Antioxidant Compound Profile and Total Flavonoid Levels of Ethanolic Extract 70% and 96% Cinnamon (*Cinnamomum Burmannii*)

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ABSTRACT

Cinnamon (Cinnamomum burmannii) is a spice plant that commonly used by the community as an additive in food processing and as one of pharmaceutical ingredient in the pharmaceutical industry. Cinnamon is known to contain secondary metabolites as bioactive compounds that can be used as a source of exogenous antioxidants that could treat various degenerative diseases. However, the obstacle that often occurs in research on natural materials is when the chemical components was extracted as bioactive compounds that contained in these natural materials. Therefore, the exploration of cinnamon as a natural antioxidant is very necessary. The purpose of this study was to determine the profile of antioxidant compounds in the ethanol extract of cinnamon bark and to determine the total flavonoid content. Extraction was carried out by maceration method using 70% and 96% ethanol as solvent. Detection of the content of antioxidant compounds in the extract was carried out using the TLC method, the resulting spot was sprayed with DPPH reagent. Total flavonoid content was determined by using quercetin as the comparison standard based on colorimetric principles and measured by UV-Vis spectrophotometer at a wavelength of 400 – 500 nm. The results of the analysis showed that each of the 5 spots on the TLC chromatogram was observed using at 254nm UV lamp. Detection of antioxidant compounds showed that there were 2 compounds that had antioxidants quality, they were the 3rd and 4th spots. The total flavonoid content of 70% ethanol extract was 49.65% or equivalent to 496.5 mgQE/g extract, while in 96% ethanol it was 29.08% or equivalent to 290.8 mgQE/g extract. Therefore, it can be concluded that the highest total of flavonoid content was obtained in 70% ethanol extract.

Keywords*: Antioxidant, extract, flavonoid, cinnamom burmannii*

INTRODUCTION

The diversity of Indonesian plant biodiversity has been widely known to have pharmacological activity. The pharmacological activity is due to the presence of bioactive compounds in the form of secondary metabolites in plants. One of the potential bioactive compounds is as an antioxidant that is useful for stabilizing free radicals. (Windono, et al., 2001)

Antioxidants are reductant compounds that are able to stop the development of oxidation reactions. Antioxidants work through binding and prevention of free radical formation and inhibiting cell damage (Rustiah & Nur 2018). Free radicals produced continuously during the metabolic cycle and the unfulfillment of endogenous antioxidants become one of the factors that cause damage to various cells in the body that can trigger various degenerative diseases (Rahman et al. 2014). Therefore, exploration of exogenous antioxidant sources from plants continues to be developed as a preventive effort in the prevention of the onset of disease.

Plants that are quite abundant in Indonesia is cinnamon (Cinnamon Burmannii L.). Empirically, cinnamon is used by the public as a healthy drink because it is known to have many properties in the treatment of a disease. Cinnamon is a species of the genus Cinnamomum with the family Lauraceae, in the form of woody plants commonly known as spices (Yulianis et al, 2011).

The largest chemical components of cinnamon barks are sinamic alcohol, coumarin, sinamic acid, synamhyde, anthocyanin and essential oils with sugar, protein, simple fats, pectin and others (Al-Dhubiab, 2012). The results of the extraction of the bark of Cinnamomum burmannii contains the main antioxidant compounds in the form of polyphenols (tannins, flavonoids) and essential oils of the phenol group. (Ervina, et al., 2016).

Flavonoids are natural phenol compounds found in almost all plants. A number of medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, antialergic, and anticancer activity. (Neldawati, et al., 2013). The antioxidant effects of these compounds are caused by the bonding of free radicals through the hydrogen atom donor of the flavonoid hydroxyl group. Flavonoid compounds are polar so they need solvents that are polar (Gillespie and Paul, 2001). But the obstacle that usually occurs in the research of natural materials is on the extraction step of chemical components as bioactive compounds contained in these natural materials.

The effectiveness of the extraction of a compound by the solvent depends largely on the solubility of the compound in the solvent, in accordance with the principle of like dissolve like i.e. a compound will be dissolved in a solvent with the same properties. Polar solvents include ethanol, methanol, acetone and water (Sudarmadji et al., 1997). There are several factors that affect the extraction process where this will affect the acquisition of levels of an active substance compound, one of which is the concentration of extracting solvents used.

Several studies have shown the effect of variations in ethanol solvent concentrations on the total flavonoid levels of the resulting extract. Research conducted by Luginda (2018) on the effect of ethanol solvent concentrations of 60%, 70%, 80% and 96% against the total flavonoid levels of beluntas leaves showed that ethanol solvents 60% obtained the highest total flavonoid levels of beluntas leaf extract. Another study conducted by Kristen and Halim (2014) on the influence of ethanol concentrations of 50%, 70%, 80% and 96% against the total flavonoid levels of corn hair extract, it is known that ethanol is 70% as the optimum solvent for the extraction of flavonoid compounds from corn hair. From these studies it is known that the concentration of solvents affects the levels of flavonoids obtained. Therefore, this study was conducted to

find out the profile of Antioxidant Compounds and Total Flavonoid Levels of Cinnamon Extract (Cinnamomum burmannii L) extracted with 70% and 96% ethanol as solvents.

RESEARCH METHODS

Materials

The materials used were thin-layer chromatography (TLC), 254 nm UV lights, rotary evaporators, freeze dryers, UV-Vis spectrophotometers, glass and non-glass apparatus, cinnamon bark, silica gel GF 60, ethanol, n-hexane, ethyl acetate, aquades, DPPH (Sigma Aldrich), Aluminum chloride, sodium acetate.

Preparation and extraction of samples

The bark of the cinnamon was washed and dried in the sun, then mashed to a uniform powder size (singed with mesh sieak 60). A fine simplisia of 500 g of cinnamon is extracted by maceration for 3 × 24 hours using a solvent of 70% ethanol and 96% ethanol respectively. Each maserat is concentrated using a rotary evaporator so that the concentrated extracts were obtained. Furthermore, the extract was dried in a freeze dryer to obtain the dried extract.

Detection of antioxidant compounds in KLT

The stationary phase was silica gel GF254 and the mobile phase was n-Hexane-ethyl acetate (9:1) (Kemenkes, 2017). The chambers were saturated with the mobile phase for 30 minutes. The test solution was made by means of a number of condensed cinnamon extracts dissolved in ethanol, then the test solution was tolerable on the silica plate gel GF254. TLC plates are diluted to the boundary mark in the saturated chamber. Once diluted, the TLC plate was removed from the chamber and dried. The plates were then observed in UV light with wavelengths of 254 and 366 nm. The resulting spot and color were calculated for the Rf value of each spot. Next the plate is sprayed with a DPPH 0.4 mM. There was a change in spot color for approximately 10 minutes. The positive result of the radical capture of DPPH by antioxidant compounds is characterized by a change in the spot area that was originally purple (after being sprayed DPPH) to yellow.

Analysis of Total Flavonoid levels

Preparation of Quercetin as Standard Solution

Standard solution of Quercetin series 10, 20, 30, 40, and 50 µg / ml as much as 10 mL was made. Each standard solution was piped as much 0.5 ml in a 10 ml volumetric flask.100 µL $AlCl_3$ solution 10% and 100 µL solution Sodium acetate 1 M , and distilled water was added to the final volume of 10 ml. Let it sit for 30 minutes and then absorption was measured at a wavelength of 400-800 nm.

Preparation of cinnamon bark extract

The concentrated extracts of 70% and 90% ethanol was weighed 40 mg each then dissolved with ethanol to a volume of 20 ml (2000 µg/ml), to make the Testing Solution sample. The testing solution samples were piped 5.0 ml and 4.0 ml respectively and transferred into a 10 ml volumetric flask. The remaining step was just exaclty the same with the standard solution aforementioned.

RESULTS AND DISCUSSIONS

Extraction Process

In this study, 70% and 96% ethanol were used as solvents. Ethanol is a polar solvent to extract phenolic compounds contained in Cinnamomum burmannii. Another advantage of ethanol as a solvent is that ethanol is a universal solvent that can attract most of the chemical compounds contained in plants. The amount of extract produced from 500 g of cinnamon bark powder extracted with 70% and 96% ethanol solvent by maceration method is 161.55 g and 139.05 g, respectively. The resulting extract is yellowish brown with a special smell.

Based on the yield of this extract it can be assumed that the compenent of bioactive compounds contained in ethanolic extracts 70% were more compared to ethanolic extract 96%. The amount of extract yield can be seen in Table 1.

Table 1. Yield of cinnamon bark extract obtained

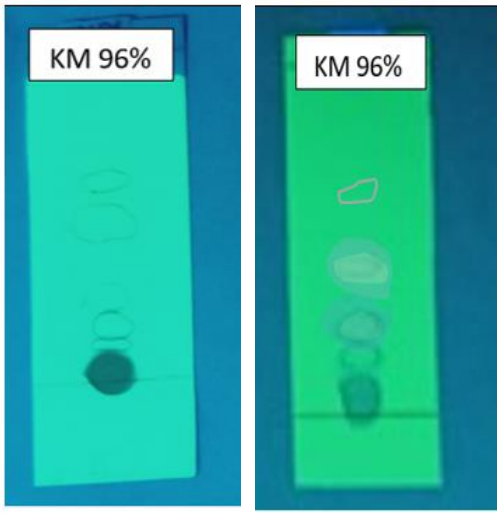
Solvent	Simplisia Weight (g)	Weight of extract obtained (g)	Yield (%)
Ethanol 70%	500	161,55	32,31
Ethanol 96%	500	139,05	27,81

Profile of Antioxidant Compounds in TLC

Detection of antioxidant compounds was done using TLC method, which aims to detect the number of compound components in the extract that can bind to DPPH free radicals. Testing is carried out by developing a TLC plate that has been tolerated by a test solution in the moble phase of n-hexan-ethyl acetate (9: 1) with an ellution distance of 10 cm. Once diluted, theTLC plate was removed from the chamber and dried. The plate was observed in UV light with a wavelength of 254 nm.

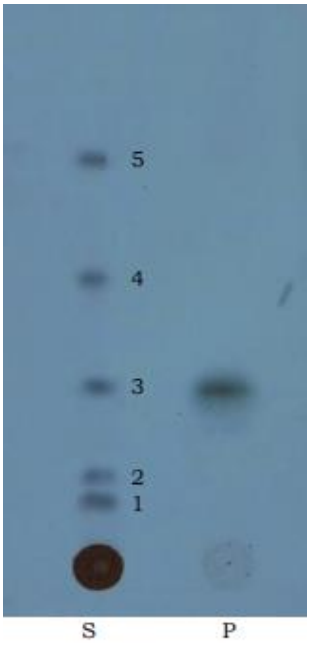
TLC results of 70% and 96% ethanolic extract of cinnamon bark indicated that there were 5 spots. The antioxidant spots were spot numbers 3 and 4 with Rf values of 0.24 and 0.26 and 0.42 and 0.48, this was indicated by the change in spot color to yellow and about the spot remains purple after being sprayed with DPPH. Based on the color and value of rf spots produced in the 2 solvents, it shows that the type of compounds that were extracted in the solvents were the same. The chromatogram results of antioxidant detection can be seen in Figure 1 and Table 2.

Chromatography profile of cinnamon extract obtained in accordance with the pattern of chromatography simplisia cinnamon listed in the Indonesian Herbal Pharmacopoeia, spot with a value of Rf 0.46 is a compound of cyamalde (Figure 2). Cyamalde is the main compound in cinnamon, Cyamalde acts as an antioxidant. The content of trans-cyamalde in cinnamon bark extract is quite high (68.65%) to be a source of antioxidant compounds with its ability to catch free radicals or radical scavengers. (Lee H.S. 2002 and Ekaprasada, 2009)



A B

Figure 1. KLT results in ethanol extract 70% and 96% cinnamon stick, A) chromatogram before spraying DPPH and B solution) chromatogram after spraying DPPH solution



Information :
S : Cinnamom simplisia
P : Cyamalde comparison
Rf of Cyamalde comparison 0,46
Rf 1. 0,25
Rf 2. 0,28
Rf 3. 0,46
Rf 4. 0,66
Rf 5. 0,87

Figure 2. Cinnamon simplisia chromatography pattern (Source: Kemenkes, 2017)

Table 2. Results of calculation of rf chromatogram value results of KLT cinnamon bark extract

Sample	Spot	Rf	UV	DPPH	Result
70% ethanol extract	1	0,12	Glowed	-	-
	2	0,16	Glowed	-	-
	3	0,24	Glowed	yellow	+
	4	0,42	Glowed	yellow	+
	5	0,54	Glowed	-	-
96% ethanol extract	1	0,1	Glowed	-	-
	2	0,18	Glowed	-	-
	3	0,26	Glowed	yellow	+
	4	0,48	Glowed	yellow	+
	5	0,6	Glowed	-	-

Information : + antiosidant compounds

\- non- antioxidant compounds

Total Flavonoid Level Test Results

The method of determining flavonoid levels was done colorimetrically using AlCl3 reagent. Tests were conducted on samples of cinnamon bark extract and Quercetin as standard using UV-Vis spectrophotometry at wavelengths of 400-500 nm (the maximum wavelength obtained is 440 nm). The total flavonoid content of bark extract cinnamon was expressed in the Quersetin equivalent (mgQE/g extract). The calibration curve of quercetin to determine total flavonoid levels can be seen in Table 3 and Figure 3

Table 3. Results of measurement of raw uptake of quercetin

No	Concentration (µg/ml)	Absorban
1	10	0,1806
2	20	0,3272
3	30	0,4937
4	40	0,6702
5	50	0,8576

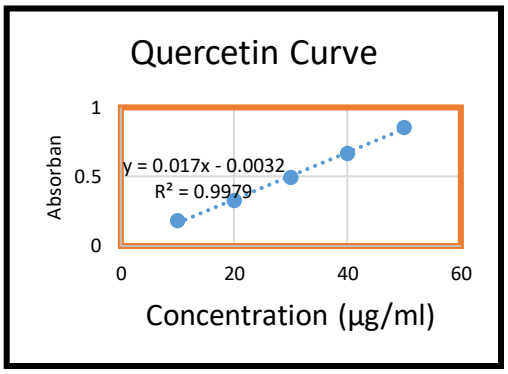


Figure 3. Raw curve of quercetin for total flavonoid determination

The total flavonoid levels in the cinnamon bark extract test sample were calculated based on the regression line equation of the raw curve of quercetin i.e. y = 0.017x - 0.0032. Based on Table 4 it is known that the total flavonoid content in ethanol extract is 70% is 49.65% or equivalent to 496.5 mgQE / g extract, while in ethanol 96% is 29.08% or equivalent to 290.8 mgQE / g extract. Based on these results it is known that the total flavonoid content of cinnamon bark extract in ethanol extract is 70% greater than ethanol extract 96%.

Table 4. Results of Measurement of Total Flavonoid Levels of Cinnamon Bark Extract

Solvent	Replication	Absorban	Initial total flavonoid content (µg/ml)	Total flavonoid content (mgQE/g extract)	Total flavonoid content (%)	Average Total Flavonoid Content (%)
Ethanol 70%	1	0,8428	49,76	497,6471	49,76	49,65
	2	0,8263	48,79	487,9412	48,79	
	3	0,8535	50,39	503,9412	50,39	
Ethanol 96%	1	0,3920	23,25	290,59	29,06	29,08
	2	0,3926	23,28	291,03	29,10	
	3	0,3924	23,27	290,88	29,09	

The difference in total flavonoid content in the extract is influenced by the polarity of the solvent used, where differences in ethanol concentration can affect the solubility of flavonoid compounds in the solvent (Prayitno, et al., 2016). The higher the concentration of ethanol, the lower the level of polarity. The high levels of flavonoids obtained in 70% ethanolic extracts of cinnamon bark in this study, showed that flavonoid compounds in cinnamon bark are polar. The greater total flavonoid content in 70% ethanol solvents was according to the number of compound components extracted in the solvent based on the yield of extracts obtained as a result of the extraction of simplisia.

CONCLUSION

The antioxidant compound profile of cinnamon bark extract showed that the types of compounds extracted in 70% and 96% ethanol solvents were the same i.e. each indicates there were 2 antioxidant compounds. The yield of the extract and the total flavonoid content of 70 % ethanolic of cinnamon bark greater than the 96 % ethanolic extract.

THANK YOU

Thank you to the Director of Poltekkes Kemenkes Makassar for this research grant fund entitled Characterization and Antioxidant Activity of Ethanol Extract, Ethyl Acetate and N-Heksan Cinnamon Bark (Cinnamomum burmannii) and Kayu Secang (Caesalpinia Sappan L.) through the Higher Education's Basic Research scheme (PDUPT)

BIBLIOGRAPHY

Al-Dhubiab, B. E. (2012). Pharmaceutical Applications and Phytochemical Profile of Cinnamomum burmannii. Pharmacognosy Reviews, 6(12), 125–131.

Ekaprasada, M. Taufik. 2009. Isolasi Senyawa Antioksidan Kulit Batang Kayu Manis (Cinnamomum burmannii Nees ex Blume). www.ekadarmun.wordpress.com.

Ervina M dkk. 2016. Comparison of In Vitro Antioxidant Activity of Infusion, Extract and Fractions of Indonesian Cinnamon (Cinnamomum Burmannii) Bark. International Food Research Journal 23(3): 1346-1350.

Gillespie, R.J. dan Paul. 2001. Chemical Bonding and Molecular Geometry. Oxford University Press, London.

Kemenkes, RI., 2017, Farmakope Herbal Indonesia, edisi II, hal 182

Kristiani, V. dan Halim, F.I., . 2014. Pengaruh Konsentrasi Etanol Dan Waktu Maserasi Terhadap Perolehan Fenolik, Flavonoid, Dan Aktivitas Antioksidan Ekstrak Rambut Jagung

Lee H S., 2002, Inhibitory activity of Cinnamomum cassiabark derived component against rat lens aldose reductase. J of Pharmacy and Pharmaceutical Sci; 5:226-23

Luginda, A.R. Lohita, B. dan Indriani, L. 2018. Pengaruh Variasi Konsentrasi Pelarut Etanol Terhadap Kadar Flavonoid Total Daun Beluntas (Pluchea indica (L.)Less) Dengan Metode

Microwave ± Assisted Extraction (MAE).

Neldawati, Neldawati, Ratnawulan dan Gusnedi, 2013, Analisis Nilai Absorbansi dalam Penentuan Kadar Flavonoid untuk Berbagai Jenis Daun Tanaman Obat. Jurnal Pillar Of Physics, Vol. 2, 76-83

Prayitno, S.A. Kusnadi, J. dan Murtini, E.S. 2016.Antioxidant activity of red betel leavesextract (Piper crocatum Ruiz and Pav.)by different concentration of solvents.Journal of Pharmaceutical, Biologicaland Chemical Science.

Rahman H., V. B. Reddy, S. Ghosh, S. K. Mistry, G. Pant, G. Sibi. 2014. Antioxidant, cytotoxic, and hypolipidemic activities of Plumeria alba L. and Plumeria rubra L., American Journal Of Life Sciences, 2 (6-1):11-15.

Rustiah, W. & Nur, U.. (2018). Uji aktivitas antioksidan pada ekstrak buah kawista (Limonia acidissima) menggunakan spektrofotometer UV-Vis. Indonesian Journal of Chemical Research. 6(1): 22-25

Sudarmadji, S., B. Haryon dan Suhardi. 1997. Prosedur Analisis untuk Bahan Makanan dan Pertanian. Penerbit Liberti, Yogyakarta

Windono, T., Soediman, S., Yudawati, U., Ermawati, E., Srielita, Erowati, T. I. Uji Peredam Radikal Bebas terhadap 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) dari Ekstrak Kulit Buah dan Biji Anggur (Vitis vinifera L.) Probolinggo Biru dan Bali. 2001. Artocarpus. 1, 34-43

Yulianis dkk. 2011. Penetapan Kadar Kumarin dari Kulit Kayu Manis (Cinnamomum burmannii Bl.) dengan Metoda Kromatografi Gas. Jurnal Sains dan Teknologi Farmasi, Vol. 16, No.2, 2011, halaman 203-208