Comparison of Antioxidant Activity of Basil Herbal Extract (Ocimum sanctum L.): Maceration Vs Reflux Using DPPH Method

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Free radicals can trigger oxidative stress that contributes to various health disorders, so that effective and safe natural antioxidant sources are needed. Basil leaves (Ocimum sanctum L.) are known to contain secondary metabolite compounds such as flavonoids and phenolics that have the potential as antioxidants. This study aims to compare the antioxidant activity of basil herbal extracts obtained through maceration and reflux methods and determine the extraction method that produces optimal activity. This study used a quantitative experimental approach. Basil herbal simplicia was extracted with 96% ethanol solvent using maceration and reflux methods, then tested for antioxidant activity by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method using UV-Vis spectrophotometry at a wavelength of 514 nm. The parameter observed was the IC50 value as an indicator of the strength of antioxidant activity. The results showed that the maceration extract had an IC50 value of 64.54 ppm, while the reflux extract was 115.37 ppm, where the maceration extract was categorized as strong and the reflux extract was categorized as moderate. The macerated extract showed higher antioxidant activity than the reflux extract. Therefore, the extraction method influences the antioxidant activity of basil leaves, and maceration is recommended to produce extracts with optimal antioxidant potential. These findings support the use of basil leaves as a natural source of antioxidants in the development of health and pharmaceutical products.
Keywords:basil leaves, antioxidant activity, maceration, reflux, DPPH.

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1. Introduction

Free radicals are unstable molecules that can trigger oxidative stress and cause various degenerative diseases such as cancer, diabetes mellitus, cardiovascular disease, and premature aging. Oxidative stress occurs due to an imbalance between the number of free radicals and antioxidants in the body. Therefore, antioxidants are needed to neutralize free radicals and prevent cell damage. Currently, natural antioxidants from plants are more in demand because they are considered safer and have fewer side effects than synthetic antioxidants [1], [2].

Basil (Ocimum sanctum L.) is a medicinal plant widely used by people as a salad and in traditional medicine. Basil leaves and roots are known to contain various secondary metabolites such as flavonoids, phenolics, tannins, alkaloids, and saponins, which have potential as antioxidants. Flavonoids and phenolic compounds can donate hydrogen atoms or electrons to stabilize free radicals, thereby inhibiting oxidative damage [4], [15].

The extraction process is an important factor influencing the quantity and quality of active compounds obtained from natural materials. Different extraction methods can produce different antioxidant activities due to the influence of temperature, extraction time, and solvent contact with the material. The maceration method is carried out without heating so it is relatively safe for compounds that are not heat-

resistant, while the reflux method uses heating which can increase the efficiency of extracting active compounds [6], [7].

Several previous studies have reported the antioxidant activity of basil extract. However, studies comparing the antioxidant activity of basil leaves and roots using maceration and reflux extraction methods are still limited. Therefore, this study was conducted to compare the antioxidant activity of basil leaf and root extracts from maceration and reflux extraction using the DPPH method and UV-Vis spectrophotometry [4], [5]. This study aims to determine the effect of extraction methods on the antioxidant activity of basil leaf and root extracts and to determine the extraction method that provides the best antioxidant activity.

2. Literature Review and Problem Statement

Antioxidants and Free Radicals

Free radicals are reactive molecules that have unpaired electrons that can cause oxidative stress and trigger various degenerative diseases. Antioxidants function to inhibit oxidation reactions by donating electrons to free radicals so that they can prevent cell damage [1], [3]. Natural antioxidants are often found in plants that contain flavonoids, phenolics, tannins, and vitamins, and are preferred because they are relatively safe compared to synthetic antioxidants [2], [12]. Antioxidant activity is generally tested using the DPPH method because it is simple, fast, and sensitive. This method works based on the color change of DPPH free radicals from purple to yellow after reacting with antioxidant compounds, then measured using UV-Vis spectrophotometry to determine the IC50 value [8], [11], [14].

Basil and Antioxidant Activity

Basil (Ocimum sanctum L.) is an herbal plant that is widely used as a food ingredient and traditional medicine. Basil leaves and roots contain various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and phenolic compounds that have potential as antioxidants [4], [15]. Flavonoid and phenolic compounds are known to be able to capture free radicals so they can inhibit oxidative damage in the body [5], [9]. Several previous studies have reported that basil leaf extract has strong antioxidant activity based on DPPH method testing [4], [5].

Extraction Method and Research Gap

The extraction method is an important factor that influences the quantity and quality of active compounds obtained from natural ingredients. The maceration method is carried out by soaking at room temperature so it is safer for compounds that are not heat resistant, while the reflux method uses heating which can increase extraction efficiency [6], [7]. However, the use of high temperatures in the reflux method also has the potential to damage flavonoid and phenolic compounds that are thermolabile [6]. Many studies have been conducted on the antioxidant activity of basil extracts, but most only use one extraction method and focus on the leaves only. Therefore, this study was conducted to compare the antioxidant activity of basil leaf and root extracts extracted from maceration and reflux using the DPPH method.

Formulation of the problem

Is there a difference in the antioxidant activity of basil leaf and root extracts from the maceration and reflux extraction methods based on the IC50 value using the DPPH method?

Hypothesis

The maceration method produces higher antioxidant activity than the reflux method.
Such brevity is sufficient and more suitable for journal article format.

3. Method

This quantitative experimental study aimed to compare the antioxidant activity of basil (Ocimum sanctum L.) herbal extracts extracted through maceration and reflux using the DPPH method with UV-Visible spectrophotometry. The study was conducted through several stages, namely sample collection, preparation of herbal preparations, extraction process, phytochemical testing, and antioxidant activity testing.

The research samples, consisting of fresh basil leaves obtained from the research site, were then wet sorted to remove dirt and other foreign materials. The samples were washed with running water and then air-dried to obtain a dry herbal medicine. The dried herbal medicine was then ground into a powder to facilitate the extraction process.

Extraction was performed using 96% ethanol using two different methods: maceration and reflux. In the maceration method, the powdered medicinal plants were soaked in 96% ethanol at room temperature for several days, with occasional stirring to optimize the extraction process. In the reflux method, extraction was performed using controlled heating, allowing the solvent to circulate continuously throughout the extraction process.

The resulting extract was then evaporated to obtain a thick extract. Next, phytochemical tests were conducted to determine the content of secondary metabolites such as flavonoids, alkaloids, saponins, and tannins in the basil extract.

Antioxidant activity testing was performed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Extract solutions were prepared in several concentrations, then reacted with DPPH solution and incubated in the dark. Absorbance measurements were performed using a UV-Visible spectrophotometer at a wavelength of 514 nm. The percent inhibition value was calculated based on the absorbance results, then a linear regression analysis was performed to determine the IC50 value. The lower the IC50 value, the stronger the antioxidant activity of the extract. The research data were analyzed quantitatively to compare the antioxidant activity of maceration and reflux extracts based on the IC50 values obtained.

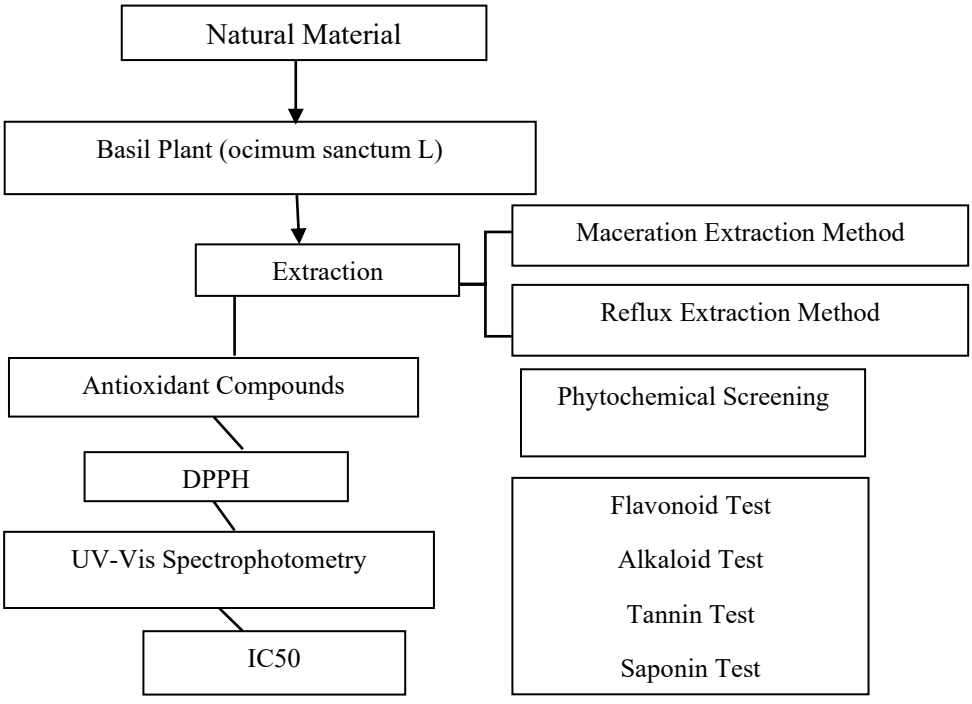

Figure 1. Research Framework

Figure 1 shows the research flow, which begins with the collection of natural ingredients in the form of basil (Ocimum sanctum L.), followed by an extraction process using maceration and reflux methods. The resulting extract was then subjected to phytochemical screening to determine the content of secondary metabolites such as flavonoids, alkaloids, tannins, and saponins. After that, antioxidant activity was tested using the DPPH method and absorbance measurements using UV-Vis spectrophotometry to obtain the IC50 value as a parameter of antioxidant activity.

4. Results and Discussion

Results of Antioxidant Activity Test of Basil Herbal Extract

Antioxidant activity testing of basil (Ocimum sanctum L.) herbal extract was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The parameter used to determine the strength of antioxidant activity is the IC50 value, which is the concentration of extract capable of inhibiting 50% of DPPH free radicals. The smaller the IC50 value, the stronger the antioxidant activity of a sample [8]. Test results showed that basil herbal extracts obtained through maceration and reflux methods had different antioxidant activities. The antioxidant activity test results are presented in Table 1.

Table 1. Results of Antioxidant Activity Test of Basil Herbal Extract

Extraction Method	IC50 value (ppm)	Antioxidant Activity Category
Maceration	64.54	Strong
Reflux	115.37	Currently

Based on these results, the macerated extract had a lower IC50 value than the refluxed extract. This indicates that the maceration method produces stronger antioxidant activity than the reflux method. The difference in IC50 values indicates that the extraction method affects the active compound's ability to scavenge free radicals.

Comparison of Antioxidant Activity of Maceration and Reflux Methods

The difference in antioxidant activity between maceration and reflux methods is influenced by the extraction process used. Maceration is performed without heating, allowing for better preservation of secondary metabolites such as flavonoids and phenolics. In contrast, the reflux method uses heating, which can potentially cause degradation of some thermolabile active compounds.

Flavonoid and phenolic compounds are known to have antioxidant properties because they can donate hydrogen atoms or electrons to stabilize free radicals [2], [3]. If these compounds are damaged during the extraction process, the antioxidant activity of the extract can decrease, resulting in a higher IC50 value.

The phenomena observed in this study indicate that the temperature used in the reflux method affects the stability of bioactive compounds. The higher antioxidant activity in the maceration method is thought to be due to the absence of heating, which maintains the flavonoid and phenolic content.

Analysis Based on Previous Research and DPPH

The results of this study are in line with the research of Nurcholis et al. [6] which stated that the extraction method affects the phenolic compound content and antioxidant activity of plant extracts. Research by Dwijayanti et al. [5] also reported that basil leaves have strong antioxidant activity because they contain flavonoids, tannins, and phenolic compounds.

The DPPH method is used because it is simple, sensitive, and commonly used to measure antioxidant activity in vitro [8]. In this method, the purple DPPH solution will turn pale yellow after reacting with the

antioxidant compound. A decrease in color intensity indicates the active compound's ability to scavenge free radicals.

Absorbance measurements were performed using a UV-Visible spectrophotometer at a wavelength of 514 nm. The absorbance values were then used to calculate the percentage of inhibition and determine the IC50 value through a linear regression equation.

Initial Conclusions of Research Results

Based on the research results, there was a difference in antioxidant activity between macerated and refluxed basil herbal extracts. The maceration method produced more optimal antioxidant activity than the reflux method, based on the IC50 values obtained.

These results indicate that the extraction method is a crucial factor in maintaining the stability of the bioactive antioxidant compounds in basil leaves. Therefore, maceration can be considered a more effective extraction method for producing basil herbal extracts with higher antioxidant activity.

5. Conclusion

This study shows that basil (Ocimum sanctum L.) herbal extracts extracted by maceration and reflux have antioxidant activity based on the DPPH method with differences in IC50 values between the two methods. The maceration extract showed higher antioxidant activity than the reflux extract. The results of this study indicate that the extraction method influences the antioxidant activity of basil extract and can affect the stability of the bioactive compounds contained therein.

This study still has limitations because the antioxidant activity test was only conducted using the DPPH method and no quantitative analysis of the active compounds that act as antioxidants was performed. Therefore, further research is recommended using other antioxidant testing methods, such as ABTS and FRAP, as well as determining total flavonoid and phenolic levels to obtain more comprehensive information regarding the antioxidant potential of basil extract.

6. References

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