Optimization of Ethanol Concentration in the Extraction of Basil Leaves (*Ocimum sanctum L.*) on Total Phenolic Content, Total Flavonoid Content, and α-Amylase Enzyme Inhibitory Activity

Danang Raharjo[1], Inanda Yuliana[1], Vivin Marwiyati Rohmana[1]
[1]Department of Pharmacy, Faculty of Health Sciences, Universitas Duta Bangsa, Surakarta, Indonesia
Correspondence**: Danang Raharjo:** Jl. K.H. Samanhudi No. 93, Sondakan, Kecamatan Laweyan, Surakarta; danang_raharjo@udb.ac.id

ABSTRACT
Type 2 diabetes mellitus remains a global health challenge that requires effective strategies to control blood glucose levels, including the inhibition of the alpha-amylase enzyme to reduce postprandial hyperglycemia. This study aimed to evaluate the influence of different ethanol concentrations; fifty percent, seventy percent, and ninety-six percent on the extraction efficiency of bioactive compounds from basil leaves (*Ocimum sanctum* L.) and on the inhibitory activity of the alpha-amylase enzyme. Basil leaves were extracted using the maceration method, and the resulting extracts were analyzed for total phenolic content using the Folin–Ciocalteu method, total flavonoid content using the aluminum chloride reagent, and alpha-amylase inhibitory activity using the iodine method. The results demonstrated that ethanol concentration significantly affected all measured parameters. The extract obtained with seventy percent ethanol produced the highest total phenolic content (108.441 ± 0.495 milligrams gallic acid equivalent per gram), the highest total flavonoid content (70.535 ± 0.393 milligrams quercetin equivalent per gram), and the strongest alpha-amylase inhibitory activity with an inhibitory concentration value of 49.255 micrograms per milliliter. A strong positive correlation was observed between the levels of phenolic and flavonoid compounds and the magnitude of enzyme inhibition. These findings indicate that seventy percent ethanol is the optimal solvent for extracting antidiabetic constituents from basil leaves, supporting the potential development of basil leaf extract as an adjuvant therapeutic agent for the management of type 2 diabetes mellitus.
Keywords: *Ocimum sanctum* L.; ethanol; phenolic; flavonoid; alpha-amylase inhibition; antidiabetic potential

INTRODUCTION
Diabetes mellitus is a major non-communicable disease and continues to pose a significant global health challenge due to its steadily increasing prevalence worldwide. The International Diabetes Federation estimates that the number of individuals living with diabetes will reach 783 million by the year 2045 if effective prevention and control strategies are not implemented [1]. Type 2 diabetes mellitus represents the most prevalent form of the disease, accounting for more than ninety percent of all global cases. This condition is characterized by chronic hyperglycemia resulting from insulin resistance and/or impaired pancreatic beta-cell function. Persistent hyperglycemia contributes to the development of serious long-term complications, including retinopathy, nephropathy, neuropathy, and cardiovascular diseases, all of which substantially increase morbidity and mortality among affected individuals [2].

One of the essential strategies in the management of type 2 diabetes mellitus is the control of postprandial hyperglycemia. Elevated blood glucose levels following food intake are known to trigger oxidative stress, chronic inflammation, and vascular injury, thereby accelerating the progression of diabetic complications [3]. Postprandial glucose control can be achieved through the inhibition of the alpha-amylase enzyme, which plays a central role in the initial stage of carbohydrate digestion. Alpha-amylase hydrolyzes starch into oligosaccharides, which are subsequently converted into glucose by the alpha-glucosidase enzyme. Therefore, inhibiting alpha-amylase activity can slow the release and absorption of glucose, helping to attenuate the rise in blood glucose levels after meals [4]. Acarbose is one of the most widely used alpha-amylase inhibitors. Although effective in reducing postprandial hyperglycemia, long-term use of acarbose is frequently associated with gastrointestinal side effects such as flatulence, diarrhea, and abdominal discomfort [5]. These limitations have increased scientific interest in identifying natural alpha-amylase inhibitors derived from medicinal plants. Medicinal plants contain diverse secondary metabolites that may offer therapeutic benefits with potentially fewer adverse effects compared to synthetic drugs.

Basil leaves (*Ocimum sanctum* L.) represent one of the promising medicinal plants that may serve as a source of antidiabetic compounds. This plant has long been used in traditional medicine for various health purposes, including the regulation of blood glucose levels [6]. Basil leaves contain a wide range of secondary metabolites such as phenolics, flavonoids, tannins, alkaloids, and terpenoids, which exhibit multiple biological activities including antioxidant, anti-inflammatory, and antidiabetic effects [7]. Several studies have demonstrated that phenolic and flavonoid compounds play a crucial role in antidiabetic activity through their ability to inhibit carbohydrate-digesting enzymes, enhance antioxidant capacity, and protect cells from oxidative stress-induced damage [8].

The successful isolation of bioactive compounds from natural materials is strongly influenced by the extraction method and the type of solvent used. Ethanol is one of the most widely applied solvents in natural product extraction due to its safety, accessibility, and ability to extract both polar and semi-polar compounds [9]. However, extraction efficiency is highly dependent on ethanol concentration, as variations in the ethanol-water ratio alter the polarity of the solvent. Different ethanol concentrations can produce extracts with distinct profiles of bioactive compounds, which in turn influence the biological activity of the resulting extract [10]. Previous studies have reported that seventy percent ethanol often yields superior extraction results for various medicinal plants because its intermediate polarity facilitates optimal extraction of phenolic and flavonoid compounds [11]. Nevertheless, information regarding the effect of ethanol concentration on total phenolic content, total flavonoid content, and alpha-amylase inhibitory activity of basil leaf extract remains limited. Most prior studies have focused on antioxidant activity or phytochemical characterization without examining the relationship between secondary metabolite content and alpha-amylase inhibition as a key antidiabetic mechanism.

Based on these considerations, the present study was conducted to analyze the effect of different ethanol concentrations; fifty percent, seventy percent, and ninety-six percent on total phenolic content, total flavonoid content, and alpha-amylase inhibitory activity of basil leaf (*Ocimum sanctum* L.) extract. This study is expected to provide scientific insight into optimal extraction conditions for obtaining basil leaf extract with the strongest antidiabetic activity, thereby strengthening the scientific foundation for developing basil leaves as a potential phytopharmaceutical candidate in the management of type 2 diabetes mellitus.

METHODS

This study was designed as a laboratory-based experimental investigation aimed at evaluating the influence of different ethanol concentrations; fifty percent, seventy percent, and ninety-six percent on the total phenolic content, total flavonoid content, and alpha-amylase enzyme inhibitory activity of basil leaf (*Ocimum sanctum* L.) extract. The research workflow consisted of several sequential stages, including sample preparation, extraction using the maceration technique, phytochemical screening, quantitative determination of total phenolic content, quantitative determination of total flavonoid content, and in vitro assessment of alpha-amylase inhibitory activity.

Fresh basil leaves were collected from local farmers in Potronayan Village, Nogosari District, Boyolali Regency (geographical coordinates: −7.489969, 110.748291). Botanical authentication was performed at the Traditional Health Service Unit, Dr. Sardjito General Hospital, Tawangmangu, under registration number TL.02.04/D.XI.6/12452.445/2025. All analytical procedures, including the determination of total phenolic content, total flavonoid content, and alpha-amylase inhibitory activity, were conducted at the Natural Products Laboratory, Faculty of Health Sciences, Universitas Duta Bangsa Surakarta.

The materials used in this study included basil leaf powder, technical grade ninety-six percent ethanol, absolute ethanol (Merck®), distilled water, gallic acid standard (Sigma-Aldrich®), quercetin standard (Sigma-Aldrich®), Folin–Ciocalteu reagent (Sigma-Aldrich®), anhydrous sodium carbonate (Sigma-Aldrich®), aluminum chloride (Sigma-Aldrich®), potassium persulfate (Sigma-Aldrich®), sodium acetate (Sigma-Aldrich®), porcine pancreas alpha-amylase enzyme (Sigma-Aldrich®), soluble starch (solani, Sigma-Aldrich®), iodine solution (Sigma-Aldrich®), and acarbose standard (Sigma-Aldrich®).

Extraction was carried out using the maceration method. A total of one hundred grams of basil leaf simplicia powder was macerated in one thousand milliliters of solvent (ratio 1:10) with ethanol concentrations corresponding to the experimental treatments (fifty percent, seventy percent, and ninety-six percent). Maceration was performed for three cycles of twenty-four hours each. At the end of every twenty-four-hour cycle, the solvent was filtered and replaced with fresh solvent. Filtrates from all three cycles were combined, filtered, and subsequently concentrated using a rotary evaporator at forty degrees Celsius until a viscous extract was obtained.

Phytochemical screening of the basil leaf extract and its fractions was conducted following established standard procedures to detect the presence of alkaloids, saponins, tannins, phenolics, flavonoids, and terpenoids/steroids [12]. The detailed procedures were as follows:

1) Alkaloids: The sample was dissolved in chloroform and tested using three different reagents. A positive result was indicated by the formation of a red precipitate (Dragendorff), a white precipitate (Mayer), or a yellow precipitate (Wagner).
2) Saponins: The sample solution was vigorously shaken with water. The formation of stable foam after thirty seconds, which persisted upon the addition of two-normal hydrochloric acid, indicated a positive result.
3) Tannins: The addition of ten percent lead acetate to the sample solution produced a white precipitate, indicating a positive reaction.
4) Phenolics: The addition of one percent ferric chloride solution resulted in a color change to dark green or black, indicating the presence of phenolic compounds.
5) Flavonoids: The sample dissolved in ethanol was reacted with magnesium powder and concentrated sulfuric acid. A positive reaction was indicated by the appearance of red, yellow, or orange coloration.
6) Steroids/Terpenoids: The sample was reacted with acetic anhydride and concentrated sulfuric acid. A brown or purple ring indicated the presence of triterpenoids, whereas a bluish-green coloration indicated steroids.

Quantitative analysis of total phenolic content was performed using a modified Folin–Ciocalteu procedure [13]. In this method, 0.5 milliliters of extract (1000 micrograms per milliliter) was mixed with 2.5 milliliters of Folin–Ciocalteu reagent (diluted ten-fold) and homogenized. After five minutes, 2 milliliters of 7.5 percent sodium carbonate solution was added. The mixture was incubated in the dark for twenty-seven minutes, after which its absorbance was measured at 735 nanometers using a UV-Visible spectrophotometer. Total phenolic content was calculated by comparing the absorbance values with a gallic acid calibration curve (10–50 micrograms per milliliter) and expressed as milligrams gallic acid equivalent per gram of extract.

Quantitative determination of total flavonoid content was conducted using the aluminum chloride colorimetric method adapted from previous literature [14]. One milliliter of sample solution (1000 micrograms per milliliter) was mixed with one milliliter of ten percent aluminum chloride reagent and one milliliter of one-molar sodium acetate solution. The reaction mixture was incubated at room temperature for fifteen minutes to allow the formation of a stable flavonoid–aluminum complex. The resulting yellow coloration was measured at 434 nanometers using a UV-Visible spectrophotometer. A quercetin calibration curve (10–50 micrograms per milliliter) was used to quantify total flavonoid content, which was expressed as milligrams quercetin equivalent per gram of dried extract.

The inhibitory activity of the alpha-amylase enzyme was assessed using a colorimetric iodine method [15]. A total of 500 microliters of alpha-amylase solution (0.5 units per milliliter) was mixed with 500 microliters of sample solution (extract at concentrations of 12.5–200 micrograms per milliliter or acarbose at 0.625–10 micrograms per milliliter) and incubated at 37 degrees Celsius for ten minutes. Subsequently, 500 microliters of one-percent starch solution in pH 6.9 phosphate buffer, preheated beforehand, was added as the substrate and incubated again at the same temperature for exactly thirty minutes. The reaction was terminated by adding 125 microliters of iodine solution and 20 microliters of one-normal hydrochloric acid. Absorbance was immediately measured at 580 nanometers. A blank inhibitor (without enzyme) and an enzyme control (without inhibitor) were prepared using the same procedure. The percentage inhibition was calculated by comparing sample absorbance with the control, and the inhibitory concentration value was determined from a regression curve plotting percentage inhibition against sample concentration.

RESULTS

Phytochemical screening results

Phytochemical screening served as an initial analytical step to detect the presence of major groups of secondary metabolites in the basil leaf extract. The procedure employed classical test-tube reactions using specific reagents that produce characteristic color

Table 1. Phytochemical screening results of basil leaf extracts at different ethanol concentrations

Compound class	Ethanol concentration (%)			Description
	50	70	96	
Flavonoids	+	+	+	Orange-yellow coloration
Saponins	+	+	+	Stable foam formation
Tannins	+	+	+	Dark green coloration
Steroids	+	+	+	Green coloration
Alkaloids				
-Mayer's reagent	+	+	+	White precipitate
-Wagner's reagent	+	+	+	Brown precipitate
-Dragendorff's reagent	+	+	+	Brick-red precipitate

changes or precipitates as indicators of compound classes. The results demonstrated that basil leaf extracts obtained using all ethanol concentrations; fifty percent, seventy percent, and ninety-six percent contained five major groups of bioactive compounds: flavonoids, saponins, tannins, steroids, and alkaloids. The consistency of these results across all solvent concentrations indicates that these metabolite groups were efficiently extracted by ethanol regardless of its polarity level. The complete phytochemical screening results are presented in Table 1.

The determination of total phenolic content was conducted using the Folin–Ciocalteu colorimetric method. The principle of this assay is based on the reduction of phosphomolybdic–phosphotungstic acid complexes in the Folin–Ciocalteu reagent by phenolic compounds under alkaline conditions, forming a blue molybdenum complex whose intensity is proportional to the phenolic concentration. Absorbance was measured at 735 nanometers [16]. Gallic acid was used as the calibration standard due to its stability, purity, and linear response to the Folin–Ciocalteu reagent, enabling accurate construction of the calibration curve [17].

Total phenolic content analysis

The results revealed significant variation in total phenolic content among extracts produced using different ethanol concentrations. The extract obtained with seventy percent ethanol exhibited the highest phenolic content (108.441 ± 0.495 milligrams gallic acid equivalent per gram), markedly higher than the other two concentrations. The ninety-six percent ethanol extract ranked second (102.338 ± 0.555 milligrams gallic acid equivalent per gram), while the fifty percent ethanol extract yielded the lowest phenolic content (65.569 ± 0.407 milligrams gallic acid equivalent per gram). These findings indicate that seventy percent ethanol provides the optimal polarity for extracting phenolic compounds from basil leaves (Table 2).

Table 2. Total phenolic content of basil leaf extracts at different ethanol concentrations

Sample	Total phenolic content (mg GAE/g)
Ethanol 50%	65.569 ± 0.407
Ethanol 70%	108.441 ± 0.495
Ethanol 96%	102.338 ± 0.555

Total flavonoid content analysis

Total flavonoid content was determined using the aluminum chloride colorimetric method, in which aluminum ions form a yellow complex with the C-4 keto group and the C-3 or C-5 hydroxyl groups of flavonoids. The intensity of the resulting yellow coloration is directly proportional to the flavonoid concentration and is measured at 510 nanometers [18]. A quercetin calibration curve was used to quantify flavonoid levels.

The extract obtained with seventy percent ethanol produced the highest total flavonoid content (70.535 ± 0.393 milligrams quercetin equivalent per gram). The ninety-six percent ethanol extract ranked second (62.233 ± 0.425 milligrams quercetin equivalent per gram), while the fifty percent ethanol extract yielded the lowest flavonoid content (36.541 ± 0.381 milligrams quercetin equivalent per gram). The results form a parabolic pattern, with seventy percent ethanol representing the optimal extraction point. These findings confirm that solvent polarity is a critical factor in flavonoid extraction (Table 3).

Table 3. Total flavonoid content of basil leaf extracts at different ethanol concentrations

Sample	Total flavonoid content (mg QE/g)
Ethanol 50%	36.541 ± 0.381
Ethanol 70%	70.535 ± 0.393
Ethanol 96%	62.233 ± 0.425

Alpha-amylase inhibitory activity

The antidiabetic potential of basil leaf extract was evaluated through an in vitro alpha-amylase inhibition assay. Alpha-amylase plays a central role in hydrolyzing starch into oligosaccharides, which are subsequently converted into glucose. Therefore, inhibiting this enzyme can slow carbohydrate digestion and help regulate postprandial blood glucose levels [19]. The assay was based on the ability of the extract or acarbose to inhibit alpha-amylase activity. The iodine colorimetric method was used, in which iodine forms a blue-black complex with unhydrolyzed starch. Active alpha-amylase reduces the amount of starch available to bind iodine, resulting in a lighter color. Conversely, stronger inhibition leads to more intense blue coloration due to higher starch retention [20].

All basil leaf extracts and acarbose exhibited concentration-dependent inhibitory activity. The seventy percent ethanol extract demonstrated the strongest inhibition, with an inhibitory concentration value of 49.255 micrograms per milliliter, significantly more potent than the ninety-six percent ethanol extract (64.727 micrograms per milliliter) and the fifty percent ethanol extract (137.881 micrograms per milliliter). At the highest test concentration, the seventy percent ethanol extract achieved an inhibition percentage of 73.276%, approaching that of acarbose (64.943%). These results align closely with the phenolic and flavonoid content analyses, indicating that these compounds likely contribute to alpha-amylase inhibition (Table 4).

Table 4. Alpha-amylase inhibition of basil leaf extracts at different ethanol concentrations

Sample	% Inhibition	IC_{50} (µg/mL)
Acarbose	43.774 48.755 52.969 58.812 64.943	2.023
Ethanol 50%	14.368 20.402 33.525 46.743 61.494	137.881
Ethanol 70%	41.475 45.019 52.203 61.494 73.276	49.255
Ethanol 96%	33.046 40.326 50.096 61.303 80.651	64.727

DISCUSSION

Type 2 diabetes mellitus is a complex metabolic disorder characterized by impaired glucose homeostasis resulting from a combination of insulin resistance and pancreatic beta-cell dysfunction. One of the recommended therapeutic approaches for controlling postprandial hyperglycemia involves the inhibition of carbohydrate-digesting enzymes, particularly alpha-amylase and alpha-glucosidase. Alpha-amylase plays a central role in the initial hydrolysis of polysaccharides into oligosaccharides, which are subsequently converted into glucose by alpha-glucosidase in the small intestine. Therefore, inhibition of alpha-amylase can slow the rate of glucose release from dietary carbohydrates, thereby reducing postprandial glucose spikes that are known to contribute to oxidative stress, chronic inflammation, and the progression of both microvascular and macrovascular complications in individuals with diabetes mellitus [21,22].

The findings demonstrate that ethanol concentration significantly influences the efficiency of extracting bioactive compounds from basil leaves. The extract obtained using seventy percent ethanol exhibited the highest total phenolic content (108.441 ± 0.495 mg GAE/g), as determined using

the Folin–Ciocalteu method, which is based on the reduction of phosphomolybdate–phosphotungstate complexes by phenolic groups to form a blue molybdenum complex detectable at 765 nm. Similarly, the highest total flavonoid content (70.535 ± 0.393 mg QE/g) was obtained using the aluminum chloride colorimetric method, in which aluminum ions coordinate with the C-4 keto group and the C-3 or C-5 hydroxyl groups of the flavonoid ring to form a yellow flavonoid–aluminum complex measurable at 510 nm. The alpha-amylase inhibitory activity also followed a consistent pattern with the bioactive compound content. The seventy percent ethanol extract exhibited the strongest inhibitory activity, with an IC_{50} value of 49.255 µg/mL, followed by the ninety-six percent ethanol extract (IC_{50} = 64.727 µg/mL) and the fifty percent ethanol extract (IC_{50} = 137.881 µg/mL).

The selection of ethanol as the extraction solvent is supported by its well-established ability to extract phenolic and flavonoid compounds and its status as a generally recognized as safe (GRAS) solvent. Solvent polarity is a critical determinant of extraction efficiency. Fifty percent ethanol, being highly polar, effectively extracts polar compounds such as phenolic acids and flavonoid glycosides through hydrogen bonding interactions. In contrast, ninety-six percent ethanol, which is relatively non-polar, is more effective for extracting semi-polar compounds such as flavonoid aglycones (e.g., apigenin, luteolin) and phenylpropanoid derivatives (e.g., eugenol, methyl eugenol) through hydrophobic interactions. Seventy percent ethanol demonstrates optimal extraction efficiency due to its unique combination of polarity and tissue penetration capability: the water component facilitates swelling of the cellular matrix, enhancing solvent diffusion, while ethanol dissolves both polar and semi-polar target compounds [23].

The results of this study are consistent with previous research reporting that ethanol extracts of basil contain high levels of phenolics, flavonoids, and condensed tannins, and exhibit alpha-amylase inhibitory activity comparable to acarbose as a positive control [24]. Other studies have also shown that seventy percent ethanol is the optimal concentration for extracting phenolic compounds from natural materials, yielding higher total phenolic content than lower or higher ethanol concentrations [11]. Additionally, comparative studies on solvent effects in extracting bioactive compounds from plants of the Asteraceae family have demonstrated that seventy percent ethanol consistently produces extracts with higher phenolic and flavonoid content than other solvents. The differences in extraction efficiency observed in this study are likely related to the polarity characteristics of both the solvent and the extracted compounds. Based on the principle of *like dissolves like*, phenolic and flavonoid compounds in basil leaves exhibit varying degrees of polarity, making extraction efficiency highly dependent on solvent polarity compatibility [9]. Seventy percent ethanol likely provides the most optimal conditions because it can dissolve both polar and semi-polar compounds more effectively than either fifty percent or ninety-six percent ethanol. Furthermore, the presence of water in the seventy percent ethanol mixture enhances cell wall swelling, facilitating the release of bioactive compounds from the plant matrix [10].

The positive correlation observed between increasing phenolic and flavonoid content and increasing alpha-amylase inhibitory activity indicates that these compound classes contribute significantly to the enzyme inhibition mechanism. Phenolic compounds are believed to inhibit alpha-amylase through hydrogen bonding and hydrophobic interactions with amino acid residues at the enzyme's active site, thereby blocking substrate access. Flavonoids inhibit alpha-amylase through similar mechanisms, as their multiple hydroxyl groups can bind to catalytic regions of the enzyme, altering its conformation and reducing its affinity for the substrate [25]. This interpretation is supported by *in silico* studies showing that flavonoid compounds can interact strongly with amino acid residues at the alpha-amylase active site through hydrogen bonding and π–π stacking interactions, potentially reducing the enzyme's catalytic activity [26]. Additionally, the inhibition mechanism may involve chelation of calcium ions (Ca^{2+}), which are essential for enzyme activity, as well as hydrogen donation that disrupts catalytic processes [27]. These findings suggest that the alpha-amylase inhibitory activity of basil leaf extract is likely the result of synergistic interactions among multiple phenolic and flavonoid compounds, producing a stronger biological effect than would be expected from any single compound alone.

CONCLUSION

The findings of this study demonstrate that varying ethanol concentrations markedly influence the extraction efficiency of bioactive compounds from basil leaves (*Ocimum sanctum* L.), as reflected in differences in total phenolic content, total flavonoid content, and alpha-amylase inhibitory activity. Among the tested solvents, seventy percent ethanol provided the most favorable extraction conditions, yielding extracts with substantially higher levels of phenolic and flavonoid constituents and exhibiting the strongest alpha-amylase inhibition. These results indicate that phenolic and flavonoid compounds play a meaningful role in the antidiabetic potential of basil leaf extract through enzyme-inhibition mechanisms associated with carbohydrate digestion. Accordingly, basil leaf extract obtained using seventy percent ethanol shows promising potential for development as a herbal raw material or supportive therapeutic agent in the management of type 2 diabetes mellitus. Nevertheless, further research is required to identify the dominant active constituents and to validate both efficacy and safety through comprehensive *in vivo* evaluation.

Ethical consideration, competing interest and source of funding

-This study involved only plant materials and laboratory reagents, without human participants or personal data. Although formal ethical approval was not required, all procedures followed responsible research practices, proper sample collection, accurate botanical identification, and standard laboratory safety guidelines.
-There is no conflict of interest related to this research.
-Source of funding is authors.

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