Phytochemical Screening Of Cinnamon Bark (*Cinnamomum Burmanii*) (C. Ness & T. Ness) C. Ness Ex Blume Ethanol Extract And Antioxidant Activity Test With DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Method

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Abstract
Cinnamon (Cinnamomum burmanii) is one of the spices that can be used as cosmetic preparations in the pharmaceutical industry because it contains potent antioxidants. Examples of cosmetic products containing cinnamon are body scrubs, body showers, and moisturizers. This study aimed to determine the antioxidant activity of cinnamon extract using the DPPH method. Cinnamon was extracted using the maceration technique with 96% ethanol solvent, and phytochemical screening was carried out. The results of phytochemical screening from cinnamon bark extract were alkaloids, saponins, tannins, triterpenoids, and flavonoids. Antioxidant activity obtained an IC_{50} value of 25.35 ppm. Based on these results, the cinnamon extract has a very strong antioxidant activity value.

Keywords: *Antioxidant, Cinnamon bark, DPPH and Free radical.*

I. INTRODUCTION

In high concentrations, free radicals (ROS / RNS) cause oxidative and nitrosative stress, which can damage biomolecules. Free radicals react to bond with electron pairs because they have unpaired electrons. Oxidation by free radicals causes membrane disintegration, membrane protein damage, and DNA mutations [1]. In diseases like diabetes mellitus, neurodegenerative diseases, rheumatoid arthritis, cataracts, cardiovascular diseases, respiratory diseases, and aging, excess ROS can damage biomolecules like lipids, proteins, and DNA, causing oxidative stress [2].Antioxidants are molecules capable of inhibiting the oxidation of free radicals. Antioxidants sacrifice their electrons to free radicals which stabilize the atoms or molecules of free radicals, thereby preventing these radicals from damaging healthy cells. Antioxidants are naturally formed in our bodies to control the reactions of free radicals [3], [4]. However, antioxidants from the outside are needed due to the small number and inability to ward off prominent free radicals ended[5]. One source of antioxidants found in nature is cinnamon (*Cinnamomum burmannii*).Cinnamon is a plant native to Southeast Asia, China, and Australia, of which there are numerous species and cultivars. Cinnamon is found in Sumatra and Java in Indonesia. Cinnamon is an Indonesian export commodity whose bark, branches, and branches can be used as spices. The bark, branches, twigs, and leaves of cinnamon all contain phytochemical compounds. Cinnamon bark can be used directly in its natural or powdered state, essential oil, and oleoresin form. Oil can be extracted from the cinnamon tree's bark, branches, twigs and leaves [6].

In the pharmaceutical industry, cosmetics, food, and traditional and modern medicine, cinnamon is used as a flavouring agent [4], [5]. In addition, its processed products have been widely used in the pharmaceutical industry.Cinnamon's uses have been studied for a very long time. One of the most widely used herbs in cooking, cosmetics, and traditional and modern medicine is cinnamon bark [7]. Cinnamon can treat canker sores, cough, shortness of breath, stomach pain, flatulence, diarrhea, rheumatism, and cancer [8]. Many chemicals, including cinnamaldehyde, cinnamic acid, coumarins, tannins, and flavonoids, are present in cinnamon bark [9]. It is known that these compounds have the potential to act as antioxidants and prevent the formation of free radicals [10].Cinnamon contains bioactive compounds such as polyphenols (including flavonoids and tannins) and volatile oil compounds phenolics and coumarins, type A proanthocyanin polymers and protonated heterodimers of flavon-3-of groups, catechins, epicatechins, procyanidin B2, quercetin, 3,4-dihydroxybenzaldehyde, and -acid cinnamate as the main antioxidant compound [7], [11]–

[13]. Compounds containing phenolics such as flavonoids, tannins, proanthocyanidins, and Coumarins are a significant source of antioxidants that can reduce free radicals' effects [14]. This is the background of research on antioxidant tests on cinnamon bark extract.

II. METHODS

2.1 Preparation of Ethanol Extract of Cinnamon Bark

Simplicia was obtained by purposive sampling in the Deli Serdang area. Simplicia extracted by maceration method using ethanol. Simplicity (500 g) soaked in 75 parts of ethanol, tightly closed, leave for five days. Stir occasionally [15]. After five days, it was filtered, and the residue was macerated again with 25 parts ethanol for two days and filtered. The macerate is then evaporated using a rotary evaporator at a temperature of 40-50°C [16].

2.2 Phytochemical Screening of Ethanol Extract of Cinnamon Bark

Screening of chemical compounds of cinnamon bark extract includes an examination of alkaloids, flavonoids, saponins, tannins, glycosides, and triterpenoids/steroid compounds[17].

2.2.1 Alkaloids Examination

500 mg of cinnamon extract was heated for 2 minutes in a water bath, then cooled and filtered. Test tubes received three drops of each filtrate. Each tube received two drops of Mayer's, Buchardat's, and Dragendrof's. Mayer reagent alkaloids produce a lumpy white or yellow precipitate. The Buchardat and Dragondroff reagents produce brown-to-black and yellow-orange precipitates, respectively, indicating tannin [18].

2.2.2 Flavonoid Examination

500 mg of cinnamon extract is heated and filtered in a water bath. Add 100 mg of magnesium powder and 1 mL of 2N hydrochloric acid to the filtrate. The formation of a yellow-to-red color signifies the presence of flavonoids. [18].

2.2.3 Saponin Examination

500 mg of cinnamon extract was mixed with 10 mL of hot water, cooled, and then vigorously shaken for 10 seconds. The foam formation indicates the presence of saponin compounds in the sample for at least ten minutes, is between one and ten centimeters in height and does not disappear when one drop of HCl 2N is added [19].

2.2.4 Tanin Examination

500 mg of cinnamon extract was dissolved in 20 mL of distilled water, and the mixture was heated. After filtering and adding a few drops of a 10% FeCl3 solution, the resulting color was observed. Tannins are indicated by forming a blue or green-black stain [20].

2.2.5 Steroid/Triterpenoid Examination

To 2 mL of chloroform, 500 mg of cinnamon extract was added. Then, 0.5 mL of acetic acid anhydrous was added. Next, 2 mL of concentrated H2SO4 was added through the test tube wall, and the resulting colour was observed. The formation of a bluish-green color signifies the presence of steroid compounds (the presence of a steroid ring). In contrast, the formation of a brownish or violet ring upon the restriction of two solvents indicates a positively charged triterpene [19].

2.3 Antioxidant Activity Test

2.3.1 Preparation of DPPH Solution

10 mg of DPPH powder was weighed and then dissolved in 50 mL of ethanol (200 ppm concentration). Then, 1 mL of the DPPH solution (concentration: 200 ppm) was transferred to a 5 mL volumetric flask, ethanol was added to the mark (40 ppm), and the mixture was left in the dark for 30 minutes [21].

2.3.2 Determination of Maximum Wavelength DPPH

The absorption of DPPH solution with a concentration of 40 ppm was measured using a wavelength of 400-800 nm with a wavelength of 515 nm.

2.3.3 Determination of Operating Time

After homogenizing the 40 ppm DPPH solution, the absorbance of the solution was measured at a wavelength of 515 nm until the 60-minute mark, and then an observation was made to determine when the solution produced a stable absorbance; the data was then used to determine the operating time [21].

2.3.4 Preparation of Standard of Stock Solution

2.3.4.1 Preparation of Standard Stock Solution of Cinnamon Bark

After measuring out a total of 10 mg of cinnamon bark extract, it was first dissolved in a volumetric flask that held 10 mL, and after that, the mark on the flask was filled with methanol (concentration 1000 ppm) [21].

2.3.4.2 Preparation of Standard Stock Solution of Vitamin C

After weighing and dissolving 10 mg of cinnamon bark extract in a 10 mL volumetric flask, the mark on the volumetric flask was filled with methanol (concentration 1000 ppm) [21].

2.3.5 Preparation of Test Solution

2.3.5.1 Preparation of Vitamin C Test Solution

Following an incubation period of thirty minutes at room temperature [21], an absorbance reading was obtained using a UV-Visible spectrophotometer with a maximum wavelength of 515 nm. To obtain the concentrations of the test solutions, which were two ppm, three ppm, four ppm, five ppm, and six ppm, respectively, it required as much as 0.1 mL, 0.15 mL, 0.2 mL, 0.25 mL, and 0.3 mL of Stok solution to be pipetted into a 25 mL volumetric flask. The total volume of the Stok solution used was as much. After that, 5 mL of DPPH solution with a concentration of 200 ppm was added to each volumetric flask, and the volume was then filled with methanol until it reached the line that had been marked. The final step was to record the results. After that, the mixture was homogenized.

2.3.5.2 Preparation of Vitamin C Test Solution

In order to obtain the concentrations of 10 ppm, 20 ppm, 30 ppm, and 40 ppm in the test solutions, the following amounts of Stok solution were pipetted into a 5 mL volumetric flask: 0.05 mL, 0.1 mL, 0.15 mL, 0.2 mL, and 0.25 mL respectively. After that, one milliliter of DPPH solution with a concentration of two hundred parts per million was added to each volumetric flask. The volume was then filled with methanol until it reached the line that had been marked, and the mixture was homogenized. After a thirty-minute incubation period at room temperature, an absorbance reading was taken with a UV-visible spectrophotometer at a maximum wavelength of 515 nm [21].

2.3.6 DPPH Free Radical Scavenging Percentage Analysis

Determination of the percentage of free radical scavenging using the 2,2-diphenyl-2- picryhydrazil (DPPH) method by compounds with an antioxidant activity using UV-VIS spectrophotometry.

2.3.7 IC_{50} analysis

The IC50 value, also known as the inhibition concentration, is the calculation that is utilized when determining the free radical scavenging activity. This value describes the test compound's concentration that can capture fifty percent of the free radicals. The lower the value of the IC50, the higher the free radical scavenging activity. In the regression equation, the results of the calculations are inputted with the concentration of the extract serving as the X-axis and the value of the percent inhibition (antioxidant) serving as the Y-axis [21].

III. RESULT AND DISCUSSION

3.1 Phytochemical Screening

Flavonoid compounds contain antioxidants. The flavonoid compounds, alkaloids, saponins, tannins, and terpenoids can all be found in the extract of cinnamon bark. Table 1 presents the findings obtained from the phytochemical analysis.

Table 1. Phytochemical Screening of Extract of *Cinnamomum burmanii*

No	Chemical Compounds	Result
1	Alkaloids	+
		+

		+
2	Flavonoids	+
3	Glycoside	-
4	Saponins	+
5	Tannins	+
6	Triterpenoids/Steroids	+ / -

Description: (+): contains a group of compounds
(-): does not contain a group of compounds

There are alkaloids, flavonoids, saponins, tannins, and triterpenoids in extract ethanol from cinnamon bark, as shown by the results of a phytochemical screening examination. Alkaloids, particularly indole, can effectively halt the chain reactions caused by free radicals. Antioxidant properties can be attributed to flavonoids. Flavonoids can reduce the number of free radicals in the body by transferring an electron to a free radical compound [22]. This makes the electrons held by free radicals more stable and less likely to react in a way that could cause damage to healthy cells. Other alkaloid compounds that have antioxidant properties include quinolones [23], caffeine, which can act as hydroxyl radical scavengers, and melatonin, which plays an essential role in protecting cells from the effects of radiation and drug toxicity [24], [25]. Quinolones are one type of alkaloid compound. Caffeine and melatonin are two other types of alkaloid compounds. On the other hand, this study did not investigate the kind of alkaloid that contributes to antioxidant bioactivity.

3.2 Antioxidant Activity Test Result

The DPPH test was carried out to determine the amount of antioxidant activity the cinnamon bark extract possessed. The 2,2-diphenyl-1-pircylhydrazil radical is used in the DPPH test, which measures an antioxidant's ability to snuff out the damaging effects of free radicals. The free radical that was used in this test is where this test got its name (DPPH). The DPPH assay is used to test compounds that act as free radical scavengers or hydrogen donors to evaluate these compounds' antioxidant activity [26]. The presence of free radicals significantly contributes to the damage done to living organisms. The molecule known as DPPH is a free radical that appears to be purple. This molecule can change into a yellow-coloured compound that is stable when it reacts with antioxidants. Antioxidants each contribute one electron to DPPH, which results in a decrease in the number of free radicals that are produced by the DPPH reaction. However, free radical scavenging activity can also be expressed as the concentration that results in a loss of 50% of DPPH activity. It is common practice to express free radical scavenging activity as a percentage of DPPH inhibition; however, free radical scavenging activity can also be expressed as the concentration that results in this loss (IC_{50}). There is a widespread consensus that the IC50 value accurately assesses the antioxidant capacity of isolated compounds or extracts [27].The DPPH test was carried out to determine the amount of antioxidant activity the cinnamon bark extract possessed. The 2,2-diphenyl-1-pircylhydrazil radical is used in the DPPH test, which measures an antioxidant's ability to snuff out the damaging effects of free radicals. The free radical that was used in this test is where this test got its name (DPPH). The DPPH assay is used to test compounds that act as free radical scavengers or hydrogen donors to evaluate these compounds' antioxidant activity [26].

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solution becomes more concentrated, the DPPH scavenging activity also increases. The absorbance value of the DPPH drops to a lower value due to the increased number of DPPH atoms that pair up with hydrogen atoms from the sample. Table 2 summarizes the findings and can be found by clicking this link.

Table 2. Extract IC_{50} Value Cinnamon Bark and Vitamin C

Sample	IC_{50} Value	Category
Cinnamon Bark Ethanol Extract	25.3567 ppm	Very Strong
Vitamin C	3.1936	Very Strong

The category of antioxidant activity can see in table 3.

Table 3. Antioxidant category based on IC_{50} value

Category	Concentration (ppm)
Very Strong	< 50
Strong	50 – 100
Currently	101 – 150
Weak	151 – 200
Very Weak	>200

The effective concentration of the extract that causes a loss of DPPH that is equivalent to fifty percent is what the IC_{50} value describes [28]. The IC_{50} value of the ethanolic extract of cinnamon bark was found to be 25.3567 ppm, while the IC_{50} value of vitamin C was found to be 3.1936 ppm in Table 3. It can be deduced from this that the combination of the ethanolic extract of cinnamon bark and vitamin C possesses highly potent antioxidant activity (IC_{50} value less than 50%). It is possible to conclude that the sample's antioxidant activity has increased because the absorbance value of the sample has decreased. The decrease in absorbance value can be attributed to the cinnamon bark ethanol extract solution's ability to neutralize DPPH [3]. This took place because the solution contributed electrons to DPPH, which caused atoms that had unpaired electrons to pair up with other electrons and stop behaving radically.

IV. CONCLUSION

The ethanol extract of Cinnamomum bark contains alkaloids, saponins, flavonoids, tannins, and triterpenoids. Based on the antioxidant test using the DPPH method, the IC_{50} value was 25.35 ppm (a very strong antioxidant).

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