ANALYSIS OF BIOACTIVE COMPONENTS AND PHYTOCHEMICAL OF POWDERS STEM AND LEAVES OF KECOMBRANG (Etlingera elatior)

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ABSTRACT

Kecombrang (Etlingera elatior) has bioactive compounds such as phenols and flavonoids as antioxidants. This study aims to determine the effect of temperature and time drying on total phenols, total flavonoids, and phytochemical compounds from stem and leaves kecombrang powder using the cabinet dryer method. This study uses a completely randomized design (CRD). Factors tested in this study were kecombrang plant parts (stems and leaves) and drying temperature (50°C, 60°C, and 70°C). The variables observed in this study were total phenols, total flavonoids, water content and phytochemical compounds from stem and leaves kecombrang powder. Quantitative research results showed that kecombrang leaf powder with a drying temperature of 70°C had total phenols, total flavonoids and water content respectively of 35.89 mg TAE / g, 2.132 mg QE / g and 5.557%. Meanwhile, kecombrang stem powder with a drying temperature of 60°C has a total phenol, total flavonoid and water content respectively of 6.030 mg TAE / g, 0.160 mg QE / g and 7.873%. The results of phytochemical testing showed that the positive kecombrang leaf powder contained alkaloid, flavonoid, saponin, steroid, tannin, phenol and negative compounds containing glycoside compounds while the positive kecombrang stem powder contained alkaloid compounds, flavonoids, triterpenoids and phenols and negatively contained glycoside compounds, while kecombrang stem powder positively contained alkaloid compounds, flavonoids, triterpenoids and phenols while negatively containing tannins, saponins and glycosides.

Keywords: bioactive component, drying, kecombrang, stem, leaf

1. INTRODUCTION

Kecombrang plant (Etlingera elatior) is a spice plant that belongs to the Zingiberaceae group and has been used in making medicine as well as
vegetables [1]. Kecombrang fruit and flowers are usually used as antioxidant and antimicobia. While kecombrang leaves and flower can be used as edible coating for sausage and fillet [2, 3, 4].

This plant is commonly found in mountainous areas or shady areas near water with a height of 800 meters above sea level. The chemical composition of kecombrang plants include polyphenols, alkaloids, flavonoids, essential oils, and saponins [1]. The bioactive component can also be used as a food and beverage additive. However, the addition of the bioactive component of the kecombrang plant in fresh form is considered to be less effective both in terms of storage and use. Therefore, the use of kecombrang plants in fresh form needs to be converted into powder form to facilitate the use of bioactive components contained in kecombrang plants through the drying process.

Drying is the process of removing water internally from the material to produce a dry product. According to [5], drying is a fairly complex product preservation method mainly due to undesirable changes in the quality of the dry product. The basic objective in drying agricultural products is to reduce water in materials to a certain extent, where spoilage microbes and damage caused by chemical reactions can be minimized so that the quality of the dry product can be maintained.

However, during the drying process, temperature and time need to be considered. The process of drying fresh materials using temperatures that are too high with a long time can actually reduce the bioactive compounds in it because the bioactive compounds are damaged [6]. However, the use of temperatures that are too low is difficult to achieve water levels that are safe to store [7]. Therefore, it is needed the right temperature and time to produce dry simplicia which has a water content according to the standard and the bioactive compounds can be maintained.

The purpose of this study was to determine the effect of treatment interactions on quantitative and qualitative variables of kecombrang stem and leaf powder.
2. METHODOLOGY RESEARCH

2.1 Experimental design

The experimental design used in this study was a Completely Randomized Design. Factors observed were kecombrang plant parts consisting of two types namely kecombrang stem and leaves and drying temperature consisting of three levels, namely 50ºC, 60ºC and 70ºC.

2.2 Material preparation

The first step of drying the kecombrang powder stem and leaves begins with wet sorting carried out to separate impurities or other foreign materials. The next step is washing carried out to remove soil and other impurities that are attached to the leaves of kecombrang so as to obtain herbs that are suitable for use. The third stage is the chopping done to simplify the process of drying, packing and grinding.

2.3 Drying Material

Drying is done using a tool that is a cabinet dryer with a predetermined combination of treatments. The temperature used are 50ºC, 60ºC, and 70ºC.

2.4 Simplisia Dry Powder

Powdering is done using a disk mill with 60 mesh sieve. It is done to simplify the subsequent analysis process.

2.5 Quantitative Analysis of Kecombrang Stem and Leaf Powder

2.5.1 Analysis of Total Phenols

400 mL of supernatant was added with 1.5 mL of 10% Folin-Ciocalteu and allowed to stand for 5 minutes at room temperature. After that added 1.5 mL of sodium bicarbonate (NaHCO3) 0.556M is shaken and left in a dark room for 90 minutes, then absorbance is measured using a spectrophotometer at a wavelength of 725 nm [8].

2.5.2 Analysis of 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% glalic acetic acid
solution) and 1 mL of 120 mM potassium acetate solution (1,176g of potassium acetate in 100 mL of distilled water). After that it was incubated for 1 hour at room temperature and measured its absorbance using a spectrophotometer at a wavelength of 435 nm [9].

2.6 Analysis of Qualitative Kecombrang Stem and Leaf Powder

2.6.1 Analysis of Alkaloid

A total of 0.05 grams of sample was added with 5 mL of 70% ethanol then shaked for 10 minutes at 200 rpm and filtered. As much as 2 mL of filtrate was added 10 mL of HCl 2% and heated on waterbath for 10 minutes at a temperature of 100ºC. Next 1 mL of filtrate was taken, put into a test tube, and added 2 drops of dragendrof reagent. The presence of red-orange deposits indicate the sample contains alkaloids [10].

2.6.2 Analysis of Flavonoid

Samples of 0.5 grams plus 10 mL of 70% ethanol are then shaken and filtered. A total of 2 mL of filtrate was taken and 0.1 gram of magnesium powder was added, 3 mL of amyl alcohol, and 1 mL of concentrated HCl. The formation of red, yellow, or orange in the amyl alcohol layer indicates the presence of flavonoids [11].

2.6.3 Analysis of Saponin

A sample of 0.5 gram was put into a test tube and then added with 10 mL of hot water. Then cooled and dropped 1 drop of 2N hydrochloric acid. Next, shake it continously for 10 seconds to form a stable white foam for about 10 minutes as high as 1-10 cm. Froth that does not disappear indicates that the simplicia contains saponins [12].

2.6.4 Analysis of Steroid /Triterpenoid

A sample of 0.5 grams was added with 10 mL of 70% ethanol. Next, shaked for 10 minutes at 200 rpm and filtered.
Filtrate of 2 mL plus 3 drops of concentrated HCl and 1 drop of concentrated H2SO4. A change in color to green indicates that a positive sample contains a steroid compound and a change in color to red or yellowish red indicates the presence of a triterpenoid [12].

2.6.5 Analysis of Tannin

A sample of 0.5 grams was added with 10 mL of distilled water, then boiled. After chilling, 5 mL FeCl3 1% (w/v) is added. If the color changes to dark blue, the sample contains tannin [12].

2.6.6 Analysis of Phenol

A total of 0.5 gram of sample was extracted with 10 mL of 70% ethanol. As much as 1 mL of the extract was added 3-4 drops of 5% FeCl3 solution. The formation of green or blue green indicates the presence of phenol compounds in the sample [12].

2.6.7 Analysis of Glycoside

1 gram of sample was added with 5 mL of anhydrous acetic acid. Then dropped with concentrated sulfuric acid. The color change to blue or green indicates the sample contains glycoside compounds [13].

2.7 Analysis of Statistic

Data were analyzed using the F test (analysis of variance) at a 95% confidence level with ANOVA. If the results of the analysis show a significant effect, then proceed with further tests using the DMRT test (Duncan Multiple Range Test) at 5% level. The software used for data analysis is SPSS 25.

3. RESULT AND DISCUSSION

3.1 Analysis of Quantitative

3.1.1 Analysis of Total Phenol

The average value of total phenol kecombrang powder of stem and leaves during drying can be seen in Figure 1.
The average total phenol in kecombrang leaf powder is higher than that of kecombrang stem powder. The difference in total phenol levels can be caused by environmental factors for different kecombrang plants such as differences in plant growth, soil composition, temperature, rainfall and UV radiation [14].

According to [14], high levels of total phenols are also influenced by other compounds present in kecombrang leaves, namely tannins. The tannin content also influences the high level of total phenol produced from kecombrang leaves because tannins which are hydrophilic are extracted in a polar ethanol solution and are read at the time of measurement.

The highest total phenol content in the leaf plant section was obtained at a 70°C drying treatment at 35.809 mg TAE / g and the lowest total phenol content was obtained at a 50°C drying treatment at 29.917 mg TAE / g. This is in accordance with [15] which states that drying with high temperatures in a short time can reduce food damage more than a longer drying time with shorter temperatures. In addition, high levels of total phenols are also caused by the use of young leaves which have soft texture and higher water content compared to old leaves. During wilting and drying, young leaves provide less heat penetration so that the polyphenol enzyme is not much damaged [16].
On kecombrang stems the highest total phenol content was obtained at a 60°C drying treatment of 6.03 mg TAE / g and the lowest total phenol content was obtained at a 50°C drying treatment which was 4.382 mg TAE / g. These results are in accordance with the literature which states that oven drying at 60°C at a fast time provides high phenolic content and the most powerful antioxidant power [17]. Drying with a treatment temperature of 50°C has the lowest total phenol because drying using temperatures that are too low can result in the decomposition of phenolic compounds by the help of the enzyme polyphenol oxidase found in plants [10].

3.1.2 Analysis of Total Flavonoid

The average total flavonoid powder of the stem and leaves of the kecombrang during drying can be seen in Figure 2.

Average total flavonoids in kecombrang leaf powder is higher than kecombrang stem powder. Based on Figure 2, the highest total flavonoid content of the kecombrang leaf section was obtained at a 70°C drying treatment temperature of 2.132 mg QE / g and the lowest total flavonoid content was obtained at a 50°C treatment temperature which was 1.81 mg QE / g. This happens because the drying process on kecombrang leaves can destroy the
waxy layer that is on the outer surface of the leaf and will further break down the cell wall so that it will facilitate the flavonoid compound to diffuse into the solvent. The higher the drying temperature causes the higher the damage to the cell wall. Carbohydrate and protein on cell wall will be broken because it, so the higher the amount of flavonoid compounds that come out with solvent [18].

On kecombrang stems the highest total flavonoid levels were obtained at 60ºC which is 0.160 mg QE / g and the lowest total flavonoid levels were obtained at 50ºC temperature treatment which was 0.104 mg QE / g. These results are in line with the literature which states that the optimal temperature used in drying simplicia is around 60ºC [19]. Drying with a treatment temperature of 50ºC has the lowest total phenol. This happens because flavonoids are polyphenol compounds that can be oxidized in the presence of the enzyme polyphenol oxidase in plants. Drying using temperatures that are too low can result in the decomposition of phenolic compounds by the help of the enzyme polyphenol oxidase found in plants [10].

3.1.3. Water Content

The average water content of kecombrang powder stem and leaves decreased with the increase in drying temperature which can be seen in Figure 3.
Based on Figure 3, the results of the water content of all treatments have the requirements as dry simplicia with a moisture content that does not exceed 10%. The lowest water content of the kecombrang leaves was obtained at a drying treatment temperature of 70°C which was 5.557% and the highest water content was obtained at a drying treatment temperature of 50°C which was 7.371% while in the kecombrang stem has the lowest water content was also obtained at a drying treatment temperature of 70°C which is equal to 7.553% and the highest water content is obtained at a treatment drying temperature of 50°C which is equal to 9.928%.

The highest drying temperature causes the evaporation of water contained in the kecombrang plant parts to be higher so that the water content is lower. This is supported by the statement of [20], the rate of evaporation besides being influenced by the humidity level is also influenced by the temperature around the dried material.

An increase in temperature on the surface of the material caused by the supply of heat energy from combustion. [21] revealed that slicing dried material will expand the surface of the
material and a broad surface can make it easier for water to come out. The composition of water in foodstuffs such as free water and bound water can affect the rate or duration of food drying.

3.2 Analysis of Qualitative

The qualitative variables observed in this study were alkaloids, flavonoids, saponins, steroids, tannins, phenols and glycosides. The results of phytochemical analysis of dried kecombrang stem and leaves can be seen in Table 1.

Table 1. Phytochemical analysis of dried kecombrang stem and leaf

| Treatment       | Stem | Leaf |
|-----------------|------|------|
| Alkaloid        | ++   | +++  |
| Flavonoid       | +++  | ++++ |
| Saponin         | -    | +++  |
| Steroid/Triterpenoid | +  | +++  |
| Tanin           | -    | ++++ |
| Fenol           | +++  | ++++ |
| glycoside       | -    | -    |

Note: - (negative), + (positive of weak), ++ (Positive), +++ (positively strong), ++++ (positively very strong)

3.2.1 Analysis of Alkaloid

Based on the results of qualitative analysis of alkaloid compounds in Table 1, it can be seen that the stem and leaf extracts of kecombrang with Dragendorff reagents contain the highest positive alkaloids of ++ for positive and positive stems +++ for leaves with strong positive marked by the formation of red brick deposits in large quantities [22].

According to [23] positive results of alkaloids in the Dragendorff test were also characterized by the formation of light brown to yellow (orange) deposits. These deposits are potassium alkaloids. The formation of orange deposits in the addition of dragendorff reagents occurs because nitrogen is used to form coordinate covalent bonds with K⁺ which is a metal ion and yellowish white deposits are formed in the addition of major
reagents because nitrogen in the alkaloids will react with K$^+$ metal ions from potassium tetraiodomerkurat (II) to form potassium complexes and form yellowish white deposits in the addition of major reagents because nitrogen in alkaloids will react with K$^+$ metal ions from potassium tetraiodomerkurat (II) to form potassium complexes. -alkaloid that settles [24].

3.2.2 Analysis of Flavonoid

Based on the phytochemical analysis of flavonoid compounds in Table 1, it can be seen that the dry simplicia of the stem and leaves of kecombrang which is extracted with ethanol solvent which is then reacted with concentrated HCl, magnesium powder and amyl alcohol produce orange-red color, so it can be concluded that the positive kecombrang stem extract contains compounds flavonoids with the highest flavonoid content of positive +++ which means strong positive and positively kecombrang leaf extract contain flavonoids with the highest flavonoid content of positive ++++ which means very positive positive. This is in line with the research of [25] which states that the positive kecombrang stem and leaf extract contains flavonoid compounds in the presence of orange-red formation.

3.2.3 Analysis of Saponin

Based on the results of phytochemical analysis of saponin compounds in Table 1, it can be seen that the stem powder extracted with distilled water solvent negatively containing saponin and kecombrang leaf powder extracted with distilled water solvent has the highest positive saponin compound content of 3 with strong positive. The chemical compound saponin in kecombrang leaf extract is characterized by the formation of froth for less than 10 minutes and is not lost on the addition of 2N hydrochloric acid. Saponins are strong surface tension-reducing compounds that cause foam when shaken in water. Saponins work as antimicrobials by
disrupting the stability of bacterial cell membranes, causing bacterial cells [25]

3.2.4 Analysis of Steroid/Triterpenoid

Based on the results of phytochemical analysis of steroid/triterpenoid compounds in Table 1 it is known that kecombrang stem extracts with Lieberman Burchard reagents produce pink formation which indicates the presence of triterpenoid compounds while kecombrang leaf extracts with Lieberman Burchard reagents produce green formation that indicates the presence of steroid compounds. This is in accordance with [26] which states that if there is a change in color to red, pink or violet, it is positive to contain triterpenoids and if the color changes to green, the sample is tested positive for steroids.

3.2.5 Analysis of Tannin

Based on the phytochemical analysis of tannin compounds in Table 1, it can be seen that the kecombrang stem extract with FeCl$_3$ reagents did not change color indicating that kecombrang stem extract negatively contained tannin compounds while the kecombrang leaf extract with FeCl$_3$ reagent formed a blackish green color with the highest tannin content of ++++ with a very strong positive statement. This is in accordance with [27] which states that kecombrang stem powder negatively contains tannin compounds and kecombrang leaf powder positively contains tannin. [25] also stated that the presence of tannins was marked by the formation of blue or blackish green.

3.2.6 Analysis of Phenol

Based on the phytochemical analysis of phenol compounds in Table 1 it can be seen that the kecombrang stems and kecombrang leaves extracted using ethanol solvent then added with FeCl$_3$ reagent turn dark green, this indicates that stem and leaf
extracts of kecombrang positively contain phenols. Color changes indicate the presence of oxidized phenol compounds. The profile of phenolic compounds in sample extracts can change with differences in the degree of polarity of the solvents used [28]. This is in accordance with [29] 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.

3.2.7 Analysis of Glycoside

Based on the results of phytochemical analysis of glycoside compounds in Table 1, it can be seen that the stem and leaves of kecombrang extracted using methanol solvent were then added to glacial acetic acid containing 1 drop of 1% FeCl₃ and also added concentrated sulfuric acid did not produce reddish brown rings. This shows that the stem and leaf samples of kecombrang do not contain glycoside compounds. The absence of glycosides in the stem and leaves of kecombrang is thought to be due to environmental factors of kecombrang growing. This is in accordance with [14] which states that differences in the content of compounds in the sample are caused by environmental factors for different kecombrang plants such as differences in plant growth, soil composition, temperature, rainfall and ultraviolet radiation.

4. CONCLUSION

Kecombrang stems and leaves were respectively dried using 60°C and 70°C temperatures to produce the best powder quality of the kecombrang plant parts as seen from the total phenols, flavonoids, water content and phytochemical analysis results. Kecombrang stem powder produces total phenols, total flavonoids, and water content respectively of 6.030 mg TAE / g, 0.160 mg QE / g, and 7.873% while kecombrang leaf powder produces total phenols, total
flavonoids, and water content respectively of 35,809 mg TAE / g, 2,132 mg QE / g, and 5.557%.

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