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Phytochemicals Profiling and Total Antioxidant Capacity of Cinnamon Bark Extract (*Cinnamomum burmanii*)

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ABSTRACT
Cardiovascular disease and stroke are the most feared disease in wide society. Both of these disease could be caused by oxidative stress. Oxidative stress occurred when there is an imbalance between ROS (*reactive oxygen species*) and antioxidants. Therefore, our body needs antioxidants to prevent it. Antioxidants can be obtained from within our body (endogen) or outside the body (exogenous). The exogenous antioxidant can be found in herbal plants, which one of them is cinnamon bark. This research was conducted to determine secondary metabolite content and total antioxidant capacity in methanol extract of cinnamon bark. In this research, extraction process by maceration using methanol as the solvent. The phytochemical profiling is according to Harborne and the total antioxidant capacity assessment was using 1,1-Diphenyl-2-Picrylhidrazyl (DPPH) according to Blois. In the phytochemical profiling, phenolics, alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, quinones, betacyans, coumarins, and cardiac glycosides are contained in cinnamon bark extract. In the total antioxidant capacity assessment, the result showed the level of IC_{50} is 42,61 µg/mL. According to the value of IC_{50}, the extract of cinnamon bark has a very strong antioxidant capacity (IC_{50} <50), so it could become an exogenous antioxidant agent.

Keywords: *Reactive oxygen species, Phytochemicals Profiling, Cinnamomum burmanii, Total Antioxidant Capacity.*

1. INTRODUCTION

Oxidative stress is a condition due to an imbalance between the production and accumulation of reactive oxygen species (ROS) in cells and tissues and the body's ability to eliminate it [1]. Reactive oxygen species are produced naturally through the body's normal metabolism. However, apart from that, ROS can be produced through inflammation that occurred in every respiration process in mitochondria, obesity, X-rays, smokes pollution, vehicle emissions, or alcoholic [2]. In oxidative stress, the production of ROS will be increased and exceed the antioxidant capacity which produced by our body, so that can cause cells and tissues' damage [1]. Oxidative stress has a role in aging and the development of several diseases such as cancer, cardiovascular disease, stroke, metabolic syndrome, and neurodegenerative disease [3]. According to the Global Alliance on Health and Pollution, in 2019, Indonesia was at 4th rank as a country with the most deaths from pollution. So this is a critical problem for Indonesia [4].

Indonesia is rich in floras and faunas. One of them is the cinnamon plant which is often used as a complementary ingredient in the culinary world. This is so unexpected that this plant has so many benefits, including the content of antioxidants and secondary metabolites. Cinnamon is a plant thought to have originated in South Asia, Southeast Asia and mainland China [5]. The plant which has the Latin name Cinnamomum burmanii contains alkaloids, phenolic

compounds, flavonoids, saponins, tannins, and antioxidants [6].

Antioxidants are compounds or substances that are useful for inhibiting the oxidation process. In addition, antioxidants are also used to eliminate free radicals or ROS by reacting with free radicals themselves, then destroying them into substances that are less active, safer, and become substances with a longer life span [7]. Antioxidants neutralize ROS by accepting or donating electrons. Due to the production of endogenous antioxidants is minimal in the body while the number of free radicals continues to increase, exogenous antioxidants are needed to eliminate these free radicals [8]. This study aims to determine the phytochemicals contents and the total antioxidant capacity of cinnamon bark extract. This research can be a hope in the future that the antioxidants capacity of cinnamon bark extract can be used to prevent the formation of free radicals, so that the risk of developing several diseases due to oxidative stress such as cardiovascular disease, neurodegenerative disease, stroke and metabolic syndrome can be reduced.

2. MATERIAL AND METHOD

In this experimental in vitro study of cinnamon bark extract which did in Biochemistry and Molecular Biology Laboratory, Medical Faculty, Tarumanagara University. The sample that we used was got directly from Kerinci Regency, Province of Jambi, Indonesia. First, we sent the sample to the Indonesian Institute of Science in Bogor, West of Java, Indonesia to been identified. Furthermore, cinnamon bark was dried and made into simplicia. We used 219 grams of cinnamon bark's simplicia in the maceration process with methanol as the solvent. Then we evaporated the result of the maceration process and we obtained 30 grams of paste that we would use in this study. We did phytochemicals profiling test (Harborne, 1998) and total antioxidant capacity test (Blois, 1958) by used diphenyl-2-picrylhidrazyl (DPPH) and vitamin C as the comparative standard.

2.1 Phytochemicals Profiling Test

In the phytochemicals profiling test, we used several methods or reagents to determine the content of phytochemicals in cinnamon bark extract. We used follin ciocalteau to determine the phenolics, NaOH to determine the flavonoids, *ferric chloride* to specified tannins, foam test to specified the content of saponins, Mayer and Wagner to determine the alkaloids, Borntrager to specified the glycosides, Salkowski test to determine the terpenoids, H_2SO_4 was used to determine the quinones, Liebermann Burchard method was used to determine the steroids, NaOH was also used to specified the anthocyanins and betacyanin, Keller Killani to determine the cardiac glycosides, and Amonia was used to specified the coumarins.

2.2 Total Antioxidant Capacity Test

In total antioxidant capacity test, we used diphenyl-2-picrylhidrazyl (DPPH) with concentration of 50 µg/mL, then the maximum wavelength and maximum absorbance were found and used to be the control absorbance. The extract of cinnamon bark with concentration of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, and 60 µg/mL were used in this test. Furthermore, ascorbic acid (vitamin C) with concentration of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL, and 10 µg/mL were used as the comparative standard. From each concentration, 0,5 mL was taken and reacted with 3,5 mL of DPPH. This result of reaction was obtained from the absorbance reading with Spectrophotometry Genesys 30 Vis.

After got the absorbance and the concentration, we calculated the percentage of inhibition (%inhibition) of cinnamon bark extract and vitamin C by used the equation below.

$$\%\text{Inhibition} = \frac{\text{control Abs.} - \text{test Abs}}{\text{control Abs.}} \times 100\% \quad (1)$$

When the %inhibition of cinnamon bark extract and vitamin C were obtained, the curves were made and the linear equation was obtained. From these linear equations, the IC_{50} of cinnamon bark extract and vitamin C were calculated and found. Data were collected from the results of the total antioxidant capacity test using the DPPH. Furthermore, GraphPad Prism V.0.9. was used in this experimental research. Data was shown in the table and graphic.

3. RESEARCH RESULTS

3.1 Percentage of Yield

This research is using 219 grams of simplicia of cinnamon bark. After maceration and evaporation processes, weight of the extract of cinnamon bark was 30 grams. So the yield was obtained 13,69% with the equation:

$$\%\text{Yield} = \frac{\text{weight of extract}}{\text{weight of simplicia}} \times 100\% \quad (1)$$

$$= \frac{30 \text{ grams}}{219 \text{ grams}} \times 100\% \quad (2)$$

3.2. Phytochemicals Content of Cinnamon Bark Extract

The phytochemicals content in the cinnamon bark extract were tested are alkaloids, betacyanin, phenolics, flavonoids, glycosides, cardiac glycosides, coumarins, quinones, saponins, tannins and terpenoids. (Table 1).

Table 1. Phytochemicals Content of Cinnamon Bark Extract

Phytochemicals	+/-	Methods/Reagents
Phenolics	+	Folin Ciocalteau
Flavonoids	+	NaOH
Tannins	+	*Ferric Chloride*
Saponins	+	Foam
Alkaloids	+	Mayer; Wagner
Glycosides	+	Borntrager
Terpenoids	+	Salkowski
Quinones	+	H_2SO_4
Steroids	-	Liebermann Burchard
Anthocyanins	-	NaOH
Betacyans	+	NaOH
Cardiac Glycosides	+	Keller Kiliani
Coumarins	+	Amonia

3.3 Total Antioxidant Capacity of Cinnamon Bark Extract

The maximum wavelength of the cinnamon bark extract was 516 nm and the absorbance was 0.572. At each concentration of cinnamon bark extract, the absorbance was checked and the percentage of inhibition was calculated using a spectrophotometer, the X-axis was the concentration of the cinnamon bark extract and the Y-axis was the percent inhibition (Table 2). Then a linear equation curve was made to find the IC_{50} value, obtained Y = 1.072X + 4.324 and R^2 = 0.9955 (Figure 1). IC_{50} of cinnamon bark extract was obtained at 42,61 µg/mL.

Table 2 Concentration, %inhibition, and IC_{50} of Cinnamon Bark Extract

Concentration of Cinnamon Bark Extract (µg/mL)	%Inhibition (%)	IC_{50} (µg/mL)
10	13,63	**42,61**
20	26,39	
30	38,81	
40	46,15	
50	57,51	
60	68,53	

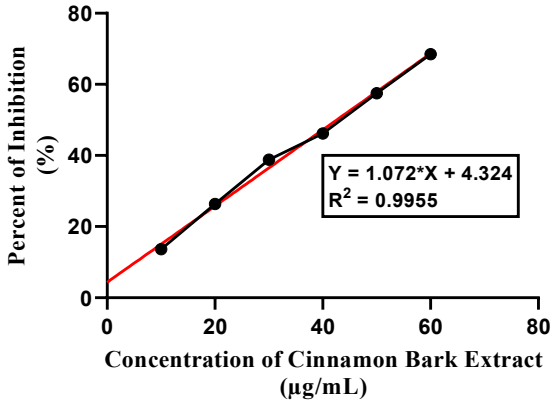

Figure 1 Curve of Total Antioxidant Capacity Test of Cinnamon Bark Extract

3.4 Total Antioxidant Capacity of Vitamin C

Measurement of absorbance at each concentration of vitamin C and percent inhibition using a spectrophotometer (Table 3). From these measurements, a standard linear regression curve for vitamin C was made. In this study, the linear equation Y = 6.934X +12.52 and R^2 = 0.9988 with the X-axis is the concentration of vitamin C and the Y-axis is the percent inhibition (Figure 2). Then from these results, the IC_{50} standard of vitamin C was 5,40 µg/mL.

Table 3 Concentration, %inhibition, and IC_{50} of Vitamin *C*

Concentration of Cinnamon Bark Extract (µg/mL)	%Inhibition (%)	IC_{50} (µg/mL)
2	26,85	**5,40**
4	39,11	
6	54,97	
8	67,87	
10	81,81	

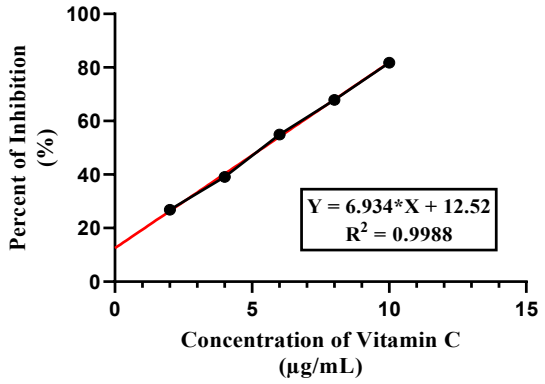

Figure 2 Curve of Total Antioxidant Capacity of Vitamin C

4. DISCUSSION

4.1 Phytochemicals Content of Cinnamon Bark Extract

According to Velavan et al [9], phytochemicals are not just macronutrients or micronutrients but have important health benefits, one of them as an antioxidant. This experimental study examined twelve phytochemicals contained in cinnamon bark extract. It was found that the phytochemicals contained in the cinnamon bark extract were phenolics, alkaloids, flavonoids, glycosides, cardiac glycosides, quinones, terpenoids, coumarins, tannins, saponins, and betacyans (Table 1). The results of this study are in line with Prahasti et al [6] that there are phenolic, alkaloid, flavonoid, saponin, and tannin content in cinnamon bark extract. Meanwhile, the research conducted by Parhusip et al [10] supports the results of this study that there are terpenoids and glycosides in cinnamon bark extract. According to Verdini et al [11] and Liang et al [12], there is coumarin content in cinnamon bark extract which means the results of both studies are similar to the results of research conducted by researchers. Research conducted by Mubarak et al [13] is also in line with this study that quinone compounds are contained in cinnamon bark extract.

According to Abdullah et al [14], steroids are triterpenoid compounds in the form of glycosides that have the potential as antibacterial. According to Priska et al [15], anthocyanins have radical scavenging abilities so that they have the potential as antioxidants. In addition, anthocyanins can also be used as neuroprotectants, anti-inflammatory, anti-aging, anti-microbial and hepatoprotective agents. According to Setiawan et al [16], apart from being a natural colorant in the food industry, betacyanins also have the capacity to act as free radical scavengers and antioxidants.

4.2 Total Antioxidant Capacity of Cinnamon Bark Extract

This study tested the total antioxidant capacity of cinnamon bark extract and ascorbic acid (vitamin C) as standard. The standard curve of ascorbic acid has an R^2 value of 0.9988 and a cinnamon bark extract of 0.9955 so that the two linear equations are reliable.

In this examination, the results were determined by calculating IC_{50}, namely the ability of cinnamon bark extract & ascorbic acid to reduce radicals in DPPH by 50%. The level of antioxidant capacity is inversely proportional to the IC_{50} value. This means, the smaller the IC_{50} value, the greater the level of antioxidant capacity in the sample. The IC_{50} value for ascorbic acid was 5.4 µg/mL while the cinnamon bark extract was 42.61 µg/mL. Based on the classification of antioxidant ability based on the IC_{50} value according to Rosidah et al [17], cinnamon bark extract has a very strong capacity and so is the capacity for vitamin C. When compared with vitamin C, the antioxidant capacity of cinnamon bark extract is indeed weaker, but according to Tasia et al [18], Cinnamon itself apart from being an antioxidant can also act as an antidiabetic. In addition, according to Pacier et al [19], excessive consumption of vitamin C can cause stomach irritation and increase the risk of kidney stones. According to Latief et al [20], the antioxidant capacity of cinnamon bark extract was 49 µg/mL. Meanwhile, according to Prahasti et al [6], the antioxidant capacity of cinnamon bark extract was 193.139 µg/mL.

5. CONCLUSION

Based on the results and discussion of the research entitled "Phytochemicals Profiling and Total Antioxidant Capacity of Cinnamon Bark Extract (*Cinnamomum burmanii*) it can be concluded that:

1. Cinnamon bark extract has a very strong total antioxidant capacity with IC_{50} value of 42.61 μg/mL

2. Cinnamon bark extract contains phenolics, alkaloids, flavonoids, tannins, saponins, glycosides, quinones, terpenoids, coumarins, cardiac glycosides, and betacyans.

AUTHOR'S CONTRIBUTION

The authors contributed equally to all aspects of the article.

CONFLICT INTERESTS

The authors declare that there is no conflict of interests.

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