid

The test results showed that all positive liquid extract treatments contained alkaloids which were characterized by red-orange deposits. These results are consistent with the literature which states that the positive kecombrang stem extract contains alkaloid compounds [2]. Sedimentation in alkaloid testing is caused by ligand changes, where nitrogen atoms in alkaloid compounds have free electron pairs so they can replace iodo ions in dragendorf and Wagner reagents [13].

3.1.2. Phenol

The phenol test results showed a positive value marked by the formation of green-blue-black color in liquid extracts of kecombrang stems and leaves. This is in accordance with [14] which states that the kecombrang stem and leaf extract contains phenol compounds. Discoloration during phenol testing was caused by the breakdown of hydroxy groups in phenol compounds and reacting with Fe³⁺ in iron salts [15]. According to [10] the color formed is derived from iron phenolics, and this reaction
can only take place in a weak or neutral acid atmosphere. The presence of high phenol components have an important role as antioxidants that can reduce free radicals [16].

3.1.3. Flavonoid

Flavonoid test is carried out in stages according to research [17]. A total of 2 mL of sample was put into a test tube and then added with magnesium powder, then added with amyl alcohol and concentrated hydrochloric acid. The formation of red, yellow or orange in amyl alcohol indicates the presence of flavonoid compounds in liquid extracts. In the flavonoid test there is a change in the color of amyl alcohol to pink which indicates that there are flavonoid compounds in liquid extracts of kecombrang stem and leaves. The results of this test are in accordance with the literature which states that liquid extracts of kecombrang stems and leaves contain flavonoid compounds [14]. According to [18] addition of HCl and magnesium powder in the flavonoid test will reduce the benzopiron nucleus contained in the flavonoid compound so that the orange-red color is formed in the solution.

3.1.4. Glycoside

The results of glycoside testing on liquid extracts of kecombrang stem showed that as many as 8 positive treatments contained glycoside compounds and 5 negative treatments contained glycosides which were marked by a blue or green discoloration when the sample was added with anhydrous acetic acid and sulfuric acid. This is consistent with the literature showing that kecombrang stem extracts contain glycoside compounds [14]. The results of glycoside testing on liquid extract of kecombrang leaves showed that as many as 13 negative treatments contained glycosides. Kecombrang stem liquid extracts contained 5 samples which showed negative results on glycoside testing. This is because testing of glycosides using anhydrous acetic acid and concentrated sulfuric acid can only test non-sugar glycoside compounds and cannot detect the presence of sugar glycoside compounds [19], so they do not show positive results at the time of testing.

3.1.5. Saponin

Saponin compounds in the study of liquid extracts of kecombrang stems and leaves are known by the test method according to [20] namely the addition of 1 drop of HCl 2 N. The extract was dropped with HCl then shaken for 10 seconds then allowed to stand for 10 minutes. The formation of a stable foam indicates the presence of saponin compounds in the extract. The test results showed that the liquid extract of the positive kecombrang stem and leaves contained saponin compounds. This is characterized by the formation of a stable foam on the surface of kecombrang extract.

According to research by [21] the emergence of foam in the saponin test also showed that the presence of glycoside compounds which have the ability to form froth in water hydrolyzed into glucose and other compounds. This is what causes the formation of micelles when shaken with water. In the micelle structure the polar group will face outward and the non-polar group will lead inward, this micelle state which looks like a foam [22].

3.1.6. Triterpenoid

Triterpenoid testing is done by adding kecombrang stem extract with 3 drops of concentrated hydrochloric acid and 1 drop of sulfuric acid, red color changes indicate the presence of triterpenoid compounds in kecombrang stem extract. Triterpenoid test results showed that from 13 total treatments, there were 8 treatments that showed that the positive kecombrang stem extract contained triterpenoid compounds, while 5 treatments showed negative. The existence of triterpenoid compounds in kecombrang stem extracts is in accordance with [14] which states that kecombrang stem extracts contain triterpenoids. This is supported by the research of [23] triterpenoid compounds can also be bound to sugar groups so that they can be extracted with solvents that are semi-polar and polar.

Negative results in testing due to triterpenoid compounds are compounds that are non-polar and cannot be extracted completely with polar water solvents such as water [24]. This is supported by
the research of [23] which states that triterpenoids are composed of C30 hydrocarbon chains which causes triterpenoid properties to tend to be non-polar and easier to extract using non-polar solvents.

3.1.7. Steroid

Based on the results of the qualitative analysis of the steroid compounds in Table 3 it can be seen that the liquid extract of kecombrang leaves which are extracted using water is then analyzed using the Lieberman Burchard reagent. There are 2 possible changes in color types, namely the formation of green for steroids and red or violet for triterpenoids. In this study, the resulting color was only green as positive as +++ with strong positive note on liquid extract of kecombrang leaves containing steroids.

Changes in color to red, pink or violet indicate positive containing terpenoids and if the color changes to green or purple then the sample is tested positive for steroids. Steroids act as antibacterial by damaging the lipid membrane, so that the liposomes leak.

3.1.8. Tanin

Tannin compounds were detected by changing the color of the extract to blue-green when added with 1% FeCl₃ [20]. In tannin testing, all samples of liquid extracts of positive kecombrang stem and leaf contain tannin compounds which are marked by changing the color of the sample to blue green. This is not in accordance with the literature which states that tannins are compounds that are not contained in kecombrang stem extracts [14].

The main properties of tannins depend on the phenolic-OH group contained in tannins. Color reactions occur when combined with iron salt. Iron salt (FeCl₃) will give a green or blackish blue color when reacted with tannin. But this test is not good because in addition to tannins that can provide a color reaction, other substances can also provide the same color reaction [15]. Other compounds that can form dark green-blue deposits are polyphenolic compounds and tannins.

3.2. Analisis Result of Qualitative Data

This study uses the expert ver.10 design program for processing statistical data. The response results from the central composite design of kecombrang stem liquid extract are presented in Table 4 and the response results from the central composite design of kecombrang leaf liquid extract are presented in Table 5

| No. | X₁ (Temperature) | X₂ (Time) | Total Phenol (mg TAE/100 g) | Total Flavonoids (mg QE/100 g) | pH |
|-----|-----------------|----------|-----------------------------|--------------------------------|----|
| 1   | 64.1421         | 4        | 23.02                       | 9.93                           | 3.8 |
| 2   | 40              | 5        | 24.96                       | 11.79                          | 3.7 |
| 3   | 50              | 2.58579  | 23.16                       | 7.34                           | 3.75|
| 4   | 40              | 3        | 28.11                       | 9.59                           | 3.8 |
| 5   | 50              | 5.41421  | 26.75                       | 10.69                          | 3.8 |
| 6   | 50              | 4        | 32.18                       | 9.30                           | 3.9 |
| 7   | 50              | 4        | 29.51                       | 7.26                           | 3.6 |
| 8   | 50              | 4        | 37.65                       | 7.84                           | 3.6 |
| 9   | 60              | 5        | 30.62                       | 9.64                           | 4.0 |
| 10  | 35.8579         | 4        | 34.84                       | 10.61                          | 3.9 |
| 11  | 60              | 3        | 35.76                       | 7.79                           | 3.8 |
| 12  | 50              | 4        | 32.95                       | 7.31                           | 3.8 |
| 13  | 50              | 4        | 37.99                       | 7.42                           | 3.8 |
Tabel 5. Response data from the central composite design of kecombrang leaf liquid extract

| No. | X1 (Temperature) | X2 (Time) | Total Phenol (mg TAE/100 g) | Total Flavonoids (mg QE/100 g) | pH  |
|-----|-----------------|-----------|-----------------------------|-------------------------------|-----|
| 1   | 50              | 5.41      | 400.29                      | 20.01                         | 4.35|
| 2   | 50              | 4         | 339.24                      | 20.64                         | 4.40|
| 3   | 50              | 2.59      | 516.09                      | 29.17                         | 4.40|
| 4   | 35.86           | 4         | 331.49                      | 12.71                         | 4.05|
| 5   | 40              | 5         | 126.55                      | 16.14                         | 4.25|
| 6   | 60              | 3         | 391.09                      | 17.81                         | 4.20|
| 7   | 60              | 5         | 224.42                      | 19.12                         | 4.30|
| 8   | 50              | 4         | 186.14                      | 17.65                         | 4.35|
| 9   | 50              | 4         | 221.15                      | 18.47                         | 4.40|
| 10  | 50              | 4         | 230.23                      | 16.61                         | 4.40|
| 11  | 40              | 3         | 226.84                      | 15.06                         | 4.50|
| 12  | 64.14           | 4         | 365.89                      | 23.31                         | 4.40|
| 13  | 50              | 4         | 289.34                      | 16.50                         | 4.30|

The Expert Design Program will provide a regression model that matches the measurement results of each response. The model obtained can be used to predict responses. The responses analyzed were total phenol levels, total flavonoids and pH.

3.2.1 Total phenol

The total phenol test was carried out by reacting the sample with 10% Focal Ciocalteu and NaHCO3 and then absorbed the sample at 725 nm wavelength. When the phenolic compounds contained in the sample are reacted with the folin-ciocaltelu reagent, the sample color changes from yellow to blue. The more concentrated blue color formed, the higher the phenolic compounds contained in the sample [25].

Based on RSM calculations, the temperature and time treatments do not have a significant effect on total phenols. Two-dimensional contour graph of the effect of temperature and time of extraction of kecombrang stem is presented in Figure 1.
The result of total phenol liquid extract of kecombrang stem provides a determination of the level of significance and suitability of the model carried out by ANOVA analysis on Design Expert ver.10. The type of regression chosen for the analysis of total phenols is a cubic model with a coefficient of determination (R2) of total phenols of 0.7812. [26] states that a value of R2 close to 1 indicates a high degree of correlation between observation and predictive value. Based on the lack of fit tests the results obtained are 0.3233 (32.33%) which indicates this model is not significant. The model will be considered appropriate if the lack of fit tests of the model are insignificant [27].

One of the possible causes of the temperature factor and duration of extraction being not affected in the liquid extraction process of kecombrang stem and leaves is due to the use of distilled aquades which are less appropriate in the extraction process. Phenols are polar compounds that have high solubility when extracted with polar solvents such as ethanol and distilled water. In the research of [28] polar solvents such as ethanol were able to extract phenols higher than other solvents. Based on these studies it is suspected that the phenol component contained in kecombrang has a polarity that approaches the ethanol polarity so that the use of ethanol solvents is more effective to dissolve phenol compounds in kecombrang. The solubility of phenol the highest is not always present in polar extracts, but depends on the structure of the phenol compound itself. The equation of the regression model obtained is as follows:

\[ Y = 340.60 - 41.79(A) + 12.67(B) - 4.97(AB) - 38.20(A^2) - 38.20(B^2) - 33.39(A^2B) + 75.10(AB^2) \]

Note:
- \( Y \) = Respons value of total phenol (mg TAE/mL)
- \( A \) = temperature (°C)
- \( B \) = time (hours)

The ANOVA model used in the total phenol response of kecombrang leaf liquid extract was a quartic model with R2 as large as 0.8924. P-values in the ANOVA model, temperature, time and interactions between temperature and time on the total phenol response were 0.0928, respectively; 0.7091; 0.2481 and 0.6136. Especially the p-value in the ANOVA total phenol model is greater than p < 5% which means that the model is not significant or does not significantly affect the response. The ANOVA total phenol model of the quartic model produces a two-dimensional color contour graph like in Figure 2.

![Figure 2. Two-dimensional contour graph of total response of phenol liquid extract of kecombrang leaves.](image)

The results of the analysis of total phenol responses by using a two-dimensional color contour graph can be seen in Figure 2. The response shows that the temperature and time of extraction did not have a significant effect on the total phenol content of liquid extract of kecombrang leaf powder. The value of total phenol content of kecombrang leaf extract ranged from 126,550 - 516,085 mg TAE /
100 g. The highest value of total phenol in the treatment combination was obtained from a treatment temperature of 50°C with a 2.6-hour duration of 516.085 mg TAE / 100 g. The lowest total phenol value in the treatment combination was obtained from the treatment temperature of 40°C with a duration of 5 hours of 126,550 mg TAE / 100 g.

The value of total phenol content in this study was lower when compared with the results of research conducted by [28], liquid extract of kecombrang leaves with ethanol solvent resulted in total phenol content values ranging from 1338.06 - 8636.15 mg TAE / 100 g. This difference in total phenol content can be caused by differences in the types of solvents used. Phenol is a polar compound so that its solubility is highest in polar solvents. Polar (ethanol) solvents are able to extract higher phenols so that the total value of the ethanol fraction is higher [29]. The bioactive component of kecombrang flower extract varies according to its polarity. The phytochemical components of hexane extract consist of steroids, triterpenoids, alkaloids, and glucosides. Phytochemical components of ethyl acetate extract are steroids, terpenoids, alkaloids, flavonoids, and glycosides, while ethanol extracts produce phenolic components, terpenoids, alkaloids, saponins, and glycosides [14].

According to [29], phenol is a polar compound so that its solubility is highest in polar solvents. Polar (ethanol) solvents are able to extract higher phenols so that the total phenol fraction value of ethanol is higher. The chlorophyll content also influences the high total phenol produced by kecombrang leaves, although it is not a phenol compound, hydrophilic chlorophyll is extracted in a polar ethanol solution and is read when measuring [28].

### 3.2.2. Total Flavonoid

The regression model chosen for the analysis of total flavonoid liquid extract of kecombrang stem is a quadratic model with a coefficient of determination (R2) of total flavonoids of 0.8578 which is close to 1. Based on the lack of fit tests, a result of 0.6808 (68.08%) is obtained, which indicates insignificant results. The equation of the regression model obtained is as follows:

\[
Y = 78.32 - 6.14(A) + 11.00(B) - 0.85(AB) - 12.37(A^2) - 6.09(B^2)
\]

Note:

- **Y** = Respons value of total flavonoid (mg QE/mL)
- **A** = temperature (°C)
- **B** = time (hours)

RSM calculation shows the influence of temperature factor and extraction time of liquid extract of kecombrang stem on total flavonoids. The regression model equation shows that the increase in total flavonoid extract response is inversely proportional to the increase in time (B) but directly proportional to the increase in temperature (A). The longer the extraction time used, the total value of the resulting flavonoids will be minimum, whereas the higher the extraction temperature, the total value of the resulting flavonoids will increase and at a certain point will be constant. This is shown by the two-dimensional graph in Figure 3.
Figure 3. Two-dimensional contour graph of total response of flavonoid liquid extract of kecombrang stem.

Determination of total flavonoid levels of kecombrang stem liquid extract in this study used quercentin as a standard solution. Quercentin is a class of flavonoids that are often found in plants that are known to have biological activities as antioxidants [30]. The highest total flavonoid value was found in the treatment temperature of 40°C with 5 hours extraction time. The use of high temperatures can increase the levels of flavonoids obtained. This is consistent with the research of [31] which states that high temperatures are able to release biocative compounds that are bound in cells due to damage to cell elements. This causes more and more compounds to be extracted.

Increased straight-line extraction time with increased flavonoid content. This is consistent with the research of [32] which states the increase in extraction time is directly proportional to the increase in the total value of flavonoids. Within a certain time the concentration of flavonoids in the solvent will not increase due to equilibrium in the extraction has been reached. So that at this point the increase in extraction time does not affect the solute [31].

At a treatment temperature of 60°C within 5 hours a decrease in the total value of flavonoids. This is because flavonoids have been damaged. Bioactive compounds such as flavonoids are generally thermosensitive and not resistant to exposure to high temperatures above 50°C, so that they undergo structural changes and produce extracts with low bioactive content [33].

The ANOVA model used in the total flavonoid response of kecombrang leaf liquid extract was a quartic model with R2 as large as 0.9436. P-values in the ANOVA model, temperature, time and interaction between temperature and time on the total flavonoid response were 0.0284; 0.0115; 0.0187 and 0.9476. Especially the p-value in the ANOVA total flavonoid model is smaller than p <5% which means that the model is significant or significantly influences the response. The ANOVA model of the total flavonoid quartic model produces a two-dimensional color contour graph like in Figure 4.
The results of the analysis of the response of the flavonoid levels of the kecombrang leaf liquid extract can be seen in Figure 4. The results of the response of the flavonoid levels showed that the temperature and time of extraction had a significant effect on the flavonoid levels of the liquid extract of the kecombrang leaf powder. The value of flavonoids of kecombrang leaf liquid extracts ranged from 12,712 - 29,178 mg QE / 100 g. The highest value of flavonoid levels in the treatment combination was obtained from the treatment temperature of 50°C with a duration of 2.6 hours at 29.178 mg QE / 100 g. The lowest value of flavonoid levels in the treatment combination was obtained from a treatment temperature of 36°C with a duration of 4 hours amounting to 12.712 mg QE / 100 g. Graph of the effect of extraction temperature on the levels of liquid extracts of kecombrang leaf extract can be seen in Figure 5.

It is seen in Figure 5, at 4 hours with a temperature of 36°C to 64°C the levels of flavonoid extract extract tends to increase. This is in accordance with research conducted by [34] the increase in extraction temperature used, can make it easier for the solvent to attract chemicals contained in the material. According to [35] extraction temperatures that were too low caused not all active compounds to be extracted from the ingredients, so the active compounds produced were low. The graph of the effect of extraction time on flavonoid levels can be seen in Figure 6.
Figure 6 shows a graph of the effect of extraction time at 50°C on the levels of flavonoids of kecombrang leaf liquid extract. At the time of 2.6 hours to 5.4 hours the level of flavonoids has decreased. Increasing the amount of time used, flavonoid levels tend to decrease. This is consistent with research conducted by [33] that the addition of the extraction time, the total flavonoids produced did not increase significantly, even decreased the total flavonoids. [35] states that the increase in temperature and extraction time used need to be considered. Because the extraction temperature is too high and the extraction time is too long and exceeds the optimum time limit can cause the loss of compounds in the solution due to evaporation.

3.2.3. pH.

The selected regression model for the variable pH of liquid extract of kecombrang stem is the cubic model. The coefficient of determination (R²) produced was 0.5204 (52.04%) with a lack of fit value of 0.9015 (90.15%) which showed insignificant results. The equation of the regression model obtained is as follows:

\[ Y = 3.74 - 0.035(A) + 0.018(B) + 0.075(AB) + 0.058(A^2) + 0.021(B^2) + 7.322E-003(A^2B) + 0.11(AB^2). \]

Note:

\[ Y = \text{Respon pH} \]
\[ A = \text{temperature (°C)} \]
\[ B = \text{time (hours)} \]

RSM calculation shows that the temperature and extraction time of the liquid extract of kecombrang stem does not significantly affect the pH value. A two-dimensional contour graph of the pH response is presented in Figure 7.
Figure 7. Two-dimensional contour graph of pH response of liquid extract of kecombrang stem.

The effect of temperature on the acidity of the extract, that the increase in temperature does not affect the pH of the extract produced. The increase in extraction time also did not affect the acidity of the extract. This is in accordance with [37] which states that the extraction time has no significant effect on pH. It is suspected that the type of solvent used in extraction can affect the acidity of the extract. In this research, distilled water is used as a solvent in the extraction process, where distilled water is not suitable for extracting phenol compounds in kecombrang stems. Phenolic compounds are acidic because of the nature of the H⁺ group that easily breaks away from its main structure [38]. This H⁺ group causes the atmosphere of the solution to become acidic and affects the pH of the solution.

The ANOVA model used in response to the pH value of kecombrang leaf liquid extract was a quartic model with R² as large as 0.9493. P-values in the ANOVA model, temperature, time and the interaction between temperature and time in response to the pH value of 0.0232 respectively, 0.0052, 0.4734 and 0.0173. Especially the p-value in the ANOVA model pH value is smaller than p <5% which means that the model is significant or significantly influences the response. ANOVA model the pH value of the quartic model produces a two-dimensional color contour graph like in Figure 8.

Figure 8. Two-dimensional contour graph of pH value of kecombrang leaf liquid extract

The results of the analysis of pH response responses can be seen in Figure 8. The response results of pH values indicate that the temperature and extraction time have a significant influence on
the pH value of liquid extract of kecombrang leaf powder. The value of flavonoids of kecombrang leaf liquid extracts ranged from 4.05 - 4.5. This is consistent with the literature. According to [14], the pH value of foodstuffs and processed foods in general ranges between 4-7. The level of acidity (pH) indicates the ability of antimicrobial compounds in food to inhibit the growth of bacteria that is influenced by the stability of the food processing of the product. The graph of the effect of extraction temperature on the pH value can be seen in Fig 9.

Figure 9. Graph of the effect of extraction temperature on the pH value of kecombrang leaf liquid extract at 3 hours

Figure 9 shows a graph of the effect of temperature at 3 hours on the pH value of the liquid extract. Addition of extraction temperature used, the pH value tends to be small or acidic. According to [2] pH will tend to decrease due to the addition of extraction time and temperature. This is due to the increasing number of elements in the material that decompose and form chemical compounds that are acidic. The graph of the effect of extraction time on the pH value can be seen in Figure 10.

Figure 10. Graph of the effect of extraction temperature on the pH value of kecombrang leaf liquid extract at temperature 50°C

Figure 10 shows a graph of the influence of time at 50°C on the pH value of kecombrang leaf liquid extract. In addition to the extraction time used, the pH value tends to be small or acidic. According to [39] pH tends to decrease due to the addition of extraction time and temperature. This is due to the increasing number of elements in the material that decompose and form chemical compounds that are acidic.

3.3 Verification of Optimization Results

After conducting research with 13 treatments, RSM recommends several optimum formulas according to the desired optimum criteria. Optimization of kecombrang stem liquid extract, the expected response criteria are in range for pH and total phenol responses, and maximize for flavonoid criteria. Based on these response criteria RSM recommends eleven formulations. From the eleven optimum formulas recommended by RSM, the formula with temperature 40°C and extraction time of
5 hours which has a high desirability of 0.964 was selected, which is presented in Table 6. Optimization of liquid extract of kecombrang stem, the expected response criteria are in range for the pH response and total phenol, and maximize for flavonoid criteria. Based on these response criteria RSM recommends eleven formulations. From the eleven optimum formulas recommended by RSM, a formula with a temperature of 51°C and a extraction time of 3 hours which has a high desirability of 0.721 was chosen, which is presented in Table 7.

Table 6. Recommended RSM formula for liquid extract of kecombrang stem.

| No. | Temperature (°C) | Time (Hours) | Total Phenol | Total flavanoids | pH  | Desirability |
|-----|------------------|--------------|--------------|------------------|-----|--------------|
| 1   | 40               | 5            | 23.51        | 11.47            | 3.694 | 0.964        |

Table 7. Recommended RSM formula for liquid extract of kecombrang leaf.

| No. | Temperature (°C) | Time (Hours) | Total Phenol | Total flavanoids | pH  | Desirability |
|-----|------------------|--------------|--------------|------------------|-----|--------------|
| 1   | 50.848           | 3            | 327.13       | 24.586           | 4.35 | 0.721        |
| 2   | 50.951           | 3            | 325.13       | 24.585           | 4.37 | 0.721        |
| 3   | 51.092           | 3            | 328.10       | 24.581           | 4.37 | 0.721        |
| 4   | 55.198           | 3            | 330.32       | 23.057           | 4.30 | 0.628        |
| 5   | 60               | 4.101        | 302.67       | 21.777           | 4.42 | 0.551        |
| 6   | 60               | 4.111        | 302.77       | 21.777           | 4.42 | 0.551        |
| 7   | 60               | 4.085        | 304.68       | 21.776           | 4.42 | 0.550        |
| 8   | 60               | 4.120        | 301.65       | 21.776           | 4.42 | 0.550        |

There are eleven optimum formulas of kecombrang stem liquid extract recommended by RSM, selected formula with a temperature of 40°C and a extraction time of 5 hours which has a high desirability of 0.964. The optimum formula produced by RSM for liquid extraction of kecombrang leaves is 8 formulations. The optimum formula was selected with a temperature of 51°C and extraction time of 3 hours which has a high desirability of 0.721. The desirability value is the value of the optimization objective function that shows the ability of the program to fulfill the desire based on the criteria set on the final product. The desirability value which is close to 1.00 indicates the ability of the program to produce the desired product more perfect [38].

The verification results of the optimum conditions recommended by RSM, obtained liquid extract of kecombrang stem with a total phenol value of 23.99 mg QE / 100 g, a total flavonoid value of 11.14 mg TAE / 100 g, and a pH value of 3.85. When compared with the predicted value by RSM (Table 8), the verification value ranges between 95% PI low and 95% PI high, so that the results of RSM optimization can be accepted.

Table 8. Verification results of kecombrang stem liquid extract

| Respon      | Verification Value | Prediction Value | PI 95% Low | PI 95% High |
|-------------|--------------------|------------------|------------|-------------|
| Total Phenol| 23.99              | 23.50            | 10.25      | 36.75       |
| Total Flavonoids| 11.14        | 11.47            | 9.16       | 13.78       |
| pH          | 3.85               | 3.69             | 3.27       | 4.12        |

The results of verification of optimum conditions of kecombrang leaf liquid extract with a total phenol value of 327.13 mg QE / 100 g, a total flavonoid value of 24,911 mg TAE / 100 g, and a pH value of 4.35. When compared with the predicted value by RSM (Table 9), the verification value ranges between 95% PI low and 95% PI high, so that the results of RSM optimization can be accepted.
| Respon            | Verification Value | Prediction Value | PI 95% Low | PI 95% High |
|-------------------|--------------------|------------------|------------|------------|
| Total Phenol      | 327.13             | 253.30           | 68.68      | 437.91     |
| Total Flavonoids  | 24.91              | 24.586           | 18.98      | 30.20      |
| pH                | 4.35               | 4.37             | 4.23       | 4.53       |

Furthermore, an independent t-test with a 95% confidence level is performed to validate the verification results with the predicted values. The test results obtained from all responses (total phenols, total flavonoids, and pH) is the absence of a significant difference between the results of verification with prediction results (F count > 0.05), so that the results of temperature optimization and liquid extraction time of stem and leaf kecombrang can be accepted because it is not significantly different from the results of RSM predictions.

4. CONCLUSION

The optimum formula of kecombrang stem liquid extract based on the response of total flavonoid value, total phenol and pH value is a temperature treatment of 40°C with extraction time of 5 hours with total flavonoid response value of 11.14 mg QE / 100 g, total phenol of 23.99 mg TAE / 100 g, and a pH value of 3.85. The optimum formula of kecombrang leaf liquid extract based on the response of total flavonoid value, total phenol and pH value is 51°C temperature treatment with extraction time of 3 hours with total flavonoid response value of 327.13 mg QE / 100 g, total phenol of 24,911 mg TAE / 100 g, and a pH value of 4.35. The total value of flavonoids, total phenols and pH values of liquid extracts of kecombrang stems and leaves did not differ greatly with RSM predictions. Kecombrang stem liquid extract contains bioactive compounds such as alkaloids, phenols, flavonoids, tannins, glycosides, saponins and triterpenoids. Kecombrang leaf liquid extract contains bioactive compounds such as alkaloids, phenols, flavonoids, saponins and steroids.

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