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The Effect of Environmental Conditions on the Alkaloid and Flavonoid Content of Basil Leaves (*Ocimum basilicum* L) Growing in Kandangan Village

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Abstract

Basil leaves have long been used as fresh vegetables. Basil leaves are used to eliminate odors and can also be used as antiseptics. Each plant can produce primary and secondary metabolites. The production of these metabolites is influenced by environmental conditions. Kandangan Village is one of the villages in Gresik Regency which has a relatively hot temperature and many industries are located around it. The purpose of this study was to determine the results of phytochemical screening tests for the presence of alkaloid and flavonoid compounds in basil leaves growing in Kandangan Village. This type of research is experimental research. Basil leaf samples were obtained from Kandangan Village. The extraction method used was maceration using 96% ethanol solvent. The test for the presence of alkaloids used the Dragendroff test method and the Mayer test. The test for the presence of flavonoids used the Wilstater test method and the Bate Smite-Metcalfe test. The results of this study indicate that basil leaves growing in Kandangan Village are positive for the presence of flavonoids with a color change to yellow for the Wilstater test and a color change to red in the Bate Smite-Metcalfe test. However, the test for the presence of alkaloids showed negative results because there was no formation of orange to red deposits for the Dragendroff test and yellowish white deposits for the Mayer test.

Keywords : Dragendorff test, Wilstater test, Mayer test, Smite-Metcalfe test, Ethanol, Maceration

INTRODUCTION

Basil leaves have the potential as antiseptics, mouth fresheners and can also be used as one of the cooking ingredients. Basil plants are popular and easy to get. Basil plants are included in the Lamiaceae family which is widely cultivated in Indonesia. Over time, people have used basil leaves as natural products or as herbal products.

In 96% ethanol extract, basil leaves positively contain flavonoid, alkaloid, saponin and tannin compounds (Kumalasari and Andiarna, 2020; Amelia *et al.*, 2022). Each active compound content in basil leaves has its own pharmacological effect. Alkaloid compounds act as antidiarrheals which have a working mechanism of suppressing intestinal peristalsis (Fadilah *et al.*, 2022). Flavonoid compounds can also function as antidiarrheals (Ujan *et al.*, 2019). Basil leaves that are positive for the presence of alkaloid, flavonoid and tannin compounds have very strong antioxidant activity (Dwijayanti *et al.*, 2023).

The production of secondary metabolites in a plant can be influenced by environmental conditions. Abiotic stress (increased temperature and CO_2 concentration) causes drastic changes in the metabolism of secondary metabolites (Austen *et al.*, 2019). In addition, climate and ecology can also affect the production of secondary metabolites (Pant *et al.*, 2021). Another factor that can also have an influence is humidity (Srivastava *et al.*, 2021).

Kandangan Village is located in Cerme District, Gresik Regency. The area has a temperature of 31 °C and humidity of 59% (https://www.bmkg.go.id/). Around Kandangan Village, there are many factories, including fabrication factories, construction services and carbon steel. In the village, basil

leaves grow a lot. Basil leaves in the area are widely used as vegetables. Seeing the relatively hot conditions of Kandangan Village and the high CO_2 pollution due to the many factories, it is important to conduct research related to screening the presence of alkaloid and flavonoid compounds in basil leaves that grow in the area. To prove whether the basil leaves growing in this area are still positive for the presence of secondary metabolites of alkaloids and flavonoids.

METHODS

Equipment

The tools used in this study include maceration vessels, analytical scales, beaker glasses, measuring cups, stirring rods, thermometers, aluminum foil, Erlenmeyer flasks, glass funnels, porcelain cups, water baths, blenders, watch glasses, wooden clamps, test tubes, droppers, ovens, and sieves no. 45.

Material

The sample in this study was basil leaves. These leaves were harvested from Kandangan village, Gresik. The materials used in this study include 96% ethanol (technical), Mayer's reagent, Dragendroff's reagent, concentrated HCl, dilute HCl, Mg powder, quarcetin, filter paper, and aluminum foil.

Method

1. Sample Preparation

Basil leaves were harvested from Kandangan Village (Gresik). The proof of the research sample is basil leaves which were tested microscopically and compared the results with data in the Indonesian Herbal Pharmacopoeia. After being harvested, basil leaves were sorted wet, washed with clean and running water, drained, chopped, dried, and sorted dry. Wet sorting is done to remove unwanted parts, such as yellow leaves, soil, and twigs (Menkes RI, 2011). The drying method used in this study was oven drying at a temperature of 40 °C. Next, the basil leaf simplicia obtained was tested for water content.

2. Preparation of Basil leaf extract

Before the extraction process, the dried basil leaves are refined using a blender. Refining also aims to expand the contact surface with the solvent, because the finer a material is, the easier it is for the cells in the material to be damaged or broken and the solvent can easily enter the material cells and secondary metabolite compounds can be easily extracted (Rifkowaty and Wardanu, 2016). To optimize the extraction process, after being ground, the basil leaf powder was sieved with a no. 45 sieve. This was done in order to standardize the size of the basil leaf powder.

Basil leaf powder was extracted using the maceration method. The solvent used in this extraction process is 96% ethanol. This extraction process requires 500 grams of basil leaf powder with a solvent amount of 1500 mL. This amount of solvent can soak the leaf powder and reach 1 cm from the top layer of the leaf powder surface. The maceration process is carried out for 5 days and is stirred every day. Stirring aims to achieve a faster balance in the concentration of the active compound group in the liquid (Sari *et al.*, 2019), so that the process of dissolving the active compound into the solvent is faster. After the fifth day, the filtering process is carried out using filter paper and the evaporation process is continued. The evaporation process uses a water bath with a temperature of 40 °C. The selection of this temperature for the evaporation process is to prevent damage to the structure of the expected secondary metabolites (Yani *et al.*, 2023). After obtaining a thick extract, the yield calculation process is continued with the following formula:

$$\% \text{ yield} = \frac{\text{weight of the extract obtained}}{\text{the weight of the extracted simplicia}} \text{ x } 100\%$$

This study also tested the quality of the extraction results, namely by measuring the water content of the extract. The procedure for determining the water content of the extract is to weigh a porcelain cup, put approximately one gram of extract into the cup, put the cup and extract in the oven at a temperature of 60 °C for ten minutes, cool it, and reweigh it. The heating process in the oven is carried out continuously until a constant weight of the cup and extract is obtained. The calculations

needed for the water content of the extract are as follows.

$$\%\ \text{The water content} = \frac{\text{Initial (cup and extract)weight} - \text{constant weight (cup and extract)}}{\text{extract weight}} x\ 100\%$$

3. Phytochemical screening

Phytochemical screening tests are carried out by adding a reagent to each compound to be tested by observing the color change and shape change in the liquid being tested (Kumalasari and Andiarna, 2020). This test is carried out 3 times in each test. The phytochemical screening tests tested in the study can be carried out in the following ways:

a. Alkaloid compound test

1. Dragendroff test

1 mL of extract is acidified with a few drops of dilute hydrochloric acid. Then Dragendroff reagent is added. Positive results containing alkaloids are indicated by the formation of an orange to red precipitate (Thilagavathi *et al.*, 2015).

2. Mayer test

1 mL of extract is acidified with a few drops of dilute hydrochloric acid. Then Mayer reagent is added. Positive results containing alkaloids are indicated by the formation of a yellowish white precipitate (Thilagavathi *et al.*, 2015).

b. Flavonoid compound test

1. Wilstater test

1 mL of extract is put into a test tube then a few drops of concentrated HCl plus a little Mg powder are added. Positive results containing flavonoids are indicated by the formation of a yellow color (Ikalinus *et al.*, 2015). The blank used in this test is quarcetin.

2. Bate Smite-Metcalfe test

1 mL of extract is put into a test tube then some concentrated HCl is added then heated. Positive results containing flavonoids are indicated by the formation of a red color (Ikalinus *et al.*, 2015). The blank used in this test is quarcetin.

RESULTS AND DISCUSSION

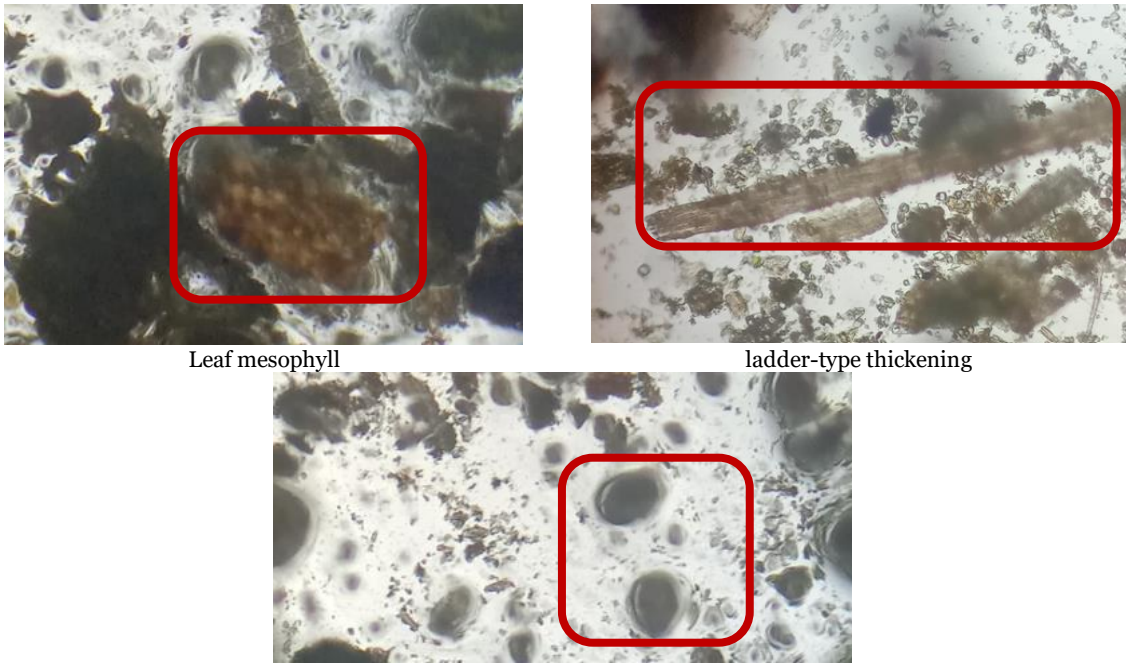
Sample Preparation (Basil Leaf Simplicia)

In this study, 6 kg of basil leaves were harvested. After the wet sorting, washing, drying, and dry sorting processes, 3 kg of basil leaf simplicia were obtained. After the refining and sieving process, 986 grams of basil leaf fine powder was obtained. The difference between the weight of fresh basil leaves and fine basil leaf powder is influenced by several things, including the removal of unwanted parts during wet sorting, parts of the leaves that must be discarded during dry sorting (because they are burnt or still wet), and possibly because there are parts that do not pass through during the sieving process.The basil leaf fine powder can be seen in Figure 1.

Figure 1. The Basil Leaf Powder

Figure 1 shows that the color of basil leaf powder is brownish green. The decrease in the intensity of the green color indicates that there has been a process of degradation of chlorophyll, a compound responsible for the formation of green color in plants (Sinaga *et al.*, 2021). The longer the drying process that occurs, the longer the water is in the simplex so that the possibility of a chlorophyll

degradation reaction to pheophytin will be greater (Purwanti *et al.*, 2018). The longer the drying process, the longer the water is in the simplex so that the possibility of a chlorophyll degradation reaction to pheophytin will be greater (Purwanti *et al.*, 2018). In this study, the oven drying process at a temperature of 40 °C was carried out for 8 hours. So it is better to reduce the duration of this drying process by reducing the size of the leaves before the drying process and turning the leaves more often during the drying process. The water content of basil leaf simples is 5%. The water content of basil leaf simplicia is 5%. This water content value meets the quality requirements of a simplicia, where the water content should not be more than 10%. In the microscopic test it can be seen in Figure 2.



Leaf mesophyll ladder-type thickening

Scale hairs

Figure 2. Microscopic results of basil leaves

In the Indonesian Herbal Pharmacopoeia, it is explained that in the microscopic test of basil leaves, there are several marker fragments, namely vascular bundles with ladder-type thickening, leaf mesophyll, and scale hairs (Menkes RI, 2017). In this research sample, these fragments were found. So this research sample can be confirmed to be basil leaves.

Extract Yield

The thick extract obtained from the evaporation process was 20,77 grams. The thick extract from the maceration method has characteristics of a dark green color and a strong basil leaf aroma. The yield results of the basil leaf extract are shown in Figure 3. Basil leaf extract has a brownish black color and a distinctive aromatic smell.

Figure 3. The Basil Leaf Extract

The calculation of the yield of the extract from the extraction process can be seen in Table 1. The extraction process produces a relatively small extract yield. In previous research, extraction of basil leaves using 96% ethanol for 3 days obtained a yield of 9,12% (Ariani et al., 2020). According to the Indonesian Herbal Pharmacopoeia, the yield of basil leaf extraction should not be less than 5,6% (Menkes RI, 2017). The yield of the extract obtained in this study was actually after the extraction process for 5 days. Actually, the addition of maceration time can increase the yield value of the simplisia (Handoyo, 2020). So there are other factors that can affect the amount of extract yield in this study.

Table 1. Results of Extract Yield Calculation

Simplicia	Solvent	The weight of the extracted simplicia (g)	Weight of the extract obtained (g)	Yield %
Basil Leaf	Ethanol 96%	500	20,77	4,15

According (Ariani *et al.*, 2020), stirring was carried out 3 times for 3 days. While in this study, stirring was carried out 3 times for 5 days. From this condition, it can be concluded that the amount of stirring during the extraction process affects the yield. Stirring can affect the IC50 value of durian fruit skin, where the IC50 value is greater for extracts extracted with stirring every 1 hour, compared to the IC50 of extracts extracted with occasional stirring (Setyowati *et al.*, 2014). There are other factors that may affect the extraction results, namely the size of the simplicia (Widwiastuti *et al.*, 2022). So it is necessary to try to change the mesh number of the sieve to a larger number. The large extract yield is also possible due to the influence of environmental conditions of the origin of the research sample. The presence of secondary compounds helps plants to adapt to the environment or protect themselves from pests and pathogens, where phenolic compounds will decrease and terpenoid compounds will increase due to heating conditions (Srivastava *et al.*, 2021).

The water content test on the extract obtained a value of 9,09%. The water content of this extract meets the quality requirements of basil leaf extract in the Indonesian Herbal Pharmacopoeia, which is no more than 12% (Menkes RI, 2017). The smaller the water content in the extract means the smaller the risk of damage to the extract due to microbes, fungi, or insects (Rosidah et al., 2020).

Phytochemical Screening

In this study, 2 phytochemical screening tests were conducted, namely alkaloids and flavonoids. Alkaloid tests used the Dragendroff test and Mayer test. Flavonoids used the Wilstater test and the Bate Smite-Metcalfe test. The results of phytochemical screening from the three tests are listed in Table 2. The Dragendroff test cannot produce orange to red precipitate, while the Mayer test also cannot produce yellowish white precipitate.

Table 2. Results of Phytochemical Screening of Alkaloids and Flavonoids

Jenis Uji	U1	U2	U3
Alkaloids			
1. Dragendroff Test	-	-	-
2. Mayer Test	-	-	-
Flavonoids			
1. Wilstater Test	+	+	+
2. Bate smith- Metcalfe Test	+	+	+

Table 2 shows that basil leaves grown in Kandangan Village are positive for the presence of flavonoids and negative for the presence of alkaloids. According to (Kumalasari and Andiarna, 2020) 96% ethanol extract of basil leaves is positive for the presence of flavonoids and alkaloids. The difference in screening results is possible due to differences in where the basil leaves grow. According to research by (Austen *et al.*, 2019) differences in compound content in a particular plant can be caused by environmental factors, namely temperature and CO_2 levels. In Kandangan Village, Gresik Regency, the temperature is very hot and there are high levels of CO_2 because there are many industries around Kandangan Village. High CO_2 levels can affect the production of secondary metabolites produced by

plants. One of them can change the chemical composition of plants, including the types of phytochemical compounds produced. There are several environmental parameters that can affect the production and accumulation of secondary metabolites in medicinal plants, including temperature, carbon dioxide, ozone, light intensity, and soil (Pant *et al.*, 2021). Of these parameters, three factors that have a large influence are soil, light intensity, and carbon dioxide. Elevated CO_2 increases carbohydrate accumulation but decreases nitrogen accumulation in plants thus affecting their C-N ratio (Rajashekar, 2018). Based on this theory, the results of phytochemical screening of the presence of alkaloids in this study showed negative. This is because the basil leaves studied grew in areas around industries that emit a lot of carbon dioxide and monoxide. Increased CO_2 can increase the concentration of phenolic compounds in leaves (Holopainen *et al.*, 2018).

CONCLUSION

The process of extracting basil leaves taken from Kandangan village (Gresik) using the maceration method and 96% ethanol solvent produced a yield of 4.15%. After testing the presence of alkaloid compounds (Dragendroff test and Mayer test) and flavonoid compounds (Wilstater test and Bate smith- Metcalfe) it can be concluded that the presence of alkaloid compounds is negative and the presence of flavonoids is positive. For further research, it is expected that the levels of flavonoid compounds can be studied.

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