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Antioxidant activity of kecombrang flower (*Etlingera elatior*) methanol extract and identification of its compounds using LC-MS/MS

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ABSTRACT

Introduction: The Kecombrang plant (*Etlingera elatior*) is a plant that has natural antioxidant potential. The antioxidant activity of a plant depends on the amount of active compound extracted and the extraction method. This study aimed to determine the active compound with LC-MS/MS.

Methods: Vacum rotary evaporator, UV-Vis Shimadzu spectrophotometer, set of tools LCMS/MS ACQUITY UPLC®H-Class System (waters, USA). Kecombrang flowers (*Etlingera elatior*) were obtained from the Soka traditional village, Tabanan and determined at the LIPI-UPT Center for Plant Conservation of the Bali "Eka Karya" Botanical Garden" Bali.

Results: Kecombrang flowers (*Etlingera elatior*) reported that the higher the methanol concentration, the higher the total flavonoids and antioxidants level. Antioxidants were carried out to determine the capacity of the active compounds in the extract to scavenge free radicals. Based on the IC_{50} value obtained, the methanol extract of kecombrang flowers with a concentration of 99% had the highest antioxidant activity with an IC_{50} value of 86.59 mg/L and was classified as a strong antioxidant. Kecombrang flowers macerated with 70% methanol and 50% methanol had moderate antioxidant activity, as indicated by the IC_{50} values of 100.32 mg/L and 123.91 mg/L, respectively.

Conclusion: Antioxidant activity test of kecombrang flowers (*Etlingera elatior*) methanol extract with concentration variation 99%,70%, and 50% the best antioxidant value 99 % low concentration solvent has a high boiling point, so that it evaporates slowly, resulting in a higher extract yield.

Keywords: Antioxidants, kecombrang flowers, *Etlingera elatior,* methanol.

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INTRODUCTION

The consumption patterns of Indonesian people, who generally like to consume foods high in fat, high in sugar, low in fiber, and antioxidants, cause various diseases.[1] Based on research conducted by the Ministry of Health (2018), as many as 95.5% of people aged ≥5 years consume vegetables and fruit under the recommended guidelines for balanced nutrition. Consumption of fruits and vegetables is very important in everyday life because fruits and vegetables function as a source of vitamins, minerals, and antioxidants to counteract free radicals.[2]

Free radicals are the result of metabolic processes in the body, such as hydroxyl, peroxyl and superoxide radicals, which are produced by the body. Free radicals in excess amounts will cause oxidative stress.[3] Oxidative stress can trigger degenerative diseases such as cancer, heart disease, cataracts, premature aging, and diabetes.[4] To prevent this, we need a compound that can prevent oxidative stress in the body by inhibiting oxidative chain reactions, namely antioxidants.[5]

Antioxidants are compounds that can absorb or neutralize free radicals so that they can prevent various degenerative diseases such as cardiovascular disease, carcinogenesis, diabetes mellitus, stroke and other diseases. Antioxidants often used are synthetic antioxidants, but because they can cause side effects to the body, it is necessary to replace synthetic antioxidants with natural antioxidants obtained from natural sources.[3] Natural sources, one of which is a plant, which has the potential as a natural antioxidant, is kecombrang plant (*Etlingera elatior*). Parts of the kecombrang plant, from roots, stems and leaves to flowers, contain secondary metabolites with the potential as antioxidants, such as phenolics, flavonoids, triterpenes, saponins, tannins, steroids, alkaloids and glycosides.[6]

Secondary metabolites in plants can be obtained by extraction; one of the extraction processes is the maceration process. Maceration is one of the simplest extraction methods carried out without heating so that compounds containing flavonoids in the material are not damaged

much.[7] The maceration process also has several other advantages, such as low process costs, and does not require special expertise in its application. This process can avoid damage to thermolabile compounds during the extraction process.[8]

Liquid chromatography mass spectrometry (LCMS) is an analytical technique that combines the physical separation capabilities of liquid chromatography with the specificity of mass spectrophotometry detection. The advantage of this tool is that it can analyze more broadly various components, such as thermally labile compounds, high polarity or high molecular mass and even proteins. Kecombrang flowers are compound flowers in the form of a hump with a 40-80 cm stalk length. The length of the stamens is approximately 7.5 cm, and the pistils are small and white. Kecombrang comes from the *Zingiberaceae* family and can broadly be found in Indonesia. Kecombrang has other names according to the area, namely *kincung* or *kencong* (North Sumatra), *honje* (Sunda), *bongkot* (Bali), and *sam throw* (West Sumatra).[9]

Kecombrang (Figure 1), among others, is edible and has antioxidant activity that can eliminate free radical inhibition and antimicrobial activity.[11]

METHODS

Tools and Materials

Kecombrang flowers (*Etlingera elatior*) were obtained from the Soka traditional village, Tabanan and determined at the LIPI-UPT Center for Plant Conservation of the Bali "Eka Karya" Botanical Garden" Bali. Ingredients included methanol 50%, 70%, and 99%, distilled water, and filter paper.

Methanol extraction of *Etlingera elatior*

Extraction of 100 grams of kecombrang flower in 500mL methanol of 99% methanol, 70% methanol and 50% methanol for 3 days filtered filtrate in a Rotary Vacuum Evaporator, viscous methanol extract of kecombrang flowers was obtained, then phytochemical tests and active compound tests were carried out using LC-MS/MS. The antioxidant activity of the kecombrang flower extract was measured using the DPPH (1,1-diphenyl-2-picrylhydrazil) method. DPPH is a chemical compound used in antioxidant tests, providing information about the ability of the compound or sample to neutralize free radicals and provide protection against oxidative damage caused by free radicals. The antioxidant activity of kecombrang flower extract is expressed in the percentage of DPPH free radical inhibition. The percentage of inhibition was obtained from the absorption ratio between the absorbance of the DPPH and the absorbance of the sample as measured by a UV-Vis spectrophotometer. The antioxidant activity of the research results is expressed in IC_{50}, which is the concentration of antioxidant substances that results in a percentage of DPPH inhibition of 50%.

Data Analysis

Descriptive data analysis was used in this study to describe the findings regarding the level of antioxidant strength, phytochemistry test results, interpretation of compound content with LC-MS/MS, and chromatogram test results of kecombrang flowers extraxt.

RESULTS

Table 1 explains the IC_{50} value is very active when <50 ppm, active when 50 ppm-100

Figure 1. Kecombrang plant.[10]

Table 1. Levels of antioxidant strength

Intensity	IC_{50} Value (ppm)
Very active	<50
Active	50-100
Currently	100-250
Weak	250-500
Not active	>500

Table 2. Phytochemistry test results of kecombrang flower methanol extract

No.	Phytochemistry	Reagents	Observations	Results	Conclusions
1	Alkaloid	*Wagner*	A brown precipitate formed	Brown	+
2	Flavonoid	Powder Mg and HCl	Red-Orange	Reddish yellow	+
3	Steroid / Terpenoid	Anhydrous acetic acid and concentrated sulfuric acid	Green Blue (Steroid) Red/purple (terpenoids)	Greenish blue	- (steroid) + (terpenoid)
4	Phenol	$FeCl_3$	Bluish black	Black	+
5	Saponin	Aquadest – HCl 2N	There was constant foam	The foam disappears	-
6	Tanin	Gelatin 1%	Turbid color	Turbid color	+

Note:
- (+): Positive contains compounds
- (-): Negative contains compounds

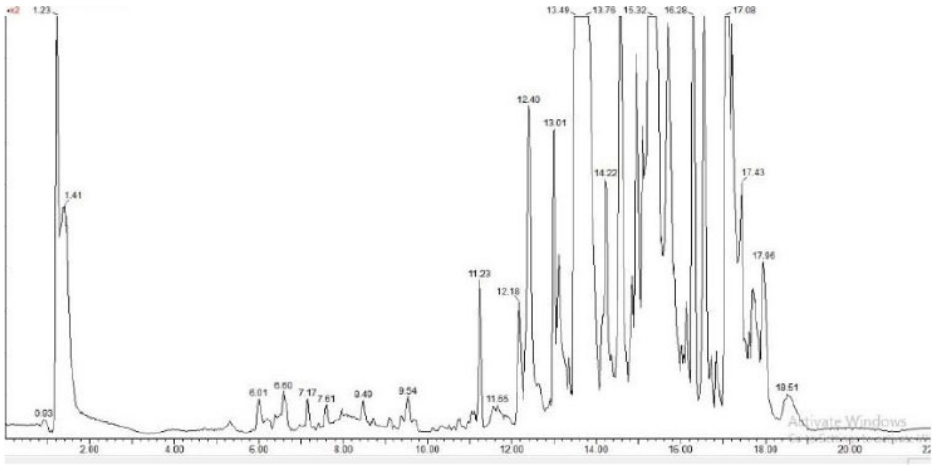


Figure 2. Chromatogram LC-MS/MS of kecombrang flowers extract.

ppm, moderate 100 ppm-250 ppm, weak when 250 ppm-500ppm, if not active >500 ppm.

The chromatogram is processed using the MassLynx V4.1 program to view the mass spectrum data from each peak and the molecular formula can be determine

The results of chromatogram analysis using the MassLynx V4.1 program are in the form of a mass spectrum displayed with the highest Fit conf % where a high score indicates high consistency with theoretical values, but there is no criterion for a specific number. Compounds with the highest fit conf % in a certain retention time shown below were matched using Pubchem or Chemspider and the results of the compounds shown in Table 3 were obtained. Some of the suspected compounds obtained were alkaloid compounds, namely piperidine.

DISCUSSION

Piperidine is one of numerous alkaloid chemicals found in the 99% extract of the kecombrang flower. This antioxidant chemical has a molecular structure that enables it to donate electrons to free radical molecules without interfering with its function in the slightest and to stop the free radical chain reaction. Based on their method of action, antioxidants are categorized into three categories: primary antioxidants, secondary antioxidants, and tertiary antioxidants. There are various methods for determining antioxidant activity, including the DPPH (1,1-diphenyl-2-picrylhydrazyl) technique. The DPPH technique for measurement is straightforward, quick, and doesn't need a lot of reagents.[12] This approach has also been demonstrated to be accurate, trustworthy, and useful. Due to the delocalization of free electrons over the whole molecule, DPPH is a stable free radical that does not produce dimers. Using UV-Vis spectrophotometry, this delocalization of free electrons also produces a purple (violet) hue at a wavelength of 517 nm. The color of the DPPH solution will change from purple to brilliant yellow and the absorbance at a wavelength of 517 nm will vanish when it is combined with a substance that can donate a hydrogen atom.[13] The decrease in color intensity is due to the reduced conjugated double bonds in DPPH, this can occur when antioxidants capture electrons and do not have resonation Figure 3.

The phytochemical test is a good first step that can help to provide an overview of the class of compounds contained in the plant being studied. The results of the phytochemical test data in Table 1 show that the methanol extract of kecombrang flowers contains various secondary metabolites including alkaloids, flavonoids, terpenoids, steroids/triterpenoids, phenols, saponins, and tannins, these are supported by the theory reported by Parthasarathi et al., 2015 where in the flower parts there are phenolic groups, namely flavonoids and tannins as well as other compounds such as alkaloids and terpenoids which have the potential as antioxidants.[14] Whereas caffeine, which has the capacity to act as a radical scavenger, hydroxy, and melatonin, which is crucial in protecting cells from the effects of radiation and drug toxicity, alkaloids, especially indole, have the ability to effectively stop reactions of free radical chain compounds as well as quinolone compounds.[15]

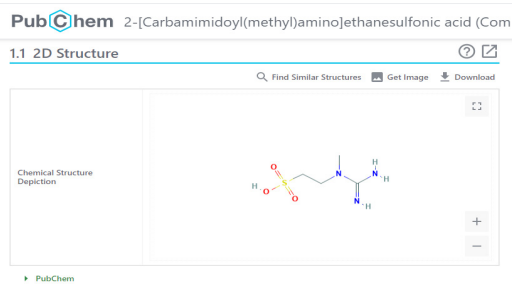
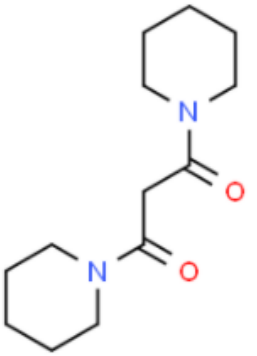
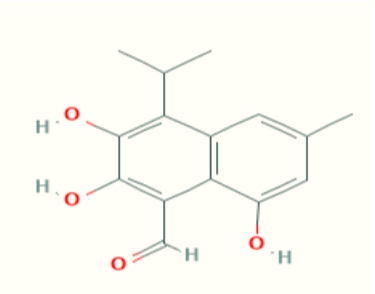
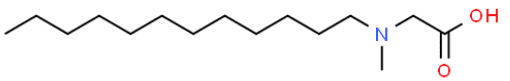
It can also be concluded that the various solvent concentrations used show different effects due to changes in polarity thereby affecting the solubility of flavonoids/alkaloids. Antioxidant activity was classified based on the IC_{50} value obtained, namely very strong (IC_{50} < 50ppm), strong (50 ppm <IC_{50}< 100ppm), moderate (100 ppm <IC_{50}< 150 ppm), weak (150 ppm < IC_{50}< 200 ppm), and very weak (IC_{50}> 200ppm).[16] Based on the IC_{50} value obtained, the methanol extract of kecombrang flowers with a concentration of 99% had the highest antioxidant activity with an IC_{50} value of 86.59 mg/L and was classified as a strong antioxidant. Kecombrang flowers macerated with 70% methanol and 50% methanol had moderate antioxidant activity as indicated by the IC_{50} values of 100.32 mg/L and 123.91 mg/L, respectively.

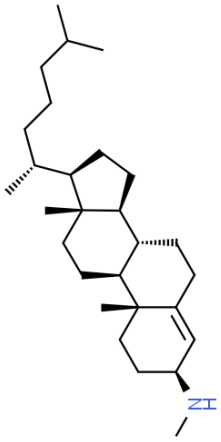
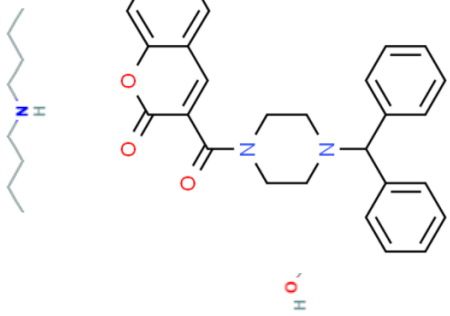
Analysis of 99% methanol extract using LC-MS/MS was used to determine the peak areas, molecular weights and possible structures of compounds present in kecombrang flower extract. Identification results using LC-MS/MS produced several chromatographic spectral peaks with time retention different retention. Before the LC-MS/MS test was carried out, separation was carried out with SPE, which aims to reduce impurities in the sample. The chromatogram obtained is shown in Figure 2. The chromatogram results have many peaks that appear, indicating that the sample is not pure and still contains many compounds present in the sample. There are a number of restrictions on this study, such as the absence of any confounding variables that may influence the findings.

CONCLUSION

Methanol, which has a concentration of 99%, has very good antioxidants. Basically, the higher the concentration of methanol solvent, the higher the levels of active compounds obtained. The compound contained in the extract of the kecombrang flowers that is likely to have antioxidant

Table 3. Interpretation of compound content with LC-MS/MS

No.	Retention Time (Minute)	Compounds	Fit conf %	Compound Structure
1.	1.23	$C_4H_{11}N_3O_3S$ 2-[carbamimidoyl(methyl)amino]ethanesulfonic acid	54.27	
2.	6.60	$C_{13}H_{22}N_2O_2$ 1,3-Di(1-piperidinyl)-1,3-propanedione	96.87	
3.	9.54	Hemigossypol; IUPAC 2,3,8-trihydroxy-6-methyl-4-propan-2-ylnaphthalene-1-carbaldehyde	92.40	
4.	13.49	$C_{15}H_{31}NO_2$ N-Dodecyl-N-methylglycine	99.78	

No.	Retention Time (Minute)	Compounds	Fit conf %	Compound Structure
5.	16.28	$C_{28}H_{49}N$ (3β)-N-Méthylcholest-4-én-3-amine	99, 52	
6.	17.08	$C_{26}H_{53}NO_2$ Dibutylammonium oleate; IUPAC: *N*-butylbutan-1-amine;(Z)-octadec-9-enoic acid	98, 60	
7.	12.40	$C_{27}H_{24}N_2O_3$ 3-{[4-(Diphenylmethyl)-1-piper azinyl]carbonyl}-2H-chromen-2-one	48.76	

activity is piperidine. Further studies are needed to validate and supplement these findings, such as other compounds in the kecombrang flower and the quantifications for each compound.

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CONFLICT OF INTEREST

There are no declared conflicts of interest that would have affected this article.

AUTHOR CONTRIBUTION

Comparatively, writers contribute to thinking by first analyzing ideas, then gathering information, researching content, verifying facts, revising papers, and finally publishing their descriptions of thinking

ETHICAL CONSIDERATION

This study was approved by Research Ethics Committee, Faculty of Medicine, Udayana University, Denpasar, Bali, Indonesia with the number of approval: 3092/UN14.5.2.VII.14/LT/2023.

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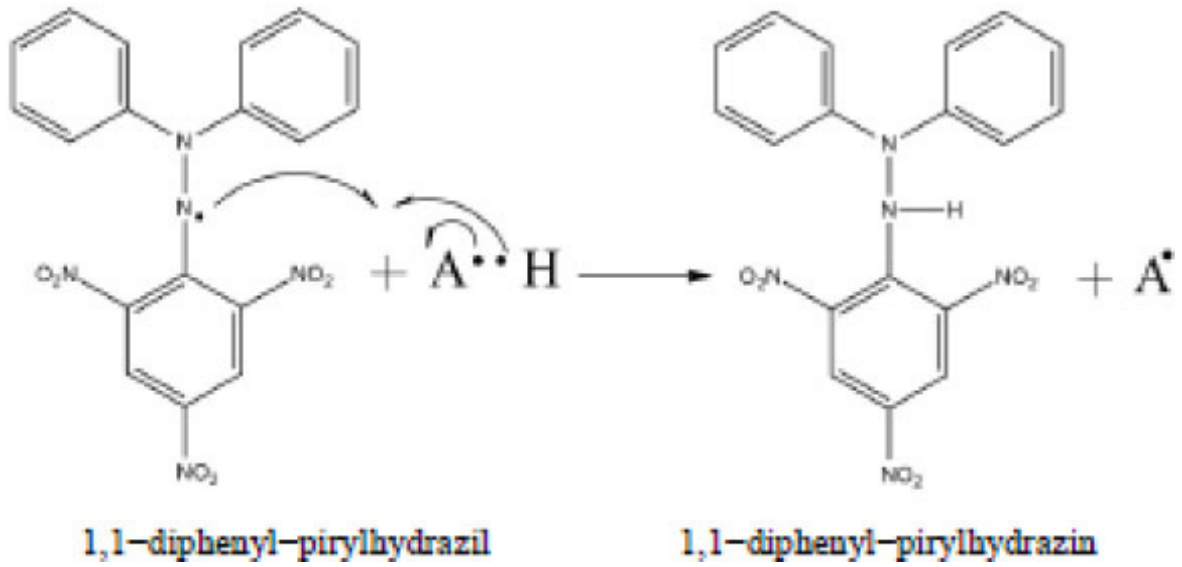

Figure 3. DPPH Reaction Mechanism with Antioxidants.

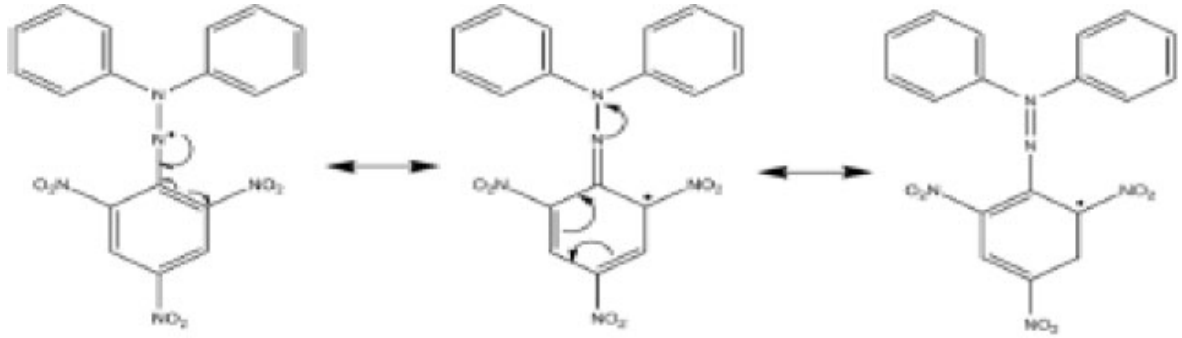

Figure 4. DPPH radical resonance reaction.

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