Medical Sains : Jurnal Ilmiah Kefarmasian
Vol. 10 No. 3, July - September 2025
https:/ojs.ummada.ac.id/index.php/iojs

MEASUREMENT OF QUALITY PARAMETERS OF SIMPLICIA AND EKSTRACT OF KECOMBRANG STEM (*Etlingera elatior*)

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Submitted: June 9, 2025 ***Revised: August 17, 2025*** ***Accepted: August 21, 2025***

ABSTRACT

Kecombrang (*Etlingera elatior*) is a type of plant known to the Indonesian people as a medicinal plant for a long time. Kecombrang stem is empirically used to treat coughs, reduce fever, act as an antiseptic, treat diarrhoea and stomach pain. Kecombrang stem has the potential to be used as a raw material for herbal medicines. Plants used as raw materials for herbal medicines must meet quality requirements in accordance with the requirements of both specific and non-specific parameters. This study aimed to determine the quality of the simplicia and 96% ethanol extract of kecombrang stem. Simplicia and 96% ethanol extract were tested for specific parameters, including organoleptic, microscopic, and phytochemical screening. Non-specific parameter testing includes the measurement of moisture content, residual solvents, and microbial contamination. Tests of specific parameters on organoleptic kecombrang stem simplicia are light brown, aromatic odor, and tasteless. Microscopically, kecombrang stem simplicia was identified with fragments consisting of transport bundles, ladder-type transport bundles, fibers, and parenchyma with secretory cells. Phytochemical screening of kecombrang stem simplicia revealed the presence of alkaloids, phenolics, flavonoids, steroids, tannins, and saponins. The results of non-specific parameter testing on 96% ethanol extract of kecombrang stem showed 1.09% moisture content, 0.48% solvent residue, 0 CFU/g bacterial contamination, and yeast/mould. Kecombrang stem is a raw material for herbal medicine that meets the quality requirements of specific and non-specific parameters.

Keywords: *Etlingera elatior*, Non-specific parameters, Simplicia quality, Specific parameters.

INTRODUCTION

Some levels of Indonesian society have long used traditional medicine for treatment and healthcare. Traditional medicine is believed to be able to treat various diseases because it has pharmacological properties, is safe, and is relatively inexpensive (Adiyasa & Meiyanti, 2021). Most traditional medicines are derived from plants. Plants used as traditional medicines contain compounds with pharmacological activity (Gunarti et al., 2022). Approximately 300 medicinal plants have been used as raw materials for traditional medicine (BPOM RI, 2023).

Kecombrang (*Etlingera elatior*) is a plant widely used in medicine. Empirically, the stem part of kecombrang is used by the Indonesian people to treat coughs, reduce fever, act as an antiseptic, and treat diarrhea and stomach pain (Saudah et al., 2022). Kecombrang stems contain alkaloid compounds, flavonoids, tannins, saponins, biondinin A, methyl kushenol C, punisic acid, and malvalic acid (Adini et al., 2023). Kecombrang stems have the potential to be used as raw materials for medicine because several studies have proven that kecombrang stems have antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Escherichia coli, and Salmonella enterica*, and have wound healing, burns, and antioxidant properties (Naufalin et al., 2021; Sahidin et al., 2019; Zunnita & Apriana, 2024).

According to Kementrian Kesehatan RI (2017), simplicia and extracts used for health must fulfill the applicable quality requirements. The quality parameters that must be qualified are specific and non-specific parameters. Specific parameter testing focuses on the identity of the sample and the compounds responsible for its pharmacological activity. In contrast, non-specific parameters aim to determine the physical, chemical, and microbiological aspects that can affect the stability of the extract and consumer safety (Triastuti, 2020). The novelty of this research is the determination of the quality standardization of simple and kecombrang extracts from Pandeglang, Banten, so that they can be used by local people as natural medicines.

This study aimed to test the quality of simplicia and 96% ethanol extract of kecombrang stems based on specific parameters, including organoleptic, microscopic, and compound content, and non-specific parameters, including moisture content, residual solvents, microbial contamination, and yeast/mold contamination.

RESEARCH METHODS

Equipment and Materials

The equipment used in this study included glassware (Pyrex), analytical scales (Fujitsu FSR- B620), grinder, microscope (Oregan), moisture analyzer (MB65), rotary evaporator (IKA MVP 10 basic), water bath (HEALTH YNC-WBE-8L), incubator (Memmert), autoclave (GEA YX-18 LM), electric stove (Maspion), and steam distillation. The materials used in this study were crude Kecombrang stem obtained from Pandeglang Regency-Banten, 96% technical ethanol, equates, Plate Count Agar (PCA) (Himedia), Potato Dextrose Agar (PDA) (Himedia), NaCl sterile, chloral hydrate, Mayer reagent, Dragendorf reagent, Wagner reagent, dichloromethane, methanol, concentrated HCl, magnesium ribbon, 3% $FeCl_3$, anhydrous acetic acid, and H_2SO_4.

Research Procedure

1. Preparation Simplicia and Extracts

Kecombrang stems were obtained from Pandeglang Regency, Banten, and plant determination was carried out at the Faculty of Applied Science and Technology, Ahmad Dahlan University. A total of 8 kg of kecombrang stem samples were collected from the inside, washed, chopped, reduced in size, dried, milled, and sieved using a No. 60 mesh sieve. The finely ground simplicia powder was then used to prepare the extract. A total of 350 g of simplicia was extracted using kinetic maceration with 96% ethanol solvent (1:1) for 2 hours; the filtrate was filtered and concentrated using a rotary evaporator until it became a thick extract (Adini et al., 2023).

2. Specific Parameters

a. Organoleptic

In this study, organoleptic testing was performed using kecombrang stem simplicia powder to observe the color, smell, and taste of the powder (Depkes RI, 2000).

b. Microscopic

The powdered kecombrang stem was placed on a glass object and dripped with chloral hydrate solution. The identification fragments were observed under a microscope at a magnification of 40×10 (Kementrian Kesehatan RI, 2017).

c. Phytochemical screening

Phytochemical screening was conducted to determine the compounds present in the kecombrang stem simplicia. The content of alkaloids, flavonoids, tannins, triterpenoids/steroids, and saponins (Hanani, 2023).

1) Alkaloid

Kecombrang stem simplicia (1 g) was extracted using 20 ml of 96% ethanol for 15 minutes and then filtered. The filtrate was evaporated to dryness and dissolved in 2 ml of methanol. The test solution was divided into 3, and Dragendorf, Wagner, and Mayer reagents were added to each. The presence of

positive alkaloids in the Dragendorf reagent is indicated by a light brown to yellow precipitate, in the Mayer reagent by a white precipitate, and in the Wagner reagent by a brown precipitate.

2) Flavonoid

Kecombrang stem simplicia (5 g) was shaken with 30 ml of dichloromethane for 15 minutes and then filtered. The filtrate was evaporated to dryness and dissolved in 2 ml of methanol. The sample was added to 100 ml of concentrated HCl and 4 pieces of magnesium ribbon. A color change to orange, pale red, and dark red indicates positive flavonoids.

3) Tannin

The kecombrang stem simplicia (2 g) was extracted using 30 ml of 96% ethanol, heated to boiling, filtered, and evaporated. The evaporation results were added to hot distilled water and stirred. After cooling, the solution was precipitated, the upper solution was collected, and 3% $FeCl_3$ was added. A color change to blackish green or blackish blue indicated positive tannins on the addition of 3% $FeCl_3$.

4) Triterpenoid/steroid

The drug (1 g) was dissolved in 20 ml of 96% ethanol in a water bath, filtered, and evaporated. The remaining filtrate was added with 1-2 ml of H_2SO_4. The formation of a red ring indicates positive terpenoids/steroids.

5) Saponin

Kecombrang stem simplicia (1 g) was placed in a test tube, 10 mL of distilled water was added, and the mixture was shaken vigorously for 30 seconds. A positive saponin sample was identified if stable foam was formed for 30 minutes, and the foam did not disappear when HCl was added.

3. Non-Specific Parameters

a. Determination of water content

Kecombrang stem extract (1 g) was placed in a moisture analyzer cup set to 105^{o}C, closed, and the water content was determined. The results were then recorded (Maharisti et al., 2022).

b. Determination of residual solvent

The extract (2 g) was dissolved in water to 25 ml and then placed into a distillation flask at a temperature of 78,5^{o}C. The distillation was performed until the distillate was obtained, which was approximately 2 mL smaller than the volume of the test liquid. Distillation was carried out for 2 hours or until the liquid no longer dripped. Water was added to a volume of 25 mL, and the specific gravity of the liquid was determined at a temperature of 25 °C. The remaining solvent was then calculated (Cahyani et al., 2019).

c. Determination of bacterial contamination (TPC)

The total plate count test was performed using the serial dilution method. A total of 1 ml of thick extract of Kersen leaves that had been diluted with distilled water in a test tube was added with 9 ml of 0,9% NaCl to obtain a concentration of 10^{-1}. Dilution was continued until a concentration of 10^{-3} was obtained. The dilution results from each tube were pipetted 1 ml and then poured into a sterile Petri dish to be given PCA media using a pour plate. Blanks were prepared by pouring 1 ml of 0,9% NaCl into a sterile Petri dish and then adding PCA using a pour plate. All Petri dishes were incubated at 37 for 24 hours. The number of colonies was calculated using a colony counter, and the Total Plate Count (TPC) was calculated (Depkes RI, 2000).

d. Determination of total yeast and mold contamination (TYMC)

The YTMC test was carried out using the serial dilution method with three dilution levels, namely concentrations of 10-1 to 10-3. The dilution results were inoculated using a pour plate on a sterile Petri dish with PDA media. Blanks were prepared by pouring 1 mL of 0,9% NaCl into a sterile Petri dish and then adding PDA

using a pour plate. All Petri dishes were incubated at 25ºC for 5-7 days. The number of colonies was observed and calculated (Depkes RI, 2000).

RESULTS AND DISCUSSION

1. Preparation Simplicia and Extracts

Based on the results of plant determination No. 009/Lab.Bio/B/I/2024, conducted at the Faculty of Applied Science and Technology, Ahmad Dahlan University, stated that the plant used was kecombrang (*Etlingera elatior*). The kecombrang stem Simplicia powder obtained weighed 412 g. The total extract obtained from 350 g of Simplicia was 7.543 g, with an extraction yield of 2.15%. This yield was lower than that reported by Adini et al. (2023), who used methanol as a solvent and obtained an extract yield of 4.44%. The difference in the yield results is due to the difference in the type of solvent used in the extraction process. Methanol solvent is more polar when compared to 96% ethanol, so compounds with high polarity are extracted more in methanol solvent than in 96% ethanol**.**

2. Specific Parameters

a. Organoleptic test results

Organoleptic testing is a simple initial identifier that uses the five senses to describe color, odor, and taste (Depkes RI, 2000). The sample used in this organoleptic test was powdered kecombrang stem simplicia. The results of the organoleptic test showed that the kecombrang stem Simplicia was in the form of a dry powder, light brown, had a distinctive aromatic odor, and was tasteless (**Table I**).

Figure 1.Kecombrang Stem Simplicia Powder

Table I. Results of Organoleptic Testing of Kecombrang Stem Simplicia

Observation	Result
Color	Light brown
Odor	Distinctive smell
Taste	Tasteless

b. Microscopic results

Figure 2. Microscopic results of kecombrang stem powder: (a) Vascular tissue. (b) Scalariform tracheids; (c) Sclerenchyma; (d) Secretory parenchyma

Microscopic testing in this study aimed to determine cell recognition fragments and cell or cell tissues in plants (Novitasari et al., 2021). The microscopic tests of kecombrang stem Simplicia with a magnification of 40×10 showed that the kecombrang stem has a vascular tissue, scalariform tracheids, sclerencyma, and secretory parenchyma (**Figure 1**).

c. Phytochemical screening

Phytochemical screening aims to detect the compounds contained in kecombrang stems. The sample used for phytochemical screening was kecombrang stem powder. Phytochemical screening is included in specific parameters because it is included in the qualitative analysis of compounds related to the pharmacological activity of an extract (Mangalu et al., 2022). Based on **Table II**, kecombrang stems contain alkaloids, flavonoids, tannins, steroids, and saponins.

Table II. The Result of The Test on The Content of The Simplicia Kecombrang Stem

Test	Reagents	Result	Description
Alkaloid	Dragendorf	No precipitate	Negative
	Mayer	White precipitate	Positive
	Wagner	Brown precipitate	Positive
Flavonoid	HCl + Magnesium ribbon	Dark red	Positive
Tanin	$FeCl_3$	Blackish green	Positive
Terpenoid/steroid	H_2SO_4	Red ring	Positive
Saponin	Distilled water + HCl	Stable foam for 30 minutes	Positive

3. Non-Specific Parameters

Table III. Results of Testing Non-Specific Parameters of 96% Ethanol Extract of Kecombrang Stems

Test	Result	Quality Requirements	Description
Water content	1,09%	≤10% (Kementrian Kesehatan RI, 2017)	Qualify
Residual solvent	0,48%	≤1% (Kementrian Kesehatan RI, 2017)	Qualify
Bacterial contaminations (Total Plate Count)	0 CFU/g	$\leq 10^4$ (BPOM RI, 2014)	Qualify
Fungal contamination (Total Yeast and Mold Count)	0 CFU/g	$\leq 10^3$ (BPOM RI, 2014)	Qualify

Water content testing was used to determine the water residue contained in the extract after the drying process. Based on **Table III**, the water content of the 96% ethanol extract of kecombrang stems was 1,09%. This result is due to the quality requirements, which is <10%. The water content is related to the purity of the extract; the greater the water content of an extract, the easier it is for the extract to be damaged or rotten due to microbial growth. In addition, a high water content causes the decomposition of active compounds in the extract due to enzymatic reactions. Therefore, the water content greatly determines the stability of an extract or preparation made from the extract (Sambode et al., 2022).

The residual solvent test aims to ensure that no residual solvent is left in the extract during extraction (Depkes RI, 2000). Based on **Table III**, the results show that the kecombrang stem extract contains a residual ethanol solvent of 0,48%. The residual solvent content obtained meets the requirement of <1%. These results show that the extract obtained can be used as a raw material for traditional medicine because it contains a low concentration of ethanol.

Microbial contamination testing is one of the tests that aims to determine the purity of the extract. Microbial testing of the extract was conducted to ensure that it does

not contain bacterial and fungal contamination exceeding the specified limits (Utami, 2020). Based on **Table III**, the results of the microbial contamination test show that the ethanol extract of kecombrang stems was not contaminated by bacteria and mould/yeast, with the TPC and TYMC tests showing 0 CFU/g. These results align with the requirements (BPOM RI, 2014): the maximum limit for TPC is 10^4 and TYMC 10^3. The results of microbial contamination were directly proportional to the water content in the ethanol extract of kecombrang stems. The lower the water content in the extract, the more difficult it is for microbes to grow.

CONCLUSION

The kecombrang stem simplicia meets the specific parameter requirements organoleptically, is light brown, has a distinctive odor, and is tasteless. The kecombrang stem simplicia has identification fragments, including vascular tissue scalariform tracheids, sclerencyma, and secretory parenchyma. Kecombrang stems contain alkaloids, flavonoids, tannins, steroids, and saponins. The 96% ethanol extract of kecombrang stems met the specific parameter requirements with a water content of 1,09%, residual solvent 0,48%, TPC and TYMC 0 CFU/g.

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