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Alpha Amylase Inhibition and Antioxidant Activity of *Phyllanthus niruri* Powder Drink.

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ABSTRACT

Phyllanthus niruri contains several bioactive components worked as alpha amylase inhibitor and antioxidant. This research was an exploration of *Phyllanthus niruri* parts (leaves, branch, roots, and whole *Phyllanthus niruri*) in making powder drink. Based on this research, it can be concluded that powder drink of *Phyllanthus niruri* contained lignans with 0.1878-0.2892 mg/ml of polyphenol, antioxidant activity was about 35.7922-64.1558%, activity of alpha amylase inhibition was 52.1977-65.7701% for condition without digesting pH simulation and 16.1452-44.2594% for condition with digesting pH simulation also energy 3.4576-3.7697 calorie.

Keywords: Alpha amylase, antioxidant, inhibition, *Phyllanthus niruri*

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INTRODUCTION

Increasing number of fast food consumption, limited exercise time, and stress of work routine as current lifestyle indicates unhealthy life. These condition smight lead to degenerative diseases such as cardiovascular, hypertension, and diabetes. Increasing number of degenerative diseases in society is result of unhealthy food and environment. In addition, there is also insufficient consumption of active compound such as antioxidant that can reduce free radical.

Diabetes Mellitus is one of leading cause of death in both developed and developing country. Critical effects of diabetes are postprandial hyperglycemia and decreasing antioxidants level. Moreover, this disease can be noticed of hyperglycemia chronic with higher sugar blood level compared (>126 mg/dl) [1] to normal condition. A global prevalence of diabetes based on every age level was about 2.8% in 2000 and it will be about 4.4% in 2030. It was predicted by [2] of increasing diabetics from 171 million in 2000 to 366 million people in 2030. Diabetes and higher sugar blood level are related to alpha amylase in hydrolizing carbohydrates into glucose. Natural compounds as alpha amylase inhibitor can be applied in production of functional product such as tea, cookies from leaves extract and fresh leaves of mulberry [3,4]. There are recent studies related to alpha amylase inhibitor from Phyllantaceae family such as *Phyllanthus virgatus*, *Phyllanthus pulcher* leaves, and *Phyllanthus amarus* [5-7].

Phytochemical screening on *Phyllanthus niruri* showed tannin, steroid, flavonoid, alkaloid, and lignans (phyllanthin and hypophyllanthin) [8,9]. Phyllanthin is an alpha amylase inhibitor [7]. Leaves, branch, and root of *Phyllanthus niruri* can reduce sugar blood level of mouse from an experiment [10]. Moreover, *Phyllanthus niruri* has antioxidant activity [11]. This research is continuation of advice on research conducted by [12]. There is still lack information about utilization of *Phyllanthus niruri* parts in making powder drink since it has antioxidant activity and also limits alpha amylase in carbohydrate hydrolyzing. In addition, powder drink has several advantages such as easy to serve and distribute also lengthens shelf life of products. Usually, *Phyllanthus niruri* was boiled in order to get its benefit. However, boiled water of *Phyllanthus niruri* is bitter and has unpleasant aroma thus it needs extra taste and aroma in powder drink making such as cassia vera.

On the other hand, cassia vera has high content of antioxidant with good taste and aroma so that it can improve taste and aroma of food and drinks. It was noted that adding cassia vera in pegagan instant powder drinks gave a good result on organoleptic [13]. In order to reduce bitter taste of product, it requires natural sweetener with high sweetness level but low in calories so that it can be consumed by diabetics. One of natural sweeteners is stevia (*Stevia rebaudiana*, Bertoni) which has higher sweetness level compared to sucrose from sugar cane. Thus, using stevia in product can be a solution for people who cannot consume sugar canesince it is safe since no carcinogen content, has low calories, and doesnot affecting product's taste [14].

MATERIALS AND METHODS

Materials

This experiment was an explorative research of *Phyllanthus niruri* parts (leaves, branch, roots, and whole *Phyllanthus niruri*) in making powder drink. Materials used in this experiment were fresh *Phyllanthus niruri*, cassia vera, stevia powder (sugar leaf), gum arabic, ethanol, alpha amylase, buffer Na-phosphate, H_2SO_4, methanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) , NaOH, HCl, acarbose, starch, aquades, dinitro salicylic acid (DNS) reagent, gallic acid, *folinciocalteau* 50% reagent and Na_2CO_3 5%. Hot air oven (YCO-N01), oven (Neycraft), pH meter, spray dryer, bomb calorimeter (IKA C 200), desiccator, spectrophotometer (UV-1800 Shimadzu), analytical balance (ABJ-NM/ABS-N), waterbath, ultrasonic (ELMA ULTRASONIC), micro pipette were used also in this experiment.

It was started by extracting *Phyllanthus niruri* and cassia vera and continued by making powder drink, product characteristics observation, and organoleptic for instant power drink. Observation was conducted on total polyphenol content, lignans, antioxidant activity. Further, condition before and after digesting pH simulation were also observed for alpha amylase inhibition.

Preparation of *Phyllanthus niruri* Dried Extract [13,15]

Five gram of fresh *Phyllanthus niruri* consisted of leaves, branch, roots, and whole *Phyllanthus niruri* were soaked in 50 ml of ethanol 80% for 15 minutes, shaken for 10 minutes and filtered as filtrate 1. Pulp from first extraction was extracted again by adding 50 ml of ethanol 80% for 10 minutes and shaken for another 10 minutes and filtered as filtrate 2. Next, pulp was washed by adding 50 ml of ethanol 96% and filtered as filtrate 3. These three filtrates from each sample were combined then evaporated using rotary evaporator at temperature less than 50°C until filtrates become thick. Arabic gum 5% (b/v) was added to thisextract of *Phyllanthus niruri* then homogenized. The extract will be dried using spray dryer at inlet temperature 150°C and 70°C for outlet temperature.

Preparation of Cassia Vera Dried Extract [16]

Cassia vera was chopped and put in desiccator after adding ethanol 96% with 1:5 ratios for 40 minutes. Solution was filtered and concentrated by rotary evaporator at temperature 55°C until dried extract of cassia vera was obtained. Next, 200 ml extract of cassia vera-ethanol and 300 ml of aquades were put in beaker glass and mixed using magnetic stirrer at 1300 ppm. Arabic gum was added in solution and mixed to homogenize. Extract solution was dried using spray dryer at temperature 150°C for inlet and 70°C for outlet.

Instant Powder Drinks from *Phyllanthus niruri*, Cassia Vera and Stevia

To produce *Phyllanthus niruri* powder drink much as 0.6 g of *Phyllanthus niruri* dry extract (leaves, branch, roots, and whole), 0.2 g of cassia vera dry extract, and 1 g of stevia dry extract combined.

Analytical Procedure for Total Phenol [17]

Gallic acid with different concentration (50, 100, 150, 200, and 250 ppm) were prepared while reagent of folin ciocalteau 50% and $Na_2CO_3$5% were also used in this analysis. It was began with dissolving 0.5 ml of standard or sample in 0.5 ml of ethanol 95%, 2.5 ml aquades and 2.5 ml reagent of folin ciocalteau 50%. Dissolved solution was incubated in dark room for 5 minutes and 0.5 ml of $Na_2CO_3$5% was added and incubated again in dark room for one hour. After incubation, solution was put on vortex. Solution absorbance was measured using a spectrophotometer (λ=725 nm).

Analytical Procedure for Phyllanthin [18]

Five grams of sample was diluted with ethanol up to 20 ml in volumetric flask. Chromatography was performed using thin layer chromatography (TLC) insilica gel consisted of acetate: methanol (9:1) and saturated in chamber glass for 7 hours. Supernatant from extract was applied on silica gel GF 254 plate using 5 µl micro capillaries. To develop spots, plate was sprayed by high concentration of sulfuric acid. Further identification for lignans can be conducted using spectrophotometer with wavelength 280-284 nm but it will be shifted to 298 nm in alkali condition.

Antioxidant Activity Assay [19]

Two milligram of sample and 2 ml methanol contained 80 ppm DPPH were mixed and put in dark room for 30 minutes. Next, solution will be observed using spectrophotometer for its absorbance at 517 nm of wavelength with methanol as blank solution. Antioxidant activity assay was calculated as DPPH scavenging activity as followed:

$$\text{DPPH } \textit{scavenging activity} = \left(1 - \frac{\text{absorbance of sample}}{\text{absorbance of blank solution}}\right) \times 100\%$$

Alpha-Amylase Inhibitory Activity Assay [20]

Sample solution (125 µl) and enzyme (125 µl) were prepared. Sample solution and enzyme were mixed and incubated at temperature 37°C for 10 minutes. Next, starch solution was added (125 µl) and put in incubator again for another 10 minutes at temperature 37°C. After incubation, 500 µl DNS reagent was added

and incubated in boiled water. After 5 minutes incubation, 5 ml of distilled water was added then absorbance of the mixture was measured using a spectrophotometer (λ=540 nm) as shown in Table 1. For positive control, sample was replaced with acarbose (0.5 mg/ml) obtained from 1 glucobay tablet contained 50 mg acarbose in 100 ml of HCl 2 N.

Percentage inhibitory activity was calculated as:

$$\%\ inhibitory = \frac{A1 - A2}{A1} \times 100\%$$

A1 = Absorbance ofcontrol A – Absorbance of blanko
A2 = Absorbanceof sample – Absorbanceof control B

Table 1: Composition of solution in Alpha Amylase Inhibition Activity Assay

Solution	Blanko (µl)	Control A (µl)	Control B (µl)	Sample (µl)	Acarbose (µl)
Sample solution	-	-	125	125	125
Buffer	250	125	125	-	-
Enzyme	-	125	-	125	125
Starch	125	125	125	125	125
DNS	500	500	500	500	500
Aquades	5000	5000	5000	5000	5000

pH for digesting simulation was conducted by controlling pH from half of sample to be similar to digestive system (stomach and intestine). pH was controlled to pH 2.0 by adding HCl for 30 minutes while adding NaOH for increasing pH to 6.8

Energy Value

Product energy was analyzed using bomb calorimeter and counted as calories.

RESULT AND DISCUSSION

Total polyphenols

Based on observation, total polyphenol from instant power drink can be shown in Table 2 as followed.

It can be noted that total polyphenol of instant power drink on average is between 0.1878-0.2892 mg/ml. These results were agreed with [21] that explained about polyphenol contents of *Phyllanthus niruri*. The highest total polyphenol of instant power drink was found on powder drink with *Phyllanthus niruri* branch extract. Moreover, it was identified by [22] that flavonoids from *Phyllanthus niruri* are quercetin, quercitrin, isoquercitrin, astragalin and rutin. Flavonoids and lignans in *Phyllanthus niruri* can be classified as polyphenol [9].

Table 2: Total Polyphenol on Average of Instant Power Drink

Type	Total Polyphenol (ml/mg)
Root Extract	0.1878±0.0167
Branch Extract	0.2892±0.0860
Leaves Extract	0.2307±0.0469
Whole Plant Extract	0.2612±0.0476

Lignans Contents

Results of lignans contents in instant powder drink from*Phyllanthus niruri* can be seen in Table 3 as followed.

As shown in Table 3, it can be indicated that all instant powder drink from different parts of *Phyllanthus niruri* contained lignans compound. Two main components of lignans from *Phyllanthus* genus are

phyllanthin and hypophyllanthin. Phyllanthin components can be utilized to identify thick extract of meniran (*Phyllanthus urinaria*) [23]. This compound can inhibit alpha amylase in hydrolyzing carbohydrates [7]. Lignans from *Phyllanthus niruri* has an activity to protect liver from toxic component or anti-hepatotoxic such as parasite, medicine, virus or bacteria [24]. Extract of *Phyllanthus niruri* in water at a dose of 3 cc can repair damaged liver cell as result of CCL_4 10% application even it was not completely. Repairing damaged liver cell is result of active component in *Phyllanthus niruri* such as lignans compound that can activate kupffer cell in regenerating liver cell [25].

Table 3: Lignans Contents in Instant Powder Drinks from *Phyllanthus niruri*

Type	Lignans Contents
Root Extract	+
Branch Extract	+
Leaves Extract	+
Whole Plant Extract	+

(+) indicates lignans contents

Antioxidant Activity

Antioxidant activity result observed on instant powder drink from *Phyllanthus niruri* are shown on Table 4 as followed.

Table 4: Antioxidant Activity of Instant Powder Drinks from *Phyllanthus niruri*

Type	Antioxidant Activity (%)
Root Extract	39.4459±15.9414
Branch Extract	40.3983±10.2233
Leaves Extract	64.1558±6.6207
Whole Plant Extract	35.7922±2.1972

It can be concluded that antioxidant activity of instant powder drinks was about 35.7922-64.1558% with the highest inhibition activity obtained from instant powder drinks of leaves extract. Antioxidant activity from this instant powder drinks is derived from its bioactive components. Based on [26], flavonoid has antioxidant activity as explained by [27] that isolated flavonoid components from *Hypericum ternum. Nirurifl avone* was a recent antioxidant compound of flavone sulfonic acid from *Phyllanthus niruri* extract. Flavonoid compound in *Phyllanthus niruri* has stronger antioxidant component than that in Vitamin E [28].

Analysis method for anti-oxidant activity was DPPH method by analyzing ability of active compound in powder drink of *Phyllanthus niruri* to capture free radical. This ability prevents free radical from outside and inside of body so that it prevents possibility of diabetes. It can be inferred that there was a correlation between anti-oxidant activities and inhibition activity of *Phyllanthus niruri* also polyphenol contents. The higher antioxidant activity means the higher inhibitor ability in *Phyllanthus virgatus,* L. also related to its polyphenol compound [5].

Alpha Amylase Inhibition Activity

Alpha amylase inhibition activity assay were conducted in condition without digesting pH simulation and in condition with digesting pH simulation as shown in Table 5.

Table 5: Activity of Alpha Amylase Inhibition of *Phyllanthus niruri* Instant Power Drink

Type	Alpha Amylase Inhibitionwithout digesting pH simulation(%)	Alpha Amylase Inhibition with digesting pH simulation(%)
Root Extract	55.7246±6.1539	32.9509±9.3760
Branch Extract	48.2644±3.1019	32.5244±4.5735
Leaves Extract	65.7701±0.1029	44.2594±4.5241
Whole Plant Extract	52.1977±2.0325	16.1452±2.9754

Even its value was smaller than glucobay inhibition activity gave (98.097%), it can be noted that leaves extract in this instant power drink gave the highest inhibition in carbohydrates hydrolyzing by alpha amylase both in condition without and with digesting pH simulation. Further, there were several components classified as alpha amylase inhibitor from *Phyllanthus niruri* such as quercetin, rutin, tannin and phyllanthin. Several studies explained that quercetin [29-31],rutin [32], tannin [33], and phyllanthin [7] have ability in alpha amylase inhibition.

Moreover, a declining on alpha amylase inhibition value in condition with digesting pH was observed. This condition might be related to changing structure of bioactive component from powder drink and it became unstable after passing in vitro of stomach pH (pH 2) for 30 minutes thencontinued into intestine condition (pH 6.8).

It is potential to develop *Phyllanthus niruri* in instant powder drink as functional drink for diabetics since it has ability to inhibit alpha amylase activity. Alpha amylase pancreatic inhibitor prevents starch hydrolysis and absorption thus it can reduce postprandial glucose level and help to maintain body weight [34,35]. Previous research found that *Phyllanthus niruri* extract was effective in reducing glucose blood level of diabetic mice (*Rattus norvegitus,* L.) [36], suppressed increasing postprandial glucose blood level of normal mice [37], while root, branch, and leaves extract from *Phyllanthus niruri* reduced glucose blood of each treatment [10].

Energy Value

Analysis for energy value of instant powder drink was obtained and displayed in Table 6 as followed.

Table 6: Energy Value in Average of *Phyllanthus niruri* Instant Power Drink

Type	Energy (Calories)
Root Extract	3.4576±0.0825
Branch Extract	3.5583±0.0094
Leaves Extract	3.6417±0.0250
Whole Plant Extract	3.7697±0.1469

It was shown in Table 6 that energy contained in instant powder drink from *Phyllanthus niruri* in average was 3.4576-3.7697 calories. Low energy in this product because the utilization of stevia as sweetener. Stevia as natural sweetener can replace sweet taste of synthetic sugar with low calories. Sweetness level of stevia is about 300 times of sucrose from sugar cane [14]. Thus, this instant powder drink can be a solution for diabetics.

CONCLUSION

From this study, it can be observed that all type of *Phyllanthus niruri* extracts using in instant powder drink contained lignans, with 0.1878-0.2892 mg/ml of polyphenol, antioxidant activity was about 35.7922-64.1558%, activity of alpha amylase inhibition was 52.1977-65.7701% for condition without digesting pH simulation and 16.1452-44.2594% for condition with digesting pH simulation also energy 3.4576-3.7697calories. As conclusion, instant powder drink from all parts of *Phyllanthus niruri* extract can be utilized as alpha amylase inhibitor with antioxidant activity.

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