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Comparison of *in vitro* antioxidant activity of infusion, extract and fractions of Indonesian Cinnamon *(Cinnamomum burmannii)* bark

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Abstract

The purpose of this research was to compare antioxidant activity of Indonesian cinnamon bark infusion, extract, its fractions and to analyse of their phytochemical constituents for antioxidant activity. The cinnamon infusion was obtained by water extraction, while the extract was by ethanol percolation. The ethanolic extract was then fractionated into n-hexane, ethyl acetate and water fractions.Their in vitro antioxidant activity was assayed semiquantitatively by using DPPH method, while the phytochemical constituents were analyzed by using TLC-autography with several spray reagents.The results showed that antioxidant activity of infusion, extract and its fractions were significantly different. Among the material tested, the cinnamon bark infusion had the highest antioxidant activity, followed by ethanolic extract, its water- and ethyl acetate- fractions with IC_{50} value of 3.03; 8.36; 8.89; and 13.51µg/mL, respectively. Their antioxidant activities were higher than rutin, with IC_{50} of 15.27 µg/mL.The phytochemical analysis results indicated that polyphenol (tannin, flavonoids) and phenolic volatile oil are the major antioxidant compounds.

Introduction

Cinnamon has been extensively researched since it has many benefits for human life. The plants spread across Southeast Asia, China and Australia with different types and varieties such as true cinnamon and *Cinnamomum zeylanicum* from Srilanka; Cassia cinnamon from China and Vietnam; *Cinnamomum tamala* from India and Myanmar (Burma); and *Cinnamomum burmannii* from Indonesia. Indonesian cinnamon is especially found in the area of Sumatra and Java islands (Ravindran *et al.*, 2004).

All parts of the cinnamon i.e. bark, branches, twigs and leaves, contain useful phytochemicals, but the bark is widely commercialized. Cinnamon bark is one of the most popular herbs utilized as a spice in cooking. In addition, its processed products in the form of essential oils and oleoresins have been widely used in pharmaceutical, cosmetic, food, beverage, and cigarette industries; also in traditional and modern medicine (Heyne, 1987; Sangal, 2011). Several compounds of cinnamon, include essential oil, eugenol, safrole, cinnamaldehyde, tannin, and calcium oxalate. Cinnamaldehyde is potential antioxidant compounds with the ability to scavenge free radicals (Thomas and Duethi, 2001). Wijayanti *et al*. (2011) reported that ethanolic extract of Indonesian cinnamon bark collected from different area possess antioxidant activity with various IC_{50} value in a range of 75.48 µg/mLand 136.88 µg/mL. In the study on beneficial of cinnamon to prevent diabetes and Alzheimer's diseases, Peterson *et al.* (2009) found that water extract of cinnamon bark contained polyphenols.

This research aim was to compare antioxidant activity of Indonesian cinnamon bark in the form of infusion, ethanolic extract and its fractions. This research also provided data of the phytochemical constituents which supposed contribute to the antioxidant activity.

Materials and Methods

Plant materials and chemicals

Dried tubular of Cinnamon bark was obtained from Materia Medica, Batu, East Java, Indonesia in August 2015. The Cinnamon bark was cleaned and observed its macroscopic (physical appearances including shape, color, specific odor, size, texture) characteristic. It was crushed into crumb with average size of 12 mm for extraction purpose, and was obtained its moisture content and total ash content, microscopic observation, and phytochemical content. All chemicals were analytical grade obtained

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from local distributor.

Preparation of infusion, ethanolic extract and its fractions

The aqueous infusion (I) was prepared by taking 10 g of the cinnamon bark crumb, put into infusion pan and 100 mL of distilled water was added, heated at 90^0C for 20 minutes, then filtered to obtain the infusion. Ethanolic extract (E) and its fractions were prepared as follows: 100 g of the Cinnamon bark crumb was weighed, moistened with 96% ethanol, transferred into a percolator, and then soaked with 96% ethanol (at ratio of 1:8) for 24 hours. After soaking, it was extracted until complete exhaustion by percolation. The solvent was then evaporated by using water bath until a semisolid ethanolic extract. It was then fractionated with n-hexane, ethyl acetate and water. 25 g of the extract was added with 50 ml of hot water; and fractionated by liquid-liquid extraction in a separating funnel. Each solvent extraction was repeated with the same procedure for three times, collected and concentrated to obtain the n-hexane fraction (Fh), ethyl acetate fraction (Fe) and water fractions (Fw).

In vitro *antioxidant activity assay*

In vitro antioxidant activity of the infusion, E (extract) and its fractions were assayed by using DPPH scavenging method. DPPH scavenging activities were assayed principally according to Džamić *et al.* (2014). 150 µL of sample solution or standard (rutin) solution in a range of concentration of 1.58 to 100 µg/mL were transferred into microplate wells, ±10 µL of DPPH 0.13% solution was added into each well and mix well, then incubated in dark at room temperature for 30 minutes then measured the absorbance at λ_{max}515 nm (as sample blank). The absorbance of the incubated mixture was measured at 515 nm using microplate reader. Blank probes were done in the same way, using methanol instead of the investigated solution (A0). The % of antioxidant activity was calculated using the equation:

$$\% \text{ Inhibition} = [(A0 - AS) / A0] \times 100 \%$$

Antioxidant activity is expressed as IC_{50}, which was calculated by using linear regression curve of absobanceat 515 nm and concentration of samples and standard solutions.

Phytochemical constituents analysis

Phytochemical compounds analysis was performed by using semiquantitative TLC-autography. as follows: 2 µL of samples (10 mg/mL) and standard (5 g/mL, cinamaldehyde (S) and rutin(R)) solutions were applied on to Silica Gel F254 and separated by using three types of mobile phase (buthanol:acetic acid:water = 4:1:5; polarity index 6.72), (ethy acetate:methanol = 1:4; polarity index 4.96) and (toluene:ethyl acetate= 7:3; polarity index 3.00). On the separated chromatogram was then sprayed with $FeCl_3$, $AlCl_3$, vanillin sulphate and 0.2% DPPH solutions to detect the potential antioxidant of the separated chemical constituents in the infusion, E (extract) and the fractions. Retention factor (Rf) of each separated constituent was calculated.

Results and Discussion

Plant material characteristics

Observation was conducted by distinguishing characteristics of the three types of cinnamon most commonly found in Indonesia, i.e *Cinnamomum burmannii, Cinnamomum cassia* and *Cinnamomum zeylanicum*. Figure 1 show the microscopic characteristics of cinnamon bark. The bark was dry, flattered to rolled shape (tubular), length of 20-40 cm, thick, outer surface: brown - reddish brown, inner surface: dark brown - blackish brown. The outer surface; pale wavy striped lengthwise, lichens are colored rather white and brown. Former fracture uneven and emit a distinctive odor. Odor: specific cinnamon (warm, sweet). These characteristics were conformed to the *Cinnamomum burmannii*, in agreement with the results reported in Materia Medika, Batu, East Java, Indonesia. Moisture content and total ash content were 11.73 ± 0.52% and 3.94 ± 0.43%, respectively, which are within the Indonesian standard of Cinnamon bark (Kementerian Kesehatan Republik Indonesia, 2008). Phytochemical screening of the bark showed that they contains alkaloid, flavonoid, saponin, tannin, quinon and steroid/ triterpenoid, which conformed with results reported in previous research on *Cinnamomum burmannii* (Guenther, 2006; Wang and Yang, 2009; Wijayanti, 2011).

The preparation methods of infusion, extract and fractions yield I, E, Fh, Fe and Fw with variable amount of 18.83%,18.78%, 3.50%, 27.29% and 69.20%, respectively. Santiago-Adame *et al.* (2015) reported that preparation of *Cinnamomum zeylanicum* infusion at 80^oC and continous stirring for 10 minutes produce a yield of about 4.75%, whereas the E of *Cinnamomum cassia* resulted in a yield of 12.73% (Yang *et al.*, 2012). Hence, different preparation methods of infusion and extract does have an affect on the yields.

Table1. Phytochemical Screening

Class of compounds	Reagents	Results	
Alkaloid	Mayer reagents	White sediment	+
	Dragendorff reagents	Orange	
Flavonoid	Wilstater's	red orange Amyl alcohol	+
Saponin	Foam formation	Stabile foam	+
Tannin	$FeCl_3$.	Black to green	+
Quinone	NaOH 1N.	Red	+
Steroid-triterpenoid	Liebermann's Burchard	Red	+

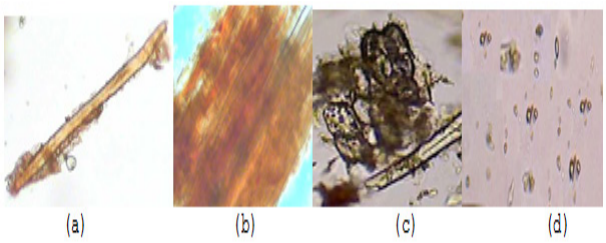

Figure 1. Microscopic characteristic of Cinnamon bark. (a: schlerenchyme fiber; b: volatile oils on parenchyma; c: stone cells; d: amylum and calcium oxalate crystals)

Semiquantitative TLC autography

The obtained infusion, extract and fractions were screened for the antioxidant potency by using semiquantitative TLC autography method with spraying solution of 0.2% DPPH. The potential antioxidants in the samples react with DPPH radical by hydrogen donation to the N radical atom of DPPH radical, resulting in a color change from purple to yellow (Molyneux, 2004). This step was used to choose samples which has potential as antioxidants sources. The results showed that infusion, E, Fe and Fw yielding color changes of the DPPH (Figure 2), while Fh and cinnamaldehyde showed weak antioxidants potential. This results indicated that those I, E, Fe and Fw were potential samples as antioxidant sources, and were further analyzed for their quantitative antioxidant activity.

Antioxidant activity

Antioxidant activity of I, E, Fe and Fw were presented in Table 2, which were express as IC_{50}. According to Blois (1958) the smaller the IC_{50} values, the higher antioxidant activity. Normal classification of a compound antioxidant activity generally follows the followings criteria: IC_{50}< 50 µg/mL is considered a very powerful antioxidant; IC_{50} 50-100 µg/mL as strong antioxidant; intermediate antioxidants (100-150 µg/mL) and weak antioxidants (IC_{50} 151-200 µg/mL). Based on these criteria, the results obtained in this study from all the samples analysed were classified as very powerful antioxidants as compare to rutin, a well known flavonoid glycoside natural antioxidant.

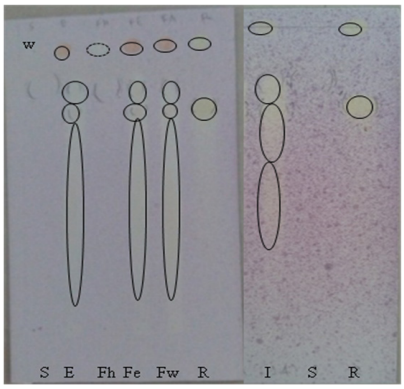

Figure 2. Semiquantitative TLC autography of I, E, Fh, Fe, Fw, and cinnamaldehyde (S) and rutin (R) reference, whole infusion/ extract/fraction respectively (w) on silica gel F254, ethyl acetate:methanol =1:4

Among the samples, IC_{50} of infusion was the lowest, followed by E, Fw and Fe. A lower antioxidant activity of *Cinnamomum zeylanicum* infusion has been reported by Santiago-Adame *et al.* (2015) with IC_{50} value of 290 µg/mL. Yang *et al.* (2012) reported the antioxidant activity of ethanolic extract of *Cinnamomum cassia* with IC_{50} value of 72 µg/mL. Other researchers also reported that ethanolic extract of Indonesian cinnamon bark possess antioxidant activity with IC_{50} values in a range of 75.48 µg/mL and 136.88 µg/mL (Wijayanti *et al*., 2011). However, Mathew and Abraham (2006) showed the higher antioxidant activity of methanolic extract of *Cinnamomum verum* with IC_{50} value of 4.21 µg/mL.The different level of antioxidant activity might be affected by the cinnamon species and the preparation method.

Antioxidant activity of infusion and ethanolic extract were higher than other samples, probably due to the synergistic effect of compounds in the infusion or extract. This results was also supported by semi quantitative TLC autography which showed that whole (w) infusion or extract was intensed yellow color than its separation spots on chromatogram after sprayed by DPPH reagents. This results suggested that the normal preparation method to obtain infusion used by the general consumers, i.e. by heating cinnamon bark at 90°Cfor 20 minutes, is sufficient

Table 2.Antioxidant activity of infusion, extract, fractions and standard

Sample	IC_{50}value (µg/mL)
Infusion	3.03 ± 0.22
Ethanolic extract	8.36 ± 0.73
Water fraction	8.89 ± 0.52
Ethyl acetate fraction	13.51 ± 0.50
Rutin	15.27 ± 0.69

and results in high antioxidant activity. On the other hand, the use of alcohol facilitates extraction for further treatment or isolation to obtain isolates of antioxidants.

Phytochemical constituents analysis

Identification of antioxidant compounds classes was analyzed by using TLC autography method with spraying chemical reagents of $FeCl_3$ (for tannin detection), $AlCl_3$(for flavonoids detection) and vanillin sulphate (for steroid-terpenoid detection) with cinnamaldehyde(volatile oil compound) and rutin (flavonoid compound) as reference compounds. The results (Figure 3) showed that the I, E, Fe and Fw of cinnamon bark was supposed to contain polyphenolic compounds (including tannin, flavonoid anf phenolic volatile oil compounds- see the DPPH, VS, $FeCl_3$ and $AlCl_3$ plates). This is indicated by the colored spots on the I, E, Fe and Fw which was compared to the color spot of rutin that is yellow after spray with $AlCl_3$ with a value of R_f of ± 0.74 (I and E), 0.76 (Fe), 0.75 (Fw) and 0.76 (rutin). The $FeCl_3$ reagent produced greenish-black spot to detect polyphenolic compounds. In Figure 3, tailing on $R_f$of 0.74 (I and E), 0.74 (Fe), 0.74 (Fw) and 0.78 (rutin) as well as reddish spots and yellow with sulfuric vanillin reagent on $R_f$of 0.74 (I), 0.79 (E), 0.78 (Fe), 0.76 (Fw) and 0.76 (rutin) can be observed. While the Fh showed gray spot, compared to the violet color for the spot with sulfuric vanillin reagent on R_f of 0.82 (cinnamaldehyde). Identification results of classes of compounds using DPPH reagent gives a yellow spot with purple background of I, E with the R_f value of 0.74 and 0.71, Fe and Fw (Rf 0.73); which was compared to rutin (Rf 0.73).

These results indicated that the I, E, Fe and Fw are compounds with potential antioxidants and these compounds are from the class of polyphenols (including flavonoids, tannin and phenolic volatile oil compounds). Brewer (2011) reported that the compounds from plants that have the potential as antioxidants in general are phenolic compounds can be from the group of phenolic acid (gallic, protocathecuic, caffeic, rosmarinic acids), phenolic diterpenes (carnosol, carnosic acid, rosmanol and rosmadial) and phenolic volatile oil (eugenol, carvacrol, thymol and menthol) or polyphenolic compounds which is including flavonoids (flavones, flavonols, isoflavones, catechins, flavonols and kalkon), tannin (Amarowics, 2007).

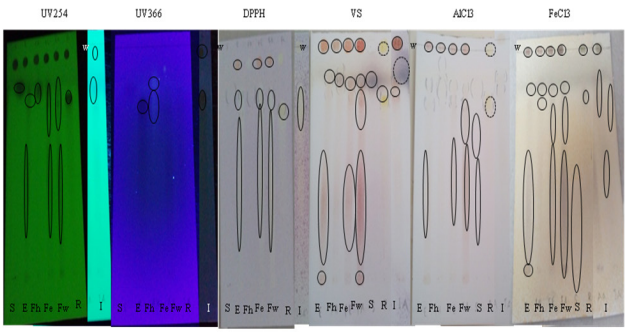

Figure 3. Phytochemical constituents of cinnamon bark on TLC-silica gel F254 with mobile phase of ethyl acetate:methanol (1:4).E, Fh, Fe, Fw, (S) cinnamaldehyde, (R) Rutin, (I) Infusion, whole infusion/ extract/fraction respectively (w), uv (ultraviolet), DPPH (1-1-diphenyl-2-pycrylhydrazyl 0.2%), VS (vannilin sulphate)

Conclusions

The results showed that antioxidant activity of infusion, extract and its fractions were significantly different. Among the material tested, the cinnamon bark infusion had the highest antioxidant activity, followed by ethanolic extract, its water- and ethyl acetate- fractions with IC_{50} value of 3.03; 8.36; 8.89; and 13.51 µg/mL, respectively. Those antioxidant activities were higher than that of standard Rutin, with IC_{50} of 15.27 µg/mL. The phytochemical analysis results indicated that polyphenols (including flavonoids, tannin) and phenolic volatile oil compounds as the major antioxidant compounds. This results suggested that the simple traditional method of preparation of infusion commonly applied by people, yielded the highest antioxidant activity.

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References

Amarowics, R. 2007. Tannins: the new natural antioxidants?. European Journal of Lipid Science Technology 109: 549-555

Blois, M.S. 1958. Antioxidant determinations by the use of a stable free radical. Nature 181: 1199-1200.

Brewer, M.S. 2011. Natural antioxidant: sources, compounds, mechanisms of actions, and potential

application. Comprehensive Reviews in Food Science and Food Safety, 10:221-247

Džamić, A.M., Soković, M.D., Ristić, M.S., Grujić, S.M., Mileski, K.S. and Marin, P.D. 2014. Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. Journal of Applied Pharmaceutical Science 4(3):1-5

Heyne, K.1987. Tumbuhan Berguna Indonesia. Jilid 3. Departemen Kehutanan. Jakarta.

Kementerian Kesehatan RI. 2008. Farmakope Herbal Indonesia. Edisi I. Jakarta: Kementerian Kesehatan RI. p. 41-45

Mathew, S. and Abraham, T.E. 2006. Studies on the antioxidant activities of cinnamon *(Cinnamomum verum)* bark extracts, through various *in vitro* models. Food Chemistry 94: 520–528.

Molyneux, P. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidantactivity. Songklanakarin Journal of Science and Technology 26(2): 211-219.

Peterson, D.W., George, R.C., Scaramozzino, F., LaPointe, N.E., Anderson, R.A., Graves, D.J. andLew, J. 2009. Cinnamon extract inhibits tau aggregation associated with Alzheimer's disease *in vitro*.Journal of Alzheimers Disease 17(3):585-597.

Ravindran, P.N., Babu, K.N. and Shylaja, M. 2004. *Cinnamon* and *Cassia*: The Genus *Cinnamomum*. CRC Press, Boca Raton.

Sangal, A. 2011. Role of cinnamon as beneficial antidiabetic food adjunct: a review. Advances in Applied Science Research 2(4):440-450.

Santiago-Adame, R., Medina-Torres, L., Gallegos-Infante, J.A., Calderas, F., Gonzalez-Laredo, R.F., Rocha-Guzman, N.E. Ochoa-Martínez, L.A. and Bernad-Bernad, M.J. 2015. Spray drying-microencapsulation of cinnamon infusions *(Cinnamomum zeylanicum)* with maltodextrin. LWT - Food Science and Technology 64: 571-577.

Thomas, J. and Duethi, P.P. 2001. Cinnamon. In: Peter, K.V., editor.Handbook of Herbs and Spices. Woodhead,Cambridge, United Kingdom.

Wijayanti, W.A., Zetra, Y. And Burhan, P. 2011. Minyak Atsiri Dari Batang Cinnamomum (Kayu Manis) Dari Famili Lauraceae Sebagai Insektisida Alami, Antibakteri Dan Antioksidan. Skripsi. Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Teknologi Sepuluh Nopember. Surabaya.

Yang, C.H., Li, R.X. and Chuang, L.Y. 2012. Antioxidant activity of various parts of *Cinnamomum cassia* extracted with different extraction methods. Molecules17: 7294-7304.