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Research Article

Antioxidant Activity Test and Phytochemical Content of *Ocimum sanctum* Extract Based on Different Extraction Methods

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ABSTRACT

Ocimum sanctum, a member of the Lamiaceae family, is widely utilized in traditional medicine due to its pharmacological properties. Among its bioactive constituents, flavonoids exhibit significant antioxidant activity, capable of scavenging free radicals and inhibiting cancer growth and proliferation. This study aimed to evaluate the antioxidant activity and phytochemical composition of O. sanctum leaf extracts obtained using two different extraction techniques: maceration and Microwave-Assisted Extraction (MAE), and to determine the most effective method. The leaves were extracted using 70% ethanol, and the resulting extracts were analyzed qualitatively for the presence of phytochemical compounds. Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, with vitamin C serving as the positive control. Absorbance measurements were performed at a wavelength of 516 nm using a visible spectrophotometer. Statistical analysis to determine the most effective extraction method was conducted using SPSS version 24. The results revealed that O. sanctum leaf extracts possess antioxidant activity, with IC_{50} values of 111.843 ± 0.14789 ppm for the maceration method (moderate antioxidant intensity) and 75.725 ± 0.4498 ppm for the MAE method (strong antioxidant intensity). Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and tannins in both extraction methods, as well as in the powdered leaf material. The MAE method yielded the highest antioxidant activity, with an IC_{50} value of 75.725 ± 0.4498 ppm, classifying it as a strong antioxidant source. Both extraction methods demonstrated the presence of key secondary metabolites, including alkaloids, flavonoids, saponins, and tannins.

Keywords:: Antioxidants; DPPH; maceration; MAE; *Ocimum sanctum*

INTRODUCTION

Antioxidants are compounds that function to prevent and repair damage to cells in the body, especially those caused by exposure to free radicals. One example of the effects of exposure to free radicals is the formation of cancer. The cancer is one of the leading causes of death in the world. The Indonesian Ministry of Health (2024) stated that in 2024 around 9.7 million deaths were caused by cancer. Antioxidants are capable of scavenging free radicals and reduce their impact. *Ocimum sanctum L.* leaves belong to the Lamiaceae family which is widely used by the community as a traditional medicine., and have antioxidant activity with an IC_{50} value of 39.771 µg/mL (Tatiana & Ria, 2020).

Research results of Bensaid et al., (2022) suggest that *O. sanctum* extracts from stems, in addition to leaves, can benefit at the nutrition-health with specific therapeutic potential. Various effective extraction methods are employed to isolate bioactive compounds from natural sources. This study aims to evaluate the antioxidant activity and phytochemical content of 70% ethanol extract of *O. sanctum* leaves using two extraction techniques: maceration and Microwave-

Assisted Extraction (MAE) and to identify the most effective method for extracting bioactive compounds from *O. sanctum* leaves.

METHODS

Tools

The tools used in this study were aluminum foil, stirring rod, beaker glass (Pyrex®), filtrate bottle, maceration bottle, spray bottle, steam cup, glass funnel, Erlenmeyer flask (Pyrex®), beaker (Pyrex®), measuring cup (Pyrex®), watch glass, rubber absorber, filter paper, exchange rate, measuring flask (Pyrex®), microwave (Samsung®), oven (Memmert®), water bath, 40 mesh sieve, tube clamp, dropper pipette, measuring pipette, volume pipette, test tube rack, rotary evaporator, UV-Vis spectrophotometer (Optizen®), furnace (Vulcan A-550®), analytical balance, extract container, water bath.

Materials

The materials used in this study were distilled water, concentrated hydrochloric acid (HCl), fresh basil leaves (*O. sanctum*), ethanol (C_2H_5OH), gelatin, 2N HCl, 3% iron (III) chloride solution ($FeCl_3$), methanol p.a. (CH_3OH), NaCl, Boucharda reagent (I2 + I-), Dragendroff reagent ($Bi(NO_3)_3$ + 3KI), Mayer reagent ($HgCl_2$ + 2KI), DPPH powder, magnesium metal powder (Mg), vitamin C ($C_6H_8O_6$).

Preparation of Plant Sample

A total of 10 kg of fresh *O. sanctum* leaves were collected and wet sorted to remove non-leaf material, followed by thorough washing under running water to eliminate soil and debris. The cleaned samples were drained and dried in a Memmert® oven at 40–50 °C until completely dry. Dry sorting was conducted to remove any remaining impurities and damaged plant parts, then the dried leaves were ground using a blender and sieved through a 40-mesh sieve.

Extraction Process of *O. sanctum*

Maceration Method

A total of 100 g of *O. sanctum* leaf powder was put into a maceration bottle and extracted with 70% ethanol at a ratio of 1:10 (w/v). First, the powder was soaked in 500 mL of solvent for 6 hours while stirring occasionally, then left for 18 hours. The mixture was allowed to settle and then filtered. The residue was re-macerated twice, each time with 250 mL of 70% ethanol solvent. The results of the three filtrates were combined and concentrated using a rotary evaporator at a temperature of 50°C until a thick extract was obtained (Bennour et al., 2020). The entire process was repeated three times.

Microwave-Assisted Extraction (MAE) Method

A total of 100 g of *O. sanctum* leaf powder was placed in an Erlenmeyer flask and extracted with 1 L of 70% ethanol. The mixture was extracted with a Samsung® microwave at 800 watts for six minutes, with alternating irradiation cycles, 1 minute of irradiation followed by 2 minutes of rest. The extract was then allowed to cool and filtered. The filtrate was concentrated with a rotary evaporator until a thick extract was obtained. This procedure was repeated three times. The extract yield was calculated as follows,

Determination of Extract Quality

Determination of Water Content

A total of 5 g of *O. sanctum* extract was placed in a previously weighed container. The sample was dried at 105°C for 5 hours, then reweighed. Drying and weighing were repeated at 1-hour intervals until the difference between successive weighings was no more than 0.25%. The total mass loss was the 'loss on drying' (Schubnell et al., 2020).

Determination of Total Ash Content

Approximately 2 g of extract was weighed into a previously ignited and tared silicate crucible. The sample was gradually incinerated in a Vulcan A-550® furnace for 3 hours until the carbonaceous material was fully combusted. After cooling, the crucible was weighed. If complete combustion was not achieved, hot water was added, and the mixture was filtered using ash-free filter paper. The residue and filter paper were then incinerated at the same temperature. The filtrate was transferred to the crucible and evaporated to dryness, followed by ignition until a constant weight was obtained. The final residue, representing total ash, was weighed and used to calculate ash content (Smith et al., 2023).

Phytochemical Screening

Alkaloid Test

A total of 500 mg of extract was weighed and placed in an Erlenmeyer flask, then added with 1 mL of 2N HCl and 9 mL of distilled water. The mixture was heated in a water bath for 15 minutes, cooled, and filtered. The resulting filtrate was subjected to alkaloid detection tests as follows:

(a) Two drops of Bouchardat reagent were added to a small amount of the filtrate on a watch glass, and a brown to black precipitate indicated a positive result.

(b) Two drops of Mayer's reagent were added small amount of filtrate, and the formation of a white precipitate indicated a positive reaction.

(c) Two drops of Dragendorff's reagent were added to a small amount of filtrate, and a positive result was shown by the appearance of an orange-brown precipitate (Hanani, 2016).

Flavonoid Test

A total of 0.5 g of extract was dissolved in 5 mL of 95% ethanol. From this solution, 2 mL was transferred into a test tube, then 0.1 g of magnesium powder was added. Next, 10 drops of concentrated hydrochloric acid were carefully added along the side of the tube and the mixture was stirred. The presence of flavonoids is indicated by the appearance of a red or orange color (Ningsih et al., 2020)

Tannin Test

Testing is done using two methods. In the first method, 0.5 g of extract or simplicia is put into a test tube, then distilled water is added until dissolved. Next, 1% gelatin is added in 10% sodium chloride. A positive result is the formation of a white precipitate. In the second method, 0.5 g of extract or simplicia is put into a test tube, then a few drops of ferric chloride (FeCl3) solution are added. A positive result is the appearance of a blue or black color (Mulyani et al., 2022).

Saponin Test

A total of 0.5 g of extract was put into a test tube and mixed with 10 mL of hot water, then cooled. Add a few drops of hydrochloric acid, then the mixture was shaken vigorously for 10 minutes. The formation of stable foam indicates a positive result (Fatwami & Royani, 2023)

Determination of Antioxidant Activity of Extract

Preparation of 1 mM DPPH Solution

DPPH powder (39.432 mg) was accurately weighed and dissolved in methanol p.a. in a 100 mL volumetric flask to prepare a 1 mM solution. The flask was covered with aluminum foil to protect it from light.

Preparation of Blank Solution

One milliliter of 1 mM DPPH solution was pipetted into a 10 mL volumetric flask and diluted to volume with methanol p.a. The solution was homogenized and incubated at room temperature (25–30 °C) for 30 minutes, protected from light using aluminum foil.

Preparation of 100 ppm Vitamin C Stock Solution

Ascorbic acid (100 mg) was dissolved in methanol p.a. in a 100 mL volumetric flask to yield a 1000 ppm stock solution. From this, 10 mL was diluted with methanol p.a. to 100 mL to obtain a 100 ppm stock solution.

Determination of Maximum Wavelength

One milliliter of 1 mM DPPH solution was added to a 10 mL volumetric flask, followed by approximately 8 mL of methanol p.a., homogenized, and brought to volume. After 30 minutes of incubation at room temperature (protected from light), the absorbance was scanned in the range of 500–600 nm using a Vis spectrophotometer to determine the maximum wavelength.

Determination of Optimum Incubation Time

One milliliter of 100 ppm ascorbic acid solution was added to a 10 mL volumetric flask, followed by 4 mL of methanol p.a. and 1 mL of 1 mM DPPH solution. The mixture was diluted to volume with methanol p.a., homogenized, and incubated. Absorbance was measured at the maximum wavelength at intervals of 10, 20, 30, 40, 50, and 60 minutes to determine the time at which the absorbance stabilized.

Preparation of Vitamin C Standard Series (Positive Control)

Standard solutions of ascorbic acid at concentrations of 2, 4, 6, 8, and 10 ppm were prepared from 100 ppm stock solution in separate 10 mL volumetric flasks. Each was mixed with 1 mL of 1 mM DPPH solution and diluted with methanol p.a., then homogenized. After incubation at optimum time, absorbance was measured at the maximum wavelength using a Vis spectrophotometer (Optizen®).

Preparation of Test Solutions

Fifty milligrams of *O. sanctum* leaf extract was dissolved in methanol p.a. in a 50 mL volumetric flask to obtain a stock solution of 1000 ppm. Then, serial dilutions of 20, 40, 60, 80, and 100 ppm were carried out in separate 10 mL volumetric flasks. Each was mixed with 4 mL of methanol p.a. and 1 mL of 1 mM DPPH solution, and homogenized. The solution was incubated at room temperature for an optimum time, protected from light, and its absorbance was measured at the maximum wavelength.

Antioxidant Activity Test Using the DPPH Method

The absorbance of the test solutions, positive control (vitamin C), and blank was measured at the maximum wavelength using a UV-Vis spectrophotometer. The antioxidant activity was evaluated based on the percentage of DPPH radical inhibition. The IC_{50} value was determined by plotting the inhibition percentage against the concentration and calculating the x-intercept from the linear regression equation ($y = bx + a$), where $y = 50$ and x represents the IC_{50} value.

1 2 3

Figure 1. *O. sanctum* Leaf Powder (1), Maceration Method (2), and MAE Method (3)

Data Analysis

To evaluate the most effective extraction method for *O. sanctum* leaf extract as an antioxidant, the data were analyzed using a Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was conducted with two extraction methods and three replications per treatment.

RESULTS AND DISCUSSION

Dry Powder of *O. sanctum* Leaf

A total of 10,000 g of fresh *O. sanctum* leaves and stems were washed, dried, and processed to obtain 905 g of dried leaf powder (Figure 1), resulting in a yield of 9.05%. Yield was calculated as the ratio of the final dried powder weight to the initial weight of the *O. sanctum* fresh leaves. The dried *O. sanctum* leaf powder was further analyzed for physicochemical characteristics, including moisture content and total ash content. Moisture content was determined gravimetrically by drying the sample in an oven at 105 °C, during which water loss was measured to calculate the final moisture percentage. The moisture content was found to be 7.93%, meeting the standard requirement of less than 10%. The total ash content, determined through incineration, was 7.27%, which also complies with the standard limit of <12.5% as specified by Krismayadi et al. (2024).

Dried and powdered *O. sanctum* leaves were evaluated for moisture and ash content. Ash content analysis was conducted to assess the total mineral content, both intrinsic and extrinsic, accumulated during harvesting, handling, and processing of the plant material. The ash content obtained in this study was 7.27%, which meets the requirement of the standard limit of <12.5%. An ash content exceeding 12.5% typically indicates a higher concentration of mineral residues within the sample.

Dry Powder and Extracts Yield

O. sanctum leaf extracts were obtained using two different extraction methods. The maceration method yielded 17.1 g of extract, corresponding to a yield of 17.1%, while the Microwave-Assisted Extraction (MAE) method produced 10.2 g of extract, with a yield of 10.2%. The physicochemical characteristics of the resulting extracts are presented in Table 1 and presented in Figure 1.

The powdered leaves of *O. sanctum* were subjected to extraction to isolate the desired extract. The primary objective of this extraction process was to separate secondary metabolites present in the plant material or its mixtures (Quero-Jiménez et al., 2020).

Phytochemical Test Results

Phytochemical screening of *O. sanctum* leaf powder and extracts was based on the color changes observed after the addition of certain reagents (Jafar et al., 2020). The results showed the presence of secondary metabolites including alkaloids, flavonoids, tannins, and saponins (Table 2).

Table 1. Characteristics of *O. Sanctum* Leaf Extract

Sample	Parameter	Extraction Method	Results (%)	Regulation (%)
Extract	Yield Extract	Maceration	17.1	
		MAE	10.2	
	Water content	Maceration	5.77	<12
		MAE	6.48	<12
	Ash Content	Maceration	5.4	<10.7
		MAE	5.98	<10.7

Table 2. Phytochemical Test of *O. sanctum* Leaf Extract

Compound content	Powder	Maceration extract	Extract MAE	Note
Alkaloid				
1. Dragendorf	+	+	+	Orange brown sediment
2. Bouchardad	+	+	+	Brown to black sediment
3. Mayer	-	-	-	White sediment
Flavonoid	+	+	+	Yellow or orange
Saponins	+	+	+	Foam
Tannin	+	+	+	Blue or blackish

Note:
(+) : Indicates the presence of secondary metabolite compounds
(-) :Shows the absence of secondary metabolites

The flavonoid test of *O. sanctum* leaf extract yielded positive results, as indicated by a red to orange color change (Table 2). Similarly, the saponin test also showed a positive result, demonstrated by the persistent formation of foam upon shaking (Table 2). This foam formation is due to the amphiphilic nature of saponins, which possess both hydrophilic and hydrophobic functional groups. During agitation, the hydrophilic moiety interacts with water while the hydrophobic moiety associates with air, stabilizing the foam. The addition of hydrochloric acid (HCl) after shaking further enhances foam stability by increasing the polarity of the solution, allowing better alignment of hydrophilic groups (Rubianti et al., 2022).

Flavonoids identified in the extract serve as antioxidants, protecting cells from oxidative stress by neutralizing free radicals. They achieve this either directly, by donating hydrogen atoms, or indirectly, through various biochemical pathways (Hassanpour & Doroudi, 2023).

The alkaloid test also confirmed a positive result, indicating the presence of alkaloids in basil leaves (Table 2). This test was performed using Dragendorff's, Mayer's, and Bouchardat's reagents. Positive reactions were indicated by brown to black precipitate in Bouchardat's reagent, orange-brown precipitate in Dragendorff's reagent, and ideally a white precipitate in Mayer's reagent. However, in this study, the Mayer reagent yielded a negative result. This variation is due to the differing sensitivities of alkaloid detection reagents, which rely on their ability to form complexes with heavy metals such as mercury, bismuth, or iodine. Mayer's reagent contains mercury(II) chloride and potassium iodide, while Dragendorff's reagent includes bismuth nitrate in acidic solution, making it more sensitive for alkaloid detection (Ningsih et al., 2020). The acidic condition created by adding HCl enhances alkaloid solubility, as alkaloids are basic (Shofa, 2020).

Tannin content was verified using the ferric chloride ($FeCl_3$) test, which produced a positive result indicated by a color change. This occurs due to the formation of a complex between tannins and Fe^{3+} ions (Rohmah et al., 2021).

Antioxidant Activity of *O. sanctum* Leaf Extract

Antioxidant capacity was quantified by determining the IC_{50} value, defined as the concentration of extract required to inhibit 50% of DPPH free radicals. A lower IC_{50} value indicates stronger antioxidant activity (Umboro et al., 2020). The IC_{50} values obtained for extracts from both MAE and maceration methods are presented in Table 3.

To determine the antioxidant activity of *O. sanctum* leaf extract, the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was employed. First, the maximum wavelength (λmax) of DPPH was determined within the 500–600 nm range to ensure accurate measurement. The maximum absorbance was recorded at 516 nm. Subsequently, the optimal incubation time was established to determine the point at which the reaction reaches stable absorbance. In this study, the 30th minute was identified as the point of stable absorbance.

Vitamin C was used as a positive control due to its well-known antioxidant properties, which include the ability to scavenge free radicals and prevent chain reactions (Ambari et al., 2021). DPPH, a stable free radical, reacts with antioxidants present in the sample by accepting hydrogen atoms, resulting in a color change that reflects radical scavenging activity.

Table 3. IC_{50} Value of MAE *O. sanctum* Leaf Extract and Maceration

Sample	Replication	IC_{50} value (ppm)	IC_{50} Average (ppm)	Antioxidant Activity Category
Extract of Maceration	1	112.035		
	2	111.820	111.843 ± 0.14789	medium
	3	111.674		
Extract of MAE	1	75.101		
	2	76.144	75.725 ± 0.4498	strong
	3	75.930		
Vitamin C		8.057		very strong

In this study, the extract obtained using the Microwave-Assisted Extraction (MAE) method exhibited higher antioxidant activity (lower IC_{50} value) than the extract obtained via maceration. Despite the maceration method yielding a higher extract quantity, yield alone did not correlate with antioxidant activity. The difference in antioxidant efficiency is attributed to the extraction mechanism.

Antioxidant compounds, particularly those containing hydroxyl (–OH) groups, form strong hydrogen bonds that require significant energy to break (Luthfiyanti et al., 2020). MAE facilitates this process by utilizing high-energy microwaves, which rapidly and uniformly heat both the solvent and plant material. This enhances the rupture of cell walls, promoting the diffusion and dissolution of bioactive compounds into the solvent.

Microwave-Assisted Extraction is known for its efficiency, offering shorter extraction times, reduced solvent consumption, and greater selectivity. Microwave energy interacts directly with polar molecules in the plant matrix, accelerating the extraction process and improving compound recovery and product quality (Lopez-Salazar et al., 2023). In contrast, maceration involves passive soaking at room temperature without the application of heat, leading to slower extraction kinetics and lower antioxidant activity than the MAE method. Based on the results, there is a significant effect of the extraction method on the IC_{50} value. The MAE extraction method is higher than the maceration method on the IC_{50} value.

CONCLUSION

The *O. sanctum leaf* extracts obtained through both maceration and Microwave-Assisted Extraction (MAE) methods demonstrated antioxidant activity, with IC_{50} values of 111.843 ppm and 75.725 ppm, respectively. The extraction method that yielded the highest antioxidant activity was MAE, followed by maceration.

Conflict of Interest

The authors declare no conflict of interest.

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