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Analysis of Phytochemical Content and Antioxidant Activity in *Phyllanthus Niruri* Linn. by Different Methods

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ABSTRACT

Phyllanthus niruri Linn. (or in Indonesian called meniran) is an herbal plant with high antioxidant activity so it possesses many health benefits. Antioxidant activity in *Phyllanthus niruri* Linn. is caused by saponins, phenolics, flavonoids, and tannins. This study aimed to analyze the effect of the brewing methods on the phytochemical content and antioxidant activity of *Phyllanthus niruri* Linn. The examined brewing methods were the Decoction Brew (DB), Cold Brew (CB), and True Brew (TB) with filter (F) and non-filter (NF). Phytochemical analysis was carried out qualitatively, while antioxidant activity was conducted using the DPPH spectrophotometric method. The results of the phytochemical analysis showed that the phytochemical content of *Phyllanthus niruri* Linn. brewed with the DBF method was higher than those brewed with other brewing methods. In addition, the CBNF method gave the highest antioxidant activity on the brewing water of *Phyllanthus niruri* Linn. with an average DPPH of 2240.37 ± 93.79 ppm. Furthermore, antioxidant activity in the six treatments was not significantly different (ρ = 0.3741). For further studies, the researchers suggested analyzing the phytochemical content quantitatively and analyzing antioxidant activity using the FRAP and FIC methods.

1. INTRODUCTION

Phyllanthus niruri Linn. (In Indonesian called meniran) is an Asian endemic plant that is widely found and used in Indonesia. The utilization of this plant as herbal medicine, standardized herbal medicine, and phytopharmaceuticals has been widely studied [1]. This plant is reported to serve as an immunomodulator, anti-viral, anti-bacterial, diuretic, anti-hyperglycemic, and hepatoprotection. This plant has been shown to have a therapeutic effect in many clinical studies either solely or in combination. The combination of this plant and metformin has been shown to improve insulin resistance in obese male rats [2]. Phytochemical analysis on this plant indicates a positive reaction to groups of polyphenols, alkaloids, flavonoids, glycosides, saponins, steroids, and tannins [3]. Furthermore, a positive relationship between consumption of beverages and foods rich in polyphenols has been widely reported in etymological uses [4]. Brewing water of herbal plants is one of the most consumed beverages by Indonesian people and has been proven to contain a lot of polyphenols [5-7], which are known to have antioxidant activity. Foods containing polyphenols and their association with antioxidant activity are reported to be able to protect body tissues from oxidative stress [8-10].

Phyllanthus niruri Linn. is generally used after being dried and served in the form of brewing water. This water contains phytochemical compounds, such as saponins, phenolics, flavonoids, and tannins. The type and amount of phytochemical content obtained depends on the extraction process of *Phyllanthus niruri* Linn. by the applied brewing method. The brew extraction method generally refers to the water absorbed by the material, the mass exchange of Material, and methods.

In this study, the researchers applied a completely randomized design (CRD) with six treatments and four replications. This study was conducted from July to November 2020. The preparation of research samples (*Phyllanthus niruri* Linn.) was carried out at the Basic Laboratory of the Faculty of Health Sciences, Siliwangi University, Tasikmalaya. Furthermore, the analysis of antioxidant activity was carried out at PT. Laboratorium Indonesia Sibaweh located at Jl. Mochamad Toha, No. 51c, Bandung, while the qualitative analysis of phytochemical compounds was carried out at the Central Laboratory of Padjadjaran University located at Jl. Raya Bandung Sumedang, Km. 21, Hegarmanah, Jatinangor, Sumedang.

2. MATERIAL AND METHODS

The main materials used in this study were distilled water (aquadest), filler bags, and all parts of *Phyllanthus niruri* Linn. which was harvested in August 2020 in Ciawi, Tasikmalaya, West Java, Indonesia. The results of research by Septhi proved that all parts of plant *Phyllanthus niruri* Linn. that can be used are leaves, stems, flowers, fruits, and roots wich are generally

called *Phyllanthus niruri* Linn. herb [11].

Phyllanthus niruri Linn. is an annual herbaceous plant, grows upright with a stem height of 30 – 50 cm. *Phyllanthus niruri* Linn. has a top root system, roots are deep and strong, the roots are yellowish white with few root branches. Stem of *Phyllanthus niruri* Linn, round, not gummy, with a hard stem base and woody, and forming branches. On the stems the leaves grow opposite and in the axils of the leaves grow shoots that form branches. *Phyllanthus niruri* Linn. has small ovoid leaves with 5 mm long and 3 mm wide. Each stalk consists pf compound leaves, opposite leaf arrangement, pinnate leaf bones, flat, and smooth leaf edges. *Phyllanthus niruri* Linn. has small flowers, greenish white, short flowers stalks, with a size of 2 mm. stamens and pistils sticking out. *Phyllanthus niruri* Linn. fruits flat round shape, 2 – 2,5 mm diameter and slippery texture. The seeds are kidney-shaped, hard, and brown [12].

In addition, the chemicals used to analyze the antioxidant activity and phytochemical content were brewing water from *Phyllanthus niruri* Linn., $FeCl_3$, HCl, H_2SO_4, NaOH, CH_3COOH, and 2, 2-diphenyl-1-picrylhydrazil. Furthermore, the tools utilized in this study were tools for brewing *Phyllanthus niruri* Linn., tools for solutes, and the separation of the brewing product with solid material. This is strongly influenced by temperature and brewing time (water contact with the herbs) [13]. During brewing, all volatile and non-volatile compounds are extracted and dissolved in water and will affect the antioxidant activity of the herbal water.

Previous studies showed that the method by brewing the herbs with boiling water for 5 minutes and then being cooled for 30 seconds (decoction brew method) and the method by brewing the herbs with distilled water and then being stored in the refrigerator at 0 – 5℃ for 8 hours (cold brew method) can provide significant results, in which the total obtained flavonoids and phenolics are higher than other methods and temperatures applied to green coffee [14]. The results of another study regarding the brewing method reported that the decoction of brewing water using the decoction brew, cold brew, and true brew methods produced significantly different amounts of phenolic compounds [15]. Therefore, this study aimed to examine the effect of the decoction brew, cold brew, and true brew methods with modification in the use of filters and non-filters on the phytochemical content and antioxidant activity of *Phyllanthus niruri* Linn. analyzing antioxidant activity, storage racks, volumetric flasks, vortices, cuvettes, test tubes, test tube racks, pipettes, and vials.

2.1 Sample preparation

The preparation process starts with cleaning, dryng, and grinding [16]. All parts of Phyllanthus niruri Linn. were rinsed and sorted to remove dirt and damaged parts. All parts of the plant were then dried at room temperature (80,6 – 84,2˚F) without sunlight and then crushed to a size of 0.5 mesh [17].

2.2 Brewing methods

For the brewing methods as the independent variable, the researchers applied modified methods developed by Muller, namely Decoction Brew (DB), Cold Brew (CB), and True Brew (TB) methods with the utilization of filter (F) and non-filter (NF). The method developed by Muller adequately describes the brewing method commonly used in the community. These methods are also different in each step, temperature, and processing time, so it will be expected to provide a different picture of the total yield of phytochemical compounds and their antioxidant activity [15]. The Decoction Brew (DB) method was conducted by preparing 1.25 g of mashed *Phyllanthus niruri* Linn. added with 100 ml of distilled water. After that, it was heated to boiling for 5 minutes, and then being stirred for 10 seconds every 1 minute. The Cold Brew (CB) method was carried out by pouring 100 ml of room temperature distilled water into 1.25 g of finely dried *Phyllanthus niruri* Linn. then stored in the refrigerator for 8 hours at a temperature of 0 – 5℃ in a closed container. Every 1 hour the container is rotated 360˚ 3 times, so that the brewing process is evenly distributed in each ingredient used. Furthermore, the True Brew (TB) method is a brewing method using 1.25 grams of mashed dry Phyllanthus niruri Linn. brewed with distilled water at a temperature of 212˚F, stirred with a stirrer 10 times in a clockwise direction, to flatten the surface of the material in contact with water, then let stand for 10 minutes. After that separate the water with *Phyllanthus niruri* Linn. Modification of the filler (F) method was carried out by putting *Phyllanthus niruri* Linn. in a dip bag before being brewed, while the modification of the non-filler (NF) was implemented by letting *Phyllanthus niruri* Linn. have direct contact with distilled water. The use of the filter aims to provide practical value and make it easier when you want to separate *Phyllantus niruri* Linn. from brewed water. However, using a filter there is a risk that the total yield of phytochemical compounds will be trapped in the filter, so two treatments are carried out to see the effect. This study used six treatments with four replications.

2.3 Phytochemical analysis

Phytochemical analysis was carried out based on the method proposed by Chigozie [18]. The identification of phenolic compounds and tannins was conducted by adding 5% and 1% $FeCl_3$ compounds. The brewing water of Phyllanthus niruri Linn. was put on a drip plate and then added with 5% $FeCl_3$ compound. After that, the color change was observed. The darker the color of the sample is, the higher the presence of phenolic compounds will be. In addition, the identification of tannin compounds was performed by putting the brewing water of Phyllanthus niruri Linn. into a test tube and reacting it with a 1% $FeCl_3$ solution. If the extract contains tannins, a blackish green or dark blue color will be formed.

The qualitative test of flavonoid compounds was executed by adding Mg metal and 4 – 5 drops of concentrated HCl to the brewing water of *Phyllanthus niruri* Linn. in a test tube. The positive result is indicated by the formation of a red or orange solution. Moreover, the presence of saponins was identified by shaking the brewing water of *Phyllanthus niruri* Linn. for 1 minute in a water bath. If foam appears, it must be added with N_1HC_l. Furthermore, if the foam persists for 10 minutes, it means that the sample is positive containing saponins. On top of that, triterpenoids and steroids were tested by putting 1 ml of the brewing water of *Phyllanthus niruri* Linn. in a test tube and then added with anhydrous acetic acid and 1 – 2 ml of concentrated H_2SO_4 through the wall of the test tube. If a brown or purple ring is formed at the boundary of the two solvents, it means that the water contains triterpenoids. Furthermore, if the results obtained has a bluish-green color, it indicates the presence of steroids.

2.4 Antioxidant activity test

Antioxidant activity was measured with the DPPH free radical scavenging method (2,2-diphenyl-1- picrylhydrazyl) [16]. Analysis of the radical scavenging activity of DPPH (antioxidant activity) was carried out by preparing a sample stock solution. The DPPH assay is a very simple assay system that provides the first indication of the radical scavenging potential of the 2, 2-diphenyl-1-picrylhydrazyl radical. This method is used to test compounds that act as free radical scavengers or hydrogen donors and evaluate their antioxidant activity [19]. Samples were made in five concentration series, namely 100, 150, 200, 250, and 300 ppm. Determination of antioxidant activity was carried out by adding 1 ml of sample from each concentration into a different test tube and then adding 4 ml of 50 M DPPH solution. The mixture was homogenized and left for 30 minutes in a dark place. After that, absorption was measured using a UV-VIS spectrometer at a wavelength of 517 nm.

The parameter used for examining antioxidant activity with this DPPH radical scavenging method as IC_{50}, which is the concentration of the test compound required to capture 50% of DPPH free radicals. The smaller the IC_{50} value is, the stronger the test compound as a DPPH radical scavenger will be.

2.5 Statistical analysis

In the statistical analysis for the different test on antioxidant activity with six treatments, the researchers applied ANOVA with a single factor by excel program. One factor analysis of variance is a special case of analysis of variance (ANOVA), for one factor of interest, and a generalization of two sample t-test.

3. RESULTS AND DISCUSSION

3.1 Clove oil composition

The results of the response analysis to the presence of (qualitative) phytochemical compounds in the brewing water of *Phyllanthus niruri* Linn. using six different brewing methods are presented in the following Table 1.

The independent variable in this study was six brewing methods with four replications. The results of the analysis of the antioxidant activity of DPPH radical scavengers are presented in Table 2.

The graph of the absorbance value of the antioxidant activity of Phyllanthus niruri Linn. according to the method of manufacture can be seen in Figures 1-6. Figure 1 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the Cold Brew Filter method.

Figure 2 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the Cold Brew Non-Filter method.

Figure 3 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the Decotion Brew Filter method.

Figure 4 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the Decotion Brew Non-Filter method.

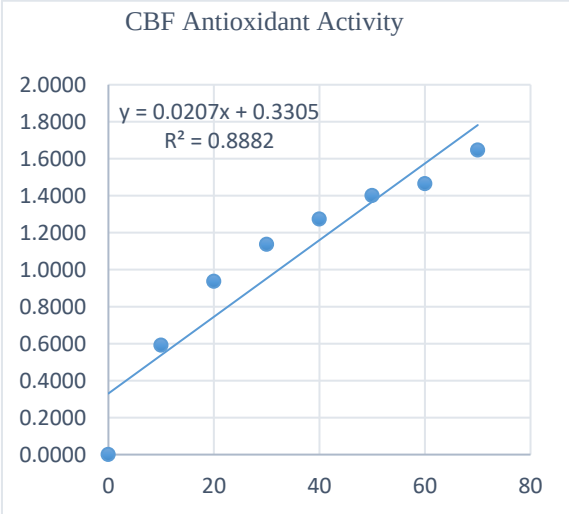

Figure 1. Absorbance graph of *Phyllanthus niruri* Linn. brewed water DPPH with CBF brewing method at 517 nm wavelength

Table 1. Response to the presence of phytochemical compound in the brewing water of *Phyllantus niruri* Linn.

Treatmentt	Phenolics 5% $FeCl_3$	Tannins 1% $FeCl_3$	Flavonoids Conc. HCl + Mg	Flavonoids 2N H_2SO_4	10 % NaOH	Saponins Heated	Triterpenoids Conc. H_2SO_4 + CH_3COOH	Steroids	Alkaloids Dragendorff
CBF	-	-	-	-	+	+	+	-	+
CBNF	-	-	-	-	+	+	+	-	+
DBF	+++	+++	-	-	+++	+	++	+	++
DBNF	+++	+++	-	-	+++	+	+	-	+
TBF	-	-	-	-	+	-	+	-	++
TBNF	-	-	-	-	+	-	-	+	++

Note: +++ = very much; ++ = quite a lot; + = a little; – = none.

Table 2. Antioxidant activity of *Phyllantus niruri* Linn. according to the applied brewing methods

Treatments	Average DPPH	F	ρ-value	Fcrit
Cold Brew Filter	2443.84 ± 21.21	1.1429	0.3741	2.7728
Cold Brew Non-Filter	2240.37 ± 93.79			
Decoction Brew Filter	2400.82 ± 95.51			
Decoction Brew Non-Filter	2341.14 ± 62.11			
True Brew Filter	2569.90 ± 143.28			
True Brew Non-Filter	2260.07 ± 442.96			

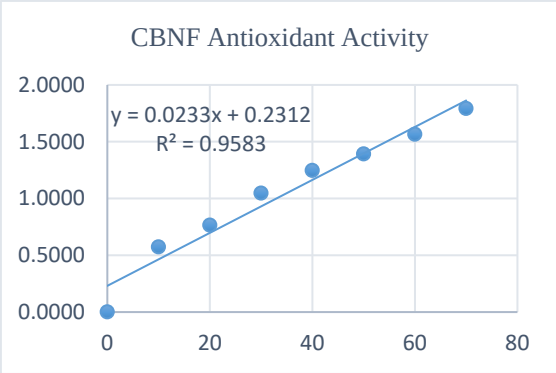

Figure 2. Graph of DPPH absorbance of *Phyllanthus niruri* Linn. brewed water with CBNF brewing method at 517 nm wavelength

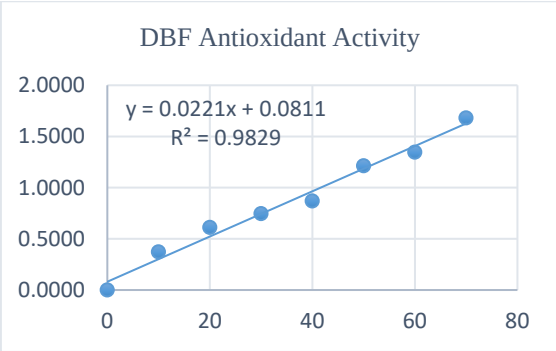

Figure 3. Absorbance graph of *Phyllanthus niruri* Linn. brewed water DPPH with DBF brewing method at 517 nm wavelength

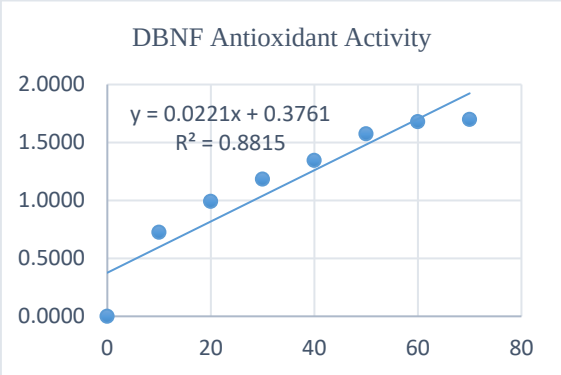

Figure 4. Absorbance graph of *Phyllanthus niruri* Linn. brewed water DPPH with DBNF brewing method at 517 nm wavelength

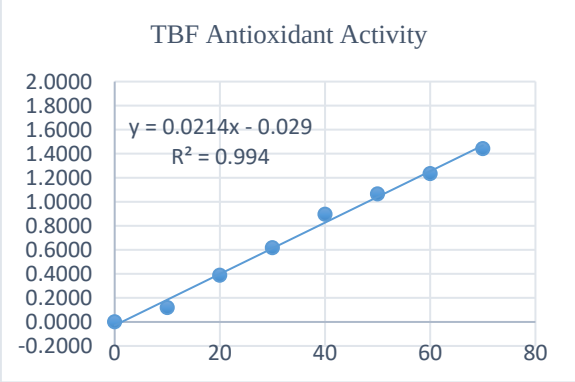

Figure 5. Absorbance graph of *Phyllanthus niruri* Linn. brewed water DPPH with TBF brewing method at 517 nm wavelength

Figure 5 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the True Brew Non-Filter method.

Figure 6 shows the antioxidant activity of *Phyllanthus niruri* Linn. by the Decotion Brew Non-Filter method.

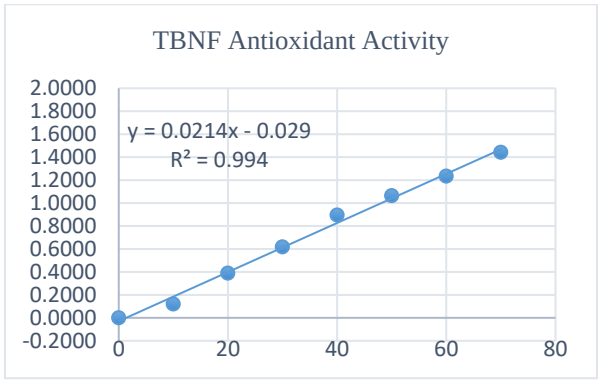

Figure 6. Absorbance Graph of *Phyllanthus niruri* Linn. Brewed Water DPPH with TBNF Brewing Method at 517 nm Wavelength

4. DISCUSSION

Phyllanthus niruri Linn. is a plant that possesses many health benefits. The results of the previous studies on the extract of *Phyllanthus niruri* Linn. showed that it can improve insulin resistance by reducing blood glucose levels, improving dyslipidemia, and inhibiting inflammation [2]. Other previous studies also had shown that this plant contains compounds that provide high antioxidant activity, such as flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins, and saponins [20-22]. In this study, the results of the response analysis to phytochemical contents for all brewing methods indicated the presence of flavonoids, triterpenoids, and alkaloids. The brewing water of *Phyllanthus niruri* Linn. that contained the most abundant compounds covering all phytochemical attributes was obtained by the DBF method. This is in line with a previous study that showed positive values for the presence of phenolics, flavonoids, steroids, saponins, and tannins in the extract of *Phyllanthus niruri* Linn [23]. In addition, the results of this study indicated that the abundance of phytochemical contents from the brewing water of *Phyllanthus niruri* Linn. using the Cold Brew (CB) and True Brew (TB) methods was low. This is consistent with the previous studies which showed positive values for the presence of phenolics, flavonoids, steroids, saponins, and tannins in the extract of *Phyllanthus niruri* Linn [20-22].

The brewing methods applied to *Phyllanthus niruri* Linn. may affect the antioxidant activity of the brewing water. This is due to the type and abundance of compounds extracted [24]. The type and abundance of extracted compounds are strongly influenced by the time and temperature during the brewing process. Therefore, different brewing methods will affect the value of the antioxidant activity of the brewing water of *Phyllanthus niruri* Linn [25, 26].

The DPPH method can be used to determine the ability of the sample to capture free radicals as an antioxidant activity feature. The principle of the DPPH method is the donation of hydrogen atoms from the sample substance to the DPPH compound which makes DPPH non-radical with a color change as the indicator. Changes in color and intensity from

purple to yellow are directly proportional to the antioxidant activity to capture the free radicals.

The measurement of the antioxidant activity value can be carried out by observing the intensity of the yellow color in the solution which is measured using a UV-Vis spectrophotometer at a wavelength of 514 nm. In this study, the measurement was carried out on 5 series of comparison stock solution concentrations and the samples of the brewing water of *Phyllanthus niruri* Linn. The absorbance value of the UV-Vis spectrophotometer was used to calculate the percent inhibition value (%). The percent value obtained was then regressed between percent and concentration. The result was a curve with the equation Y = a + bX. Furthermore, it was then continued by finding the IC50 value.

The data presented in Table 2 showed that the antioxidant activity of the brewing water of *Phyllanthus niruri* Linn. had a value between 2240.37 and 2569.90 ppm. The highest antioxidant activity of the brewing water of *Phyllanthus niruri* Linn. was obtained from the CBNF method with an average IC50 value of 2240.37 ± 93.79 ppm. This Hantioxidant activity was lower than that possessed by the brewing water of *Phyllanthus niruri* Linn. extracted using 96% ethanol, which was 237.33 ± 17.26 ppm [21, 25]. This was influenced by the type and abundance of phytochemicals that can be extracted by certain brewing methods. The optimization value of the brewing water of *Phyllanthus niruri* Linn. was also influenced by water temperature and contact time. Based on a previous study conducted by Nikniaz [27] on brewing black tea, the best temperature for brewing tea was 80°C with a contact time of 5 minutes. This combination between temperature and time gave higher antioxidant activity compared to the brewing water of black tea with the time under 5 minutes. It will be different if the tea is processed by being not fermented like green tea. Furthermore, the temperature applied and the optimal time for brewing tea, which is 95°C in no more than 30 minutes, has better antioxidant content compared to those more than 30 minutes.

5. CONCLUSION

This study showed that the brewing method on *Phyllanthus niruri* Linn. has an effect on the phytochemical qualitative parameters and antioxidant activity. The results of the qualitative analysis of the brewing water of *Phyllanthus niruri* Linn. indicated that those containing the most phytochemicals were those using the decoction brew with filter (DBF) and non-filter (DBNF) methods. Meanwhile, the true brew with non-filter (TBNF) method gave the highest antioxidant activity although the results of ANOVA with a single factor proved that there was no significant difference in antioxidant activity in all samples of the brewing water of *Phyllanthus niruri* Linn. using six different methods. For further studies, the researchers suggested carrying out the quantitative analysis of the phytochemical content on *Phyllanthus niruri* Linn. and analyze antioxidant activity using methods other than DPPH for comparison.

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