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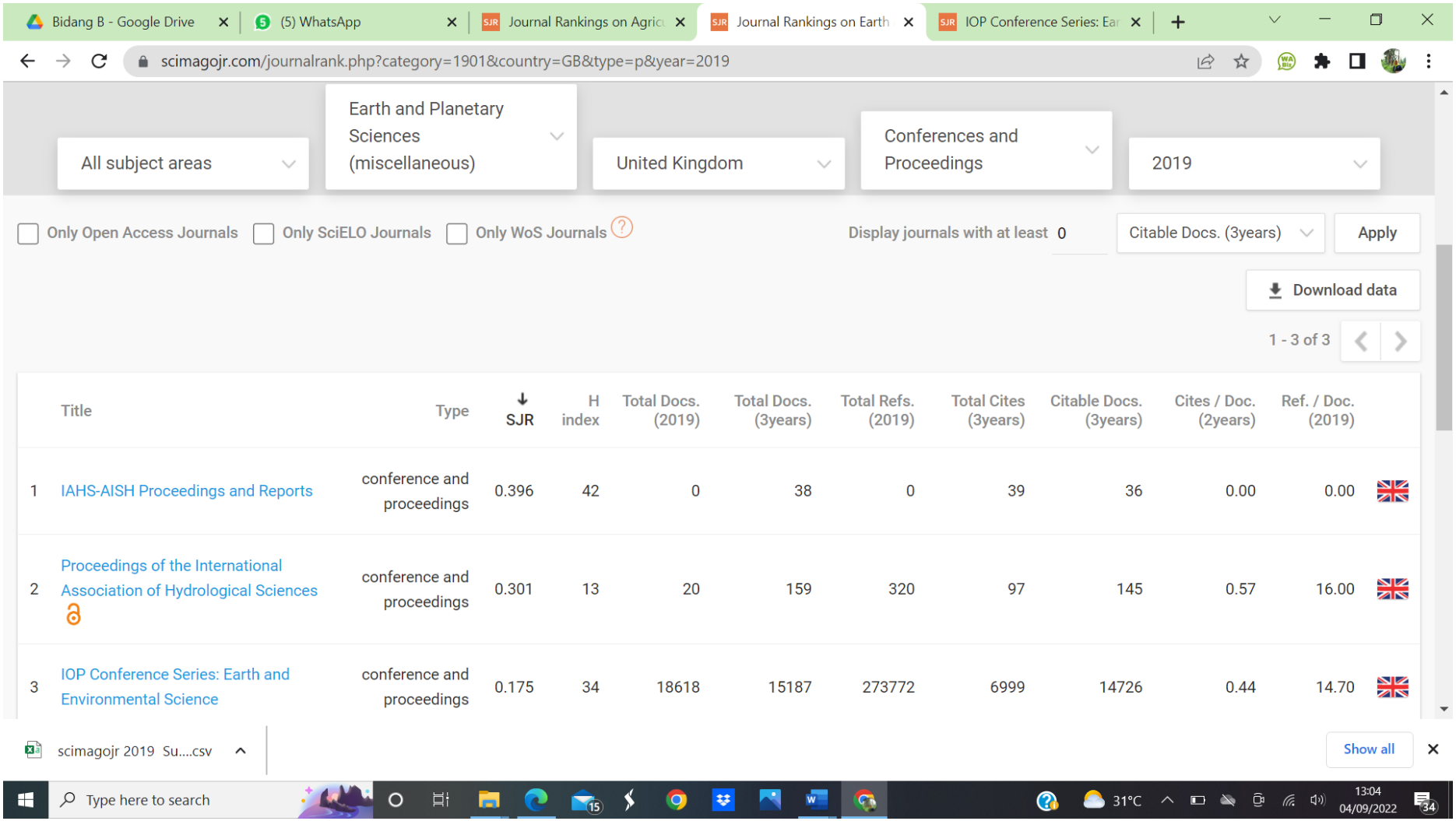
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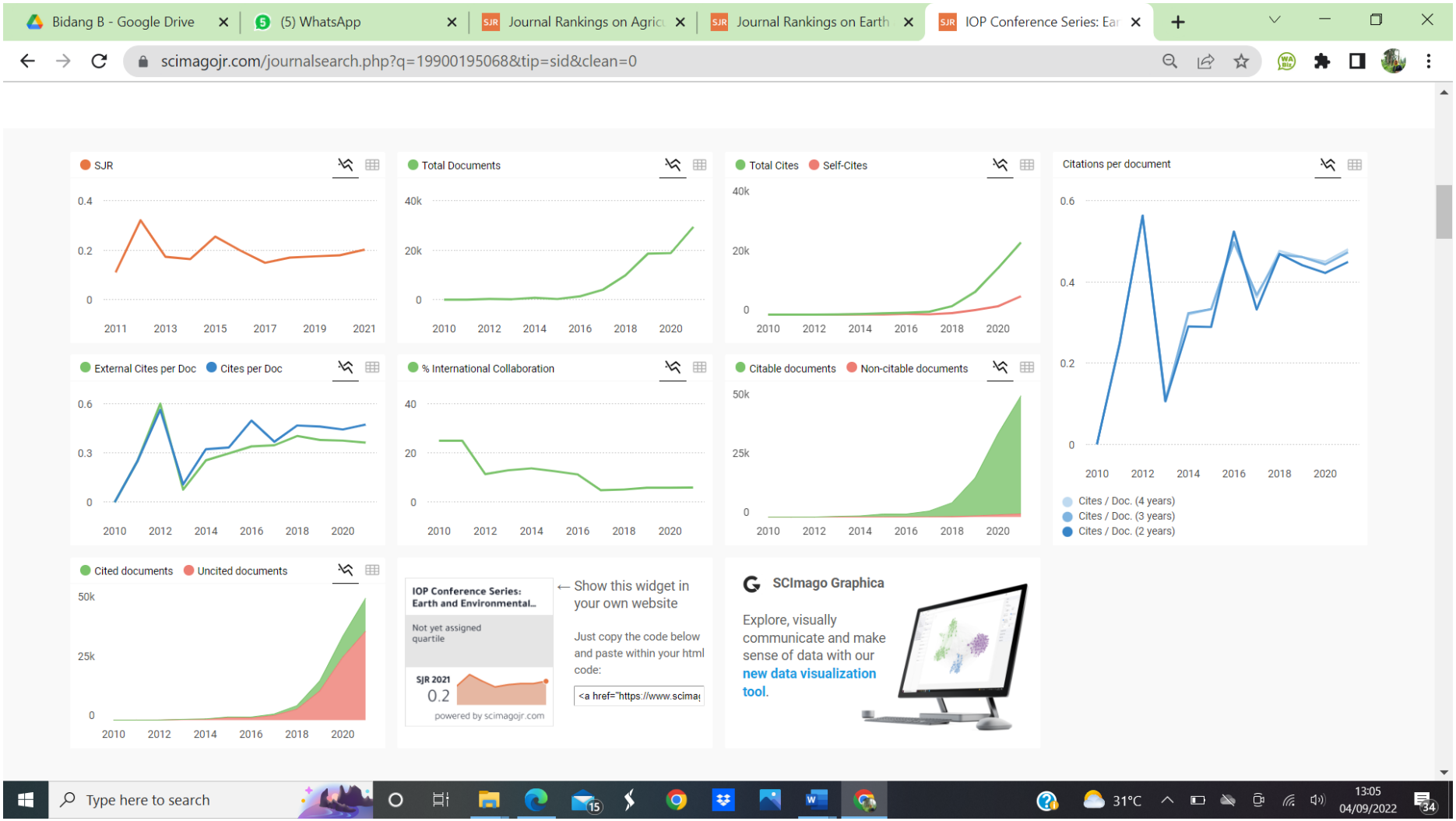
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QUALITY OF SIMPLICIAN BIOACTIVE COMPONENTS AND LIQUID EXTRACT OF KECOMBRANG FLOWER POWDER FROM TEMPERATURE AND TIME OPTIMIZATION RESULTS

Dwiana Intan Pertiwi, Rifda Naufalin*), Poppy Arsil, Erminawati, Rumpoko Wicaksono and Taslimatul Auliya

Food Science and Technology Study Program, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto 53122
Corresponding author : rifda.naufalin@unsoed.ac.id

Abstract. Kecombrang flower has benefits as an antioxidant and antimicrobial. This capability can be maximized by processing through drying and liquid extraction. In the drying and extraction of liquid, the temperature and time that is not right can affect the content of bioactive components of the product. This study aims to determine the quality of bioactive components of powder and liquid extract of kecombrang flowers quantitatively and qualitatively based on temperature and time. The experimental design used in kecombrang flower powder is a Completely Randomized Design (CRD) with 9 treatment and 3 replication as long as the liquid extract of kecombrang flowers using the Responsse Surface Method (RSM) method with Expert Design 10 software. Central Composit Design (CCD) produced 13 optimization formulas. Factors that were tested on drying are 50^{o}C, 60^{o}C, 70^{o}C, and time is 4, 5 and 6 hours. Liquid extraction temperature is 40oC, 50oC, 60oC and time is 3, 4 and 5 hours. Based on the results of the study the highest total phenol simplisia and liquid extract of kecombrang flowers namely 27.93 TAE / 100 g sample and 64.458 mg TAE / 100 g. The highest total of flavonoids is simplicia and liquid extract of kecombrang flower which is 2.073 QE / 100 g sample and 35.120 mg QE / 100 g and pH value (3.9). The compounds in the optimum formula product are phenolic, flavonoids, alkaloids, terpenoids and tannins.

Keywords: kecombrang flower, liquid extraction, temperature and time drying.

1. INTRODUCTION

One of the herbs and medicinal plants that have potential as functional foods in antioxidants and antibacterial is kecombrang (*Etlingera elatior*) [1, 2]. Kecombrang is one type of spice plant that has long been known and used by humans as food ingredients. Plant parts that are commonly used are flowers and stems. Compounds contained in kecombrang flowers are alkaloids, flavonoids, polyphenols, terpenoids, steroids, saponins, and essential oils [3, 4, 5].

The ability of kecombrang flowers can be maximized by further processing by drying. Drying aims to reduce the water content of the kecombrang flowers to the limits of the development of microorganisms and enzyme activities that can cause decay is inhibited or even stopped altogether. Thus, the dried material has a longer shelf life [6]. During the drying process, several things must be considered such as drying temperature and drying

time. The drying process plays a very important role. If the drying temperature is too high it will cause a decrease in the active compound and change the color of the dried product. Meanwhile, if the temperature used is too low it is difficult to reach the desired water content.

The use of kecombrang flower powder can still be maximized by processing it in liquid extract form. Extraction is the activity of withdrawing soluble chemicals so that they are separated from insoluble substances by using a liquid solvent [7]. The method used in making liquid extract of kecombrang flower powder is by extraction using a water solvent.

Generally the solubility of the extracted active substance will increase in size with increasing temperature. However, the increase in extraction temperature also needs to be considered. This is because temperatures that are too high can cause damage to the material being processed [8]. Besides temperature, the use of extraction time also needs to be considered. This is in accordance with [9], which states that the right extraction time will produce optimal compounds. Extraction time that is too long will cause hydrolyzed extracts, while extraction time that is too short causes not all active compounds to be extracted from the ingredients.

Therefore, this study aims to determine the quality of the quantitative bioactive components of powder and liquid extract of kecombrang flowers, namely total phenols, total flavonoids and pH values as well as qualitative, namely phenolic compounds, flavonoids, alkaloids, triterpenoids, tannins, saponins, and glycosides based on temperature and time.

2. RESEARCH METHODOLOGY

2.1. Experimental design

The experimental design used in kecombrang flower powder is a Completely Randomized Design (CRD) with 9 treatment combinations and was repeated 3 times, as far as the liquid extract of kecombrang flowers using the Responsse Surface Method (RSM) method with Expert Design 10 software. Central Composit Design (CCD) with two factors and two analytical tests tested produced 13 optimization formulas. The factors that were tried in this study are temperature and time. The flower drying temperature consists of three levels, 50°C, 60°C, 70°C, and time consists of three levels, 4, 5 and 6 hours. The liquid extract temperature consists of three levels, namely 40°C, 50°C, 60°C and time consists of three levels namely 3, 4 and 5 hours.

2.2. Sample preparation of kecombrang flower powder

The stage before conducting research is to prepare a sample of kecombrang flower powder. The kecombrang flower is first selected and a flower crown is taken and then washed with water. Furthermore, kecombrang flower crowns are reduced in size so that the drying process is more optimal. After that, the flower slices are dried using a cabinet dryer with the appropriate treatment temperature that is 50°C, 60°C and 70°C with a time of 4, 5 and 6 hours. After the flowers are dried, they are ground using a disk mill. Flower powder that is formed into 60 mesh is used as material in the study of temperature optimization and liquid extraction time.

2.3. Liquid Extraction of Kecombrang Flower Powder

The extraction method used in this study is maceration using a water solvent. At first, a 60 mesh flower powder was put into the extractor. Then, it is mixed with a water solvent whose temperature is adjusted to the treatment factor which is 40°C, 50°C and 60°C through the extractor cover pipe. Comparison of powder: water used is 1:14. After that, the extractor is closed then turned on, set the temperature according to the treatment and agitator speed of 60 rpm. The extraction time used in accordance with the treatment factor is 3, 4 and 5 hours. After extraction is complete, the extract is then allowed to stand in a closed container and dark conditions for 19 to 24 hours. Next, the sample is filtered using a press to separate the yield of the powder and the filtrate. Separate powder, then dried using a cabinet dryer with a temperature of 50°C and a time of 4 hours. After drying, the extraction residual powder is first extracted again in a ratio of 1:14 using the same temperature and time. The filtrate sample used is a combination of liquid extract in the first extraction and replicates.

2.4. Quantitative Test

2.4.1. Total Phenol

400 µL supernatant was added with 1.5 mL of Folin Ciocalteu and allowed to stand for 5 minutes at room temperature. Next, added 1.5 mL sodium bicarbonate (NaHCO3) 0.556 M, shaken and left in a dark room for 90 minutes, then absorbance was measured using a spectrophotometer at a wavelength of 725 nm [10].

2.4.2. Total Flavonoid

100 mL or 0.1 mL sample supernatant was added with 1 mL of 2% AlCl 3 (2 g AlCl 3 in 100 mL of 5% glacial acetic acid solution) and 1 mL of 120 mM potassium acetic acid solution (1,176 g 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 [11].

2.4.3. Water Content

Samples were weighed as much as 0.5 g in a moisture analyzer cup. Moisture analyzer is set at 105°C. Moisture analyzer is closed and awaited the results of its water content. The results of the water content are recorded. Moisture content of 10% [12].

2.4.4. pH Value

The pH measurement is carried out using a pH meter. Before use, the pH meter is calibrated first using a buffer solution of pH 4 and pH 7. The way to measure pH is to insert the pH meter electrode in the sample, wait a while until the pH stabilizes, so that the pH value is measured. After completion, the electrodes are removed and rinsed with distilled water [13].

2.5. Qualitative Test

2.5.1. Phenolic Compounds

As much as 2 mL of the extract sample was added 2 drops of 5% FeCl3 solution. The formation of green or blue green indicates phenol compounds in the ingredients [14].

2.5.2. Alkaloid

0.1 gram extract was added with 10 mL chloroform and a few drops of ammonia were added. The chloroform fraction is separated and acidified with a few drops of concentrated H2SO4. Acid fraction is taken and divided into 3 tubes, then Dragendorff, Meyer and Wagner reagents are added. The presence of alkaloids is characterized by the formation of white deposits in Meyer reagents, red deposits in Dragendorff reagents, and brown deposits in Wagner reagents [14].

2.5.3. Steroid/triterpenoid

A total of 1 g of sample was dissolved with 25 mL of 50 ° C hot ethanol, then filtered into a Porcelain cup and evaporated to dryness. The residue is dissolved with ether and transferred into a test tube, then added 3 drops of anhydrous acetic acid and 1 drop of concentrated H2SO4 (Lieberman Burchard Test). Red or purple indicates triterpenoids and green or blue indicates steroids [14].

2.5.4. Saponin

Saponins can be detected by foam testing in hot water. The foam is stable for 10 minutes and does not disappear with the addition of 1 drop of HCl 2 N indicating saponin [14].

2.5.5. Tannin

Extract as much as 10 mL boiled. After cooling, the filtrate was added with 5 mL FeCl3 1% (w / v). If the color changes to dark blue, it means that the sample contains tannin [14].

2.5.6. Flavonoid

A sample of 2 mL was put into a test tube. Then 100 mg of magnesium powder and 3 mL of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol of equal volume) and 2 mL of hydrochloric acid are added, then the mixture is shaken. The formation of red, yellow or orange in the amyl alcohol layer shows the results of flavonoids [15].

2.5.7. Glycoside

It is done with the Lieberman-Buchard reaction. Kecombrang flower bubk extract was dissolved in ethanol solvent, evaporated on a water bath and then dissolved in 5 mL acetic acid anhydride and then added 10 drops of concentrated sulfuric acid. The formation of blue or green indicates glycosides [16].

2.6. Data Analysis

Kecombrang flower powder data were analyzed using the F test (analysis of variance) at a 95% confidence level with ANOVA. If the analysis results show a real effect, then proceed with the DMRT test (Duncan Multiple Range Test) at the level of 5%. Kecombrang flower liquid extract data were analyzed using the Response Surface Method (RSM) to obtain the optimal response of liquid extraction methods. The results of the analysis of quantitative optimization variables are visualized in the form of two-dimensional images to show the

effect of factors on responses. The recommended optimum formula is then verified and validated using an independent t test to find out whether there is a difference between the predicted value and the product after treatment.

3. RESULT AND DISCUSSION

3.1. Quantitative

3.1.1. Total Phenol

The average value of total phenol kecombrang flower in powder and liquid extract can be seen in Figure 1.

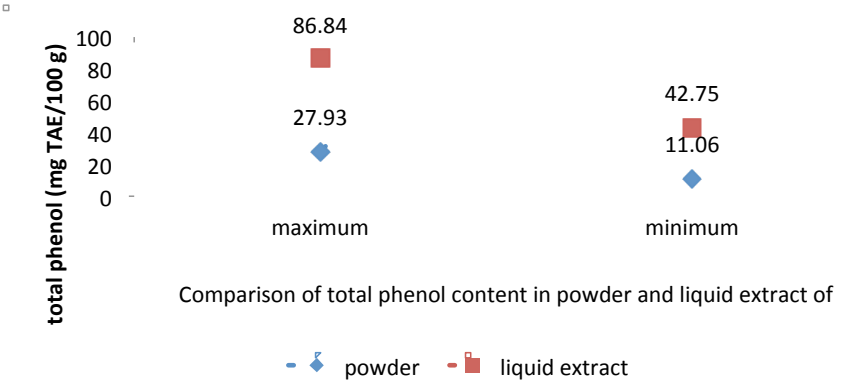

Figure 1. Comparison of total phenol content in powder and liquid extract of kecombrang flowers.

Based on Figure 1, the highest value of total phenol liquid extract of kecombrang flower is 86.84 mg TAE / 100 g and the lowest value is 42.75 mg TAE / 100 g. While the highest total value of phenol kecombrang flower powder was obtained in the amount of 27.93 mg TAE / 100 g and the lowest value was 11.06 mg TAE / 100 g. The total value of phenol liquid extract of kecombrang flower is better than kecombrang flower powder. This is seen based on the total phenol value of liquid extract which is higher than the total value of phenol in kecombrang flower powder, the higher total value of kecombrang flower phenol in the form of liquid extract because of the bioactive component especially phenol has separated from the insoluble material with liquid solvent while still in powder form. Therefore, the total phenol content of kecombrang flowers in liquid extracts is higher than when it was still in powder form.

According to research conducted by [17, 18] differences in total phenol levels due to phenol damage can be caused by environmental factors such as light, temperature and oxygen. This statement is reinforced by research [19] that the total levels of phenol compounds decreased due to oven drying. The higher drying temperature results in an increase in the enzyme inactivation process of the polyphenol oxidase enzyme, so that the enzyme activity will be lower and the damage to the polyphenol compound less, but if the drying temperature exceeds the optimum temperature, the stability of the polyphenol compound will be disturbed so as to cause a decrease in the content of polyphenol compounds in the material [20].

According to [21], extraction is the activity of withdrawing soluble chemicals so that they are separated from insoluble materials with liquid solvents. During extraction, optimal use of temperature and time is also one of the reasons for the higher total phenol content produced. According to [22] the longer the extraction time, the greater the chance of the solvent interaction to dissolve the material so that the results also increase to the saturation point of the solution. Contact between the sample and the solvent can be improved if it is assisted with shaking. This is useful so that contact between the sample and the solvent is more frequent, so that the extraction process is more perfect.

3.1.2. Total Flavonoid

The average value of total flavonoids of kecombrang flowers in powder and liquid extract can be seen in Figure 2.

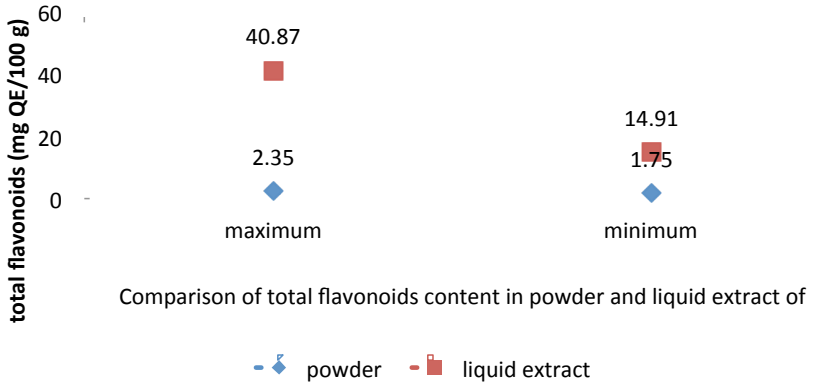

Figure 2. Comparison graph of flavonoid content in powder and liquid extract of kecombrang flower

Based on Figure 2, the highest value of total flavonoid kecombrang flower powder was obtained at 2.35 mg QE / 100 g and the lowest flavonoid value was 1.75 QE / 100 g. The highest value of total flavonoids of kecombrang flower extract is 40.87 mg QE / 100 g and the lowest total value of flavonoids is 14.91 mg QE / 100 g. The value of flavonoids in liquid extract of kecombrang flower powder is higher than in kecombrang flower powder, the higher total value of kecombrang flower flavonoids in liquid extract form is presumably because the flavonoid compound has separated from the insoluble material with liquid solvent while still in powder form. Therefore, the total flavonoid content of kecombrang flowers in liquid extracts is higher than when it was still in powder form.

Flavonoids are polyphenols with the basic structure of phenols which have already oxidized and sensitive to heat treatment so that the drying process will affect the levels of flavonoids in the sample [23]. Flavonoid analysis was carried out using ethanol as a polar solvent. According to [21], extraction is the activity of withdrawing soluble chemicals so that they are separated from insoluble materials with liquid solvents. Polar flavonoids can be extracted well using solvents so that the kecombrang flowers produce flavonoids.

An increase in temperature and time extraction need to be considered. Extraction temperatures that are too high and extraction times that are too long and exceed the optimum

time limit can cause the loss of compounds in the solution due to evaporation. Therefore, the extraction treatment temperature of 60°C with a length of 3.735 hours can provide maximum results because between the temperature and extraction time are not maximized both [24].

3.1.3. Water Content

The average water content of kecombrang flower powder can be seen in Figure 3.

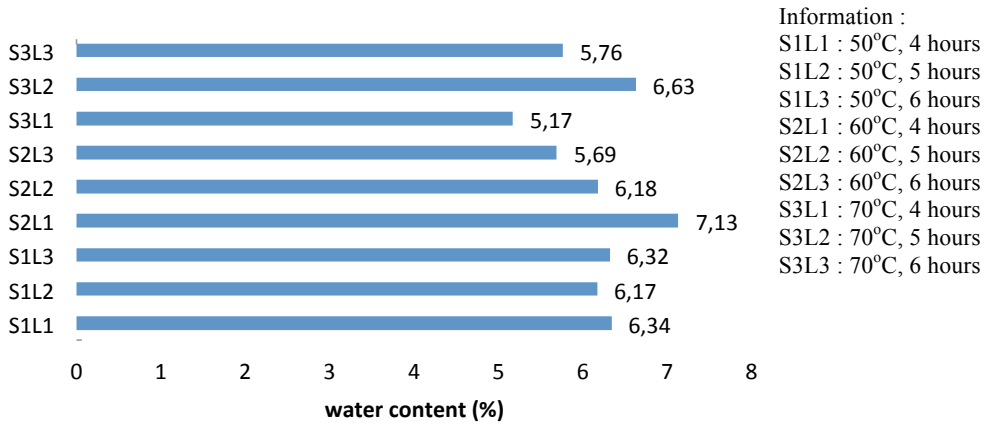

Figure 3. Water content of dried simplicia kecombrang flowers with various treatments

Based on Figure 3, it shows that the value of the water content of simplicia kecombrang flowers ranges between 5.17 - 7.13%. According to the Indonesian Herbal Pharmacopoeia [25] and the Minister of Health Decree No. 661 / Menkes / SK / VII / 1994 regarding Requirements for Traditional Medicines, the standard water content of calcium at simplicia is 10%. Fresh kecombrang flowers have high water content which is around 90%, so they need to be dried to extend their shelf life. The purpose of drying is to reduce the water content so that the material is not easily overgrown with molds and other microorganisms and stop enzymatic reactions that can further decompose the active ingredient [26].

Decreased water content in kecombrang flowers is influenced by water evaporation from temperature and time drying [27]. The longer the drying process causes the evaporation of water which is found in the kecombrang flowers, the lower. According to the literature, the higher the temperature and duration of drying, have a great influence on the speed of water transfer in the material. The ability of the material to release water from the surface will be even greater with the increasing temperature of the drying air used and the length of the drying process [28].

3.1.4. pH Value

Temperature and time did not affect the pH value according to [29] that if the concentration (%) of ethanol increases, the pH obtained also increases, but if the extraction

temperature increases the resulting pH remains. The pH value in the optimization formula of liquid extract of kecombrang flower powder (60°C for 3.735 hours) was 3.9. According to [2] kecombrang flowers have a pH value of 3.89.

The decrease in pH is characterized by an increase in color density. Increasing the pH value can cause a decrease in color intensity and concentration of *flavilium cations*. There are several factors that can affect the color of the extract [30]. The process of extracting spices, the composition, color, aroma and rendeman produced will be influenced by the type, size, level of maturity of the raw material, type of solvent, temperature and time of extraction and the extraction method [31].

3.2. Qualitative

Qualitative variables in this study consisted of phenols, flavonoids, alkaloids, triterpenoids, tannins, saponins, and glycosides. The results of testing the qualitative variable of powder and liquid extract of kecombrang flower are presented in Table 1.

Table 1. Qualitative test results of kecombrang flower powder and liquid extract

Treatment	Phenolic	Flavonoids	Alkaloids	Triterpenoid	Tannins	Saponins	Glycosides
Powder	++++	+++	+++	+++	-	++	-
Liquid extract	++++	+++	+++	+++	++	+	-

Information : - = negative, + = weak positive, ++ = positive, +++ = strong positive, ++++ = very strong positive

3.2.1. Phenol

Phenol compounds can function as antioxidants because of their ability to eliminate free radicals and peroxide radicals so that they are effective in inhibiting lipid oxidation [2]. The presence of phenolic is marked in blue to green which is formed when the sample is subjected to FeCl3. The more phenols contained, the more dense the resulting color. This is in accordance with [32] which explains that phenol testing is carried out by reacting 1 mL samples with 5% FeCl. The presence of phenol compounds is characterized by the formation of a bluish-green color to black.

Based on the phenolic test data in Table 1, it shows that the liquid powder and extract of kecombrang flower contains phenol which is very strong due to the change in bluish-green to blackish color. The use of high temperatures and a long time in extraction can reduce levels of phenolic compounds. [33] explained that phenolic compounds are thermosensitive substances, thus enabling hydrolysis and percentage reduction at high temperatures. The profile of phenolic compounds in sample extracts can change with differences in the degree of polarity of the solvents used [34]. This is in accordance with [35] which states that the phenol component contained in the fraction of kecombrang plant parts is thought to have a polarity close to ethanol polarity, so that the use of ethanol solvents is more effective to dissolve phenol compounds.

3.2.2. Flavonoid

Flavonoids are compounds consisting of C6-C3-C6. Flavonoids are commonly found in plants as glycosides. The sugar group has 11 compounds in one or more phenolic hydroxyl groups. The purpose of flavonoids for plants is to attract insects that help the pollination process and to attract the attention of animals that help spread seeds. For humans, flavonoids in small doses work as stimulants in the heart and capillaries [36].

Based on data from the flavonoid test results in Table 1, it shows that the liquid powder and extract of kecombrang flowers contain strong flavonoids because they are marked by the red color formed. The more concentrated the color, the more flavonoid content is. This is also in accordance with [37] that a positive result is indicated by the appearance of dark red (magenta) within 3 minutes. From the analysis, according to [38], the addition of magnesium powder and hydrochloric acid in the testing of flavonoids will cause the reduction of existing flavonoid compounds, causing a red reaction which is a characteristic of the presence of flavonoids.

3.2.3. Alkaloid

Alkaloids are chemical compounds produced by secondary metabolites that are formed based on the principle of formation of a mixture. Alkaloids are divided into three parts, namely N-containing elements involved in the formation of alkaloids, elements without N found in alkaloid molecules and reactions that occur for the typical binding of elements to the alkaloids [36].

Based on the alkaloid test data results in Table 1, it shows that the powder and liquid extract of kecombrang flowers contain strong alkaloids because it produces a red dregs when dropped with Dragendorff reagents. Alkaloid testing is done by adding HCl before adding reagents. This is because alkaloids are alkaline so they are extracted with solvents that contain acids [16]. Alkaloid test results are characterized by the formation of orange to red dregs at the bottom of the tube. The positive alkaloid results in the Dragendorff test were marked by the formation of orange dregs. These dregs are metal complexes with alkaloids. In the manufacture of Dragendorff bismuth nitrate reagents react with potassium iodide to form bismuth (III) iodide dregs which then dissolve in excess iodide to form potassium tetra iodobismunate. In the alkaloid test with Dragendorff's reaction, the lone pair of electrons in nitrogen is used to form covalent bonds with bismuth producing an orange to red dregs [39].

3.2.4. Triterpenoid

Triterpenoids are compounds whose carbon skeletons originate from six isoprene units and are biosynthetically derived from acyclic C30 hydrocarbons, namely squalene. Triterpenoids are colorless, crystalline compounds, often with high melting points and optically active, which are generally difficult to distinguish because there is no chemical reactivity. Triterpenoids are classified into four groups, namely actual triterpenes, steroids, saponins, and cardiac glycosides [16].

Based on the alkaloid test results in Table 1, it shows that the liquid powder and extract of kecombrang flowers contain strong triterpenoids. Test results of liquid powder and extract of kecombrang flower show the formation of red color. This indicates that the liquid

extract of kecombrang flowers contained compounds namely terpenoids. The test of terpenoids and steroids using Lieberman Burchard reagents [40].

3.2.5. Tannin

Tannins are polyphenol compounds that can form polyphenols that form complex compounds that do not dissolve with protein. The formation of green or blue color in the sample after the addition of FeCl3, possibly because tannin compounds will form ion Fe^{3+} complexes [16].

Based on the phenolic test results in Table 1, it shows that the kecombrang flower powder does not contain tannin while the liquid extract of kecombrang flower contains tannin. Kecombrang flowers basically do not contain tannins. However, the results of tannin green to blackish blue test were formed in all samples of liquid extract of kecombrang flower extract. This is because FeCl3 can react with other compounds with the same color. The main properties of tannins depend on the phenolic-OH group contained in tannins. Color reactions occur when combined with iron salt. Iron salt ($FeCl_3$) 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 [20].

Green to blackish blue color that is formed is thought to be due to $FeCl_3$ which reacts with phenolic compounds. On kecombrang flowers, the results of phenolic compounds test produce positive results with the formation of green to blackish blue. Phenol testing is carried out by reacting 1 mL samples with 5% FeCl. The presence of phenol compounds is characterized by the formation of a bluish-green color to black [32].

3.2.6. Saponin

Saponins are glycosides that are found in plants, consisting of sugar groups that bind to sapogenin aglycones. Saponin has the characteristics of a foam because when reacted with water and shaken will make a foam. Saponins are classified into two types, namely steroid saponins with carbohydrate molecules, whereas triterpenoid saponins are composed of a triterpenoid core with carbohydrate molecules [41].

Saponin testing is characterized by the formation of foam when the sample is shaken. formation of a stable foam as high as ± 1.5 cm, which indicates a positive result. In general, if the results are positive, the addition of 2N HCl aims to increase polarity so that the hydrophilic group will bind more stable and the froth formed becomes stable [42].

Based on the alkaloid test results data in Table 1, it shows that the positive kecombrang flower powder and liquid extract contain saponins, but the saponin content in flower powder is more than liquid extracts. Saponins are compounds that have hydrophilic and hydrophobic groups. Saponins are formed when foam is formed due to the presence of hydrophilic groups that bind to water, while hydrophobes will bind to air. In micelle structures, the polar group faces outward while the non-polar group faces inward. This situation affects the formation of foam, but the liquid saponin extract produced is very little because of the foam form [43].

3.2.7. Glycoside

Glycosides are natural compounds consisting of carbohydrate parts and non-carbohydrate parts. The most non-carbohydrate parts found are triterpenes, steroids, and flavonoids, while the most commonly found carbohydrate molecules are glucose, galactose, xylose, and arabinose. These monosaccharides can be bound to one or more C atoms in the non-carbohydrate part. The word glycoside means carbohydrate or sugar which is generally an oxidizing agent called glycone, while non-sugar is called aglycone. Chemical bonds formed by glycosides resemble ether so that chemically in the process of its formation always releases water or H_2O [44].

The presence of glycoside compounds is characterized by the formation of blue or green rings. The glycoside test is carried out with the Lieberman-Buchard reaction. Itchy leaf extract is dissolved in ethanol solvent, evaporated on a water bath and then dissolved in 5 mL acetic acid anhydride then 10 drops of concentrated sulfuric acid are added. The formation of blue or green color indicates the presence of glycosides [45]. However, the testing of powder and liquid extract of kecombrang flower powder did not form the color ring. This is presumably due to the glycoside compounds found in kecombrang flowers, namely the type of sugar glycoside compounds. [46] explains, testing 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.

4. CONCLUSION

Optimization, verification and validation results of the optimum formula showed that the liquid extract of kecombrang flower powder had a phenol response value of 64.458 mg TAE / 100 g, flavonoids 35.120 mg QE / 100 g, and a pH value (3.9). While the total phenol value of kecombrang flower powder is 27.93 mg TAE / 100 g, and the flavonoid value is 2.35 mg QE / 100 g. The compounds contained in the optimum product formula are phenolic, flavonoids, alkaloids, terpenoids and tannins.

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