JHSS (Journal of Humanities and Social Studies)
https://jhss-lppmunpak.id
Volume 10, Number 01, Page 247-250
e-ISSN: 2598-120X; p-ISSN: 2598-117X

EFFECT OF 70% ETHANOL AND 70% METHANOL SOLVENTS ON TOTAL PHENOL CONTENT OF BEETROOT EXTRACTS

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Article history: received 11 January 2026; revised 22 January 2026; accepted 10 February 2026 *DOI: https://doi.org/10.33751/jhss.v10i1.62*

Abstract: Antioxidants are known to be present in beetroot tubers. Antioxidant activity found in plants comes from the amount of phenolic chemicals present, including phenolic acids and polyphenols. To identify the existence of phenolic compounds in plants, the first step that needs to be done is extraction. Extraction is one of the factors that affect the content of bioactive compounds in the extract, because it can directly affect the process of taking phytochemical compounds from plants. The use of solvents during extraction must be in accordance with their polarity. This study's goal is to determine the impact of using 70% ethanol and 70% methanol solvents on beet tuber extracts' overall phenol content. The method used was using Folin-Ciocalteu reagent and measured by UV-Vis spectrophotometry. The outcomes of the study revealed that the overall amount of phenol content with 70% ethanol solvent had higher results compared to 70% methanol solvent. Since the results of the ANOVA test differ just slightly, P≥0.05 indicates that there is no significant difference.

Keywords: beetroot tubers, solvents, ethanol, methanol, and total phenol

I. INTRODUCTION

For traditional medicinal uses, some people still use plants, particularly in rural areas where species variety is great. Traditional plant-based medicine's affordability, accessibility, and lack of adverse effects as compared to contemporary pharmaceutical therapies are some of its primary benefits. One plant that has many medicinal properties is the beetroot [1]

Beet tubers serve as a source of antioxidants. Antioxidant compounds have a role in fighting or neutralizing free radicals, thus reducing the risk of degenerative diseases such as cardiovascular disorders and cancer [2] In general, antioxidant activity found in plants comes from the information contained in phenolic compounds, both in the form of polyphenols and phenolic acids. Previous research shows that phenolic compounds have the ability to increase the immune system against free radicals through antioxidant activity [3]. To identify Phenolic compounds' presence in plants, the first step that needs to be done is the extraction process.

Since extraction has a direct impact on the process of obtaining phytochemical substances derived from plants, it is one of the elements that affects the amount of bioactive components in extracts. The extraction process is the separation of mixed materials using a suitable solvent. The choice of solvent for extracting compounds must be based on the level of polarity. The level of polarity of a solvent is determined by its dielectric constant, the higher the dielectric constant value, the more polar the solvent [4]

According to research conducted by [5], methanol solvent is more effective in extracting phenol from gadung tubers because it has a higher phenol content compared to extracts using water and ethanol solvents. From these findings, it can be concluded that the type of solvent used can affect the extraction process and the collection of chemical compounds contained in plants. The author has an interest in examining the effect of using 70% ethanol and 70% methanol solvents on the total phenol content in beet root extracts.

II. RESEARCH METHODS

Equipment and Materials: The instruments utilized are test

Tubes (Iwaki®), stirring rod (Pyrex®), glass funnel (Pyrex®), beaker glass (Pyrex®), volumetric flask (Pyrex®), measuring flask (Iwaki®), erlenmeyer flask (Pyrex®), 1 liter reagent bottle, porcelain cup, dropper (OneMed®), micropipette (Eppendorf Research®), analytical balance scales (OHAUS®), oven (Memmert©), rotary evaporator (IKA® RV10 Digital), UV-Vis spectrophotometry (Thermo Scientific©), shaker (DLAB SK-L330-Pro), blender (Panasonic©), moisture analyzer (AND® MX-50), spatula, 100 mesh sieve, aluminum foil (Klin Pak®), filter paper (Whatman paper number 1.), plastic wrap (Klin Pak®), and label paper. The materials used are beet tubers, 70% ethanol, 70% methanol, gallic acid, Folin-Ciocalteu and Na2CO3.

Methods

Sample Preparation

The beet tubers used were obtained from Singosari, Malang Regency. The skin of the beetroot tubers was peeled off and the flesh was taken. The flesh of the beetroot tubers was then dried in the sun, after which it was baked for nine hours at 50˚C. After the oven process, the dried beetroot flesh was pulverized using a blender until it reached a smooth texture and then filtered with a sieve until a simplisia powder was obtained.

Water Content Testing

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e-ISSN: 2598-120X; p-ISSN: 2598-117X

Measurement of simplisia water content is carried out by weighing 1 g of sample. The sample is then analyzed using a moisture analyzer for 10 minutes. It is recommended that simplisia's moisture content ought to be less than 10% [6]

Extract Preparation

Put 100 g of beet simplisia into glass jars 1 and 2. Add 1 L of 70% ethanol solvent to glass jar 1 and 1 L of 70% methanol solvent to glass jar 2. Next, cover the glass jar tightly with aluminium foil and shaker for 2 hours at 130 rpm for 3x24 hours. Store the glass jar in a closed place protected from sunlight. To obtain a thick beet extract, filter the beet sample and use a rotary evaporator to extract the filtrate for 2 hours at 55-65˚C.

Calculation of Extraction Yield:

The bit extraction result is calculated by the formula.

$$\%Yield = \frac{Extract\ of\ weight\ (gram)}{Simplisia\ of\ weight\ (gram)} \text{ X } 100$$

Determination of Total Phenol Content:

1. Preparation of Gallic Acid Master Solution

   Ten milligrams of gallic acid were dissolved in absolute ethanol to make a stock solution with a 1000 ppm concentration.
2. Finding the Maximum Wavelength

   A volumetric flask was filled with 0.1 mL of gallic acid mother solution at a concentration of 100 ppm. One milliliter of Folin-Ciocalteu reagent was then added. One milliliter of Na2CO3 was added to the solution after it had been Five minutes were spent incubating. Following a vortex to homogenize the mixture, it was left to stand at room temperature in a dark place for 90 minutes. Measurements of absorbance were made between 600 and 850 nm in wavelength.
3. Making a Standard Curve for Gallic Acid

   By diluting a 1000 ppm gallic acid mother solution, gallic acid Standard solutions containing 20, 40, 60, 80, and 100 parts per million were created. One milliliter of Folin-Ciocalteu reagent was then added, and after five minutes of incubation, one milliliter of Na2CO3 was added, and everything was vortexed until it was homogenous. Using UV-Vis spectrophotometry, it was possible to identify the absorbance at a predefined wavelength after For ninety minutes, the mixture was incubated in a dark environment. A calibration curve illustrating the correlation between absorbance and gallic acid content was then created.
4. Determining the extract of solution

   To create a mother solution with a 1000 ppm concentration, 10 mg of beet tuber extract was weighed, diluted in 10 mL of 70% ethanol and 70% methanol solvents, and then placed in a volumetric flask. 0.1 mL of the 1000 ppm mother liquor was extracted, followed by the addition of 1 mL of Folin-Ciocalteu reagent and a 5-minute incubation period. Next, 1 mL of Na2CO3 was added. After homogenizing the solution using a vortex, it was incubated for ninety minutes in a dark room. Using UV-Vis spectrophotometry, three measurements of the extract solution's absorbance at the maximum wavelength were made.

Data Analysis

Total phenolics were analyzed quantitatively with y values entered into the gallic acid standard curve equation, where x is the milligram equivalent of gallic acid per gram of extract (GAE). Data analysis was performed using ANOVA to determine if there were significant differences between the different solvents.

III. RESULT AND DISCUSSION

Water Content

The results of the water content test showed a value of 9.09% which met the standard quality of simplisia.

Beetroot Extract Yield

Calculation of yield results is carried out to determine the ratio between the weight of raw materials and the extract material produced can be seen in Table 1.

Table 1. Yield results of beet root extract

Solvents (%)	Simplisia Weight (g)	Extract Weight (g)	Yield (%)
Ethanol 70	100	21,042	21,042
Methanol 70	100	47,391	47,391

Total Phenol Content

The calibration curve for gallic acid with concentration changes of 20, 40, 60, 80, and 100 ppm is shown in Figure 1.

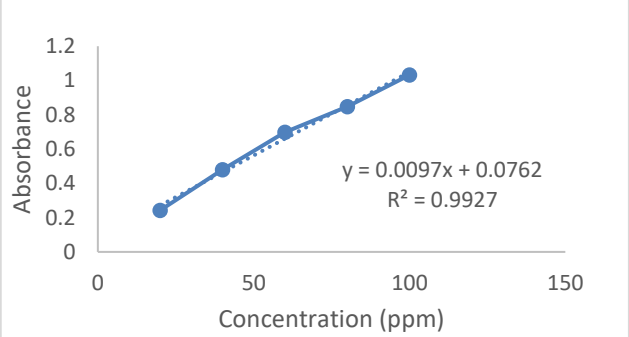

Figure 1. Adjustment the Curve of Gallic Acid

The findings of three repeats of a UV-Vis spectrophotometric measurement of the total phenol content of 70% ethanol and 70% methanol extracts of beetroot tubers at a concentration of 1000 ppm are displayed in Table 2.

Table 2. Measurement results of total phenol content of 70% ethanol and 70% methanol extracts of beet tubers

Solvent	Absorbance			Average	KTF (mgGAE/gram)
	U1	U2	U3		
Ethanol 70%	0,206	0,188	-0,043	0,117	8,83
Methanol 70%	0,178	-0,05	0,21	0,113	7,89

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Figure 2 uses the ANOVA test to analyze data and displays the graph of the differences in total phenolics of beet tubers between 70% ethanol and 70% methanol solvents.

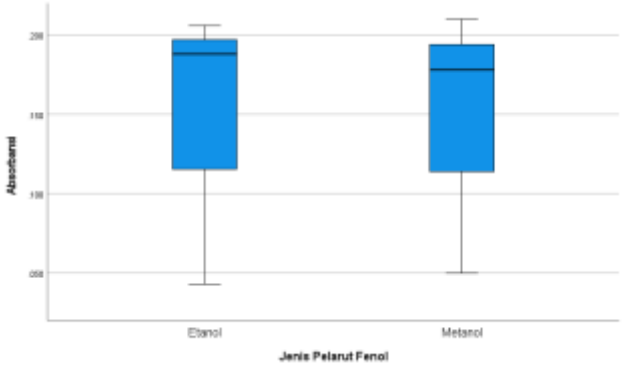

Figure 2. Graph of Solvent Type Differences in Total Phenolics

The study's findings indicate that beetroot simplisia has a moisture content of 9.09%, which is within the acceptable range for simplisia. Based on the Indonesian Ministry of Health (2017), the standard standard for simplisia is a water content of no more than 10%. Finding the lowest threshold for water content in simplisia is the aim of the water content test. The more water there is in a material, the easier it is for the material to become a medium for the growth of fungi and molds that can reduce the biological activity of simplisia during storage [7]

The maceration method was employed for the extraction process in this investigation. One benefit of the maceration extraction method is its simplicity and lack of heating requirements. It is possible to use this method on non-heat-resistant materials [8]. After soaking the beetroot, a liquid extract is produced, which is then concentrated by employing a rotary evaporator to evaporate each solvent. The process of evaporation can yield a viscous extract. According to Table 1, the yields for methanol extraction of beet fruits were 47.391% and ethanol extraction of beet tubers were 21.042%. The minimum yield limit for raw materials is set at >10%, so 70% ethanol and 70% methanol extracts of beet tubers can be considered to meet these criteria. In this case, yield and the number of active compounds in the raw material are significantly correlated; as yield rises, so does the amount of active compounds in the sample [9]. Different types of solvents can affect the amount of extract produced. Methanol solvents produce higher yields than ethanol solvents. This shows that the compounds in the beet extract are more compatible with methanol, because the compound extraction process is highly dependent on the level of polarity compatibility between the extracted compounds and the solvent used.

Total phenol testing in beetroot extract was carried out to measure the amount of phenol in it using the Folin-Ciocalteu method and gallic acid as a comparison. Gallic acid absorbance was tested with concentration variations of 20, 40, 60, 80, and 100 ppm in order to assess the calibration curve's linearity. After measuring the absorbance at each concentration, a linear regression equation was created, which would be utilized to ascertain the beetroot's overall phenol content. The results of the gallic acid calibration curve can be seen in Figure 1. The R^2 value obtained a result of 0.9927. If the linearity or R value is close to one or equal to one, the equation is getting better and positively correlated or linear (Winata et al., 2023).

In Table 2. The outcomes indicate revealed the 70% methanol extract of beet tubers had a total phenol concentration of 7.89 GAE/g, and The ethanol extract of beet tubers has a total phenol concentration of 8.83 mg GAE/g. The findings demonstrated that phenol chemicals may be extracted from beet tubers more successfully using a 70% ethanol solvent. The solubility level of ethanol solvent shows compatibility with phenol compounds contained in beetroot tubers, resulting in extracts with high phenol content. This result is consistent with previous studies [11]It claims that the amount of polyphenols in cocoa bean extract indicates how well the polarity level with ethanol solvents works. Ethanol is therefore a more effective solvent than methanol or acetone for the extraction of polyphenolic chemicals.

Figure 2. Shows the graph of the difference between the types of solvents 70% ethanol and 70% methanol using data analysis with ANOVA test. $P \geq 0.05$, the result of the ANOVA test, indicates that the total phenol content of beet tubers is not significantly affected by the use of 70% ethanol and 70% methanol solvents. Although ethanol solvent is slightly superior compared to methanol solvent, the difference is not too significant because the results are almost identical. It can be seen that both ethanol and methanol solvents are equally effective in dissolving phenol, based on the absorbance values obtained. This is consistent with studies carried out by [12]which claims that there is no discernible effect of the solvent type's interaction with vegetables ($P \geq 0.05$) on the amount of phenol produced. In addition, [13]mentioned that the difference in value between ethanol and methanol is not significant, this is due to the similar nature of both as universal polar solvents.

The use of solvents with different polarity levels also affects the number and type of compounds that can be extracted[14]. The choice of solvent type must consider various factors such as selectivity, efficiency in extracting target compounds, toxicity level, ease of evaporation, and economic aspects such as solvent prices [13]. Due to its capacity to break down cell walls, ethanol can dissolve phenolic compounds, allowing bioactive substances to be released from plant cells more readily. The hydroxyl group in ethanol can also interact with the hydrogen groups in phenolic compounds, increasing the solubility of phenolic compounds in solvents [15].

IV. CONCLUSION

It is possible to draw the conclusion from the research that the test of total phenol content of beetroot extract with 70% ethanol and 70% methanol solvents has no significant difference between the two solvents because it has almost identical results.

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