Jurnal Akademika Kimia, 11(4): 247-252, November 2022
ISSN (online) 2477-5185 | ISSN (print) 2302-6030
http://jurnal.fkip.untad.ac.id/index.php/jak/

OPEN ACCESS

Comparison of the Composition of *Cinnamomum verum* J. Presl and *Zingiber officinale* Roscoe to the IC_{50} Value

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Received 28 September 2022, Revised 25 October 2022, Accepted 18 November 2022
doi: 10.22487/j24775185.2022.v11.i4.pp247-252

Abstract

Antioxidants are substances that can reduce and prevent free radical damage or prevent oxidative damage. The combination of two or more plant species can result in a greater potential for antioxidant activity. The purpose of this study was to determine the IC_{50} value of the combination of Cinnamomum verum J. Presl and Zingiber officinale Roscoe with a ratio of 1:2, 1:1, and 2:1. Antioxidant activity was determined by the amount of DPPH absorption inhibition (% Inhibition) and the value (50% Inhibition Concentration). The IC_{50} value in antioxidant activity measurement of ethanol extract combination of Cinnamomum verum J. Presl and Zingiber officinale Roscoe in the ratio of 1:2, 1:1, and 2:1, was 372.3078 ppm, 354,3077 ppm, and 344.0863 ppm respectively. The ethanol extract of Cinnamomum verum J. Presl and Zingiber officinale Roscoe at a ratio of 2:1 was the best combination with total phenolic content of 5.63 mgGAE/100 g and produced an IC_{50} value of 344.0863 ppm.

Keywords: Antioxidant, cinnamon, IC_{50}, ginger, phenolic

Introduction

Indonesia has a variety of plants that have biological activity for treatment, one of which is as an anti-free radical. Free radicals are reactive molecules because they have unpaired electrons. Free radicals can trigger chronic tissue damage and cause various diseases such as Alzheimer's, hypertension, rheumatoid arthritis, cancer, diabetes mellitus, asthma, stroke, immunodepression, etc (Arifin et al., 2019). Antioxidants are substances that can reduce and prevent damage caused by free radicals or inhibit oxidative damage (Mahantesh et al., 2012).

Antioxidants stabilize free radicals by complementing the electron deficiency of free radicals and inhibit the chain reaction of the formation of free radicals that can cause oxidative stress (Wibawa et al., 2020). Natural antioxidants can be obtained from plants or fruits that contain secondary metabolites in the form of flavonoids and phenols which are useful as free radical scavengers. Phenolics are secondary metabolites that are widely distributed in plants where these compounds have a role in antioxidant activity. The greater the content of the phenol group compounds, the greater the antioxidant activity (Nur et al., 2019). Phenol compounds have hydroxyl groups that can donate hydrogen atoms so that they can neutralize free radical compounds to become more stable compounds.

The combination of two or more plant species can result from greater potential for antioxidant activity. Each plant has secondary metabolites that can interact with each other. These interactions have the effect of increasing potency at small concentrations or may weaken each other (Marianne et al., 2018). Several studies of antioxidants by combining plants have been carried out to increase antioxidant potentials such as soursop leaves and guava leaves extract (Wicaksono & Ulfah, 2017), ant-house plants, and rat taro plants (Wimpy & Harningsih, 2017), roses flowers and jicama tubers (Husna et al., 2018). Ginger contains flavonoids, polyphenols, malic acid, oxalic acid, and essential oils. The active substances in essential oils include shogaol, gingerol, and zingerone (Aryanta, 2019) so they have the potential as antioxidants. Besides ginger, cinnamon also has the potential as an antioxidant because it contains alkaloids, flavonoids, polyphenols, and saponins (Sufiana & Harlia, 2014).

Based on the description above, the researchers measure the total phenolic content and determine the antioxidant activity of the extract combination of *Cinnamomum verum* J. Presl and

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Zingiber officinale Roscoe. The combination of cinnamon bark extract and ginger rhizome was made in a ratio of 1:2, 1:1, and 2:1. Total phenolic content assay was carried out using the Folin-Ciocalteau reagent and Visible Spectrophotometry method. While the antioxidant activity of the sample was determined by calculating the percentage of DPPH absorption inhibition (% inhibition) and the IC_{50} value (50% Inhibition Concentration). IC_{50} is the concentration of an antioxidant substance that can cause 50% of DPPH to lose its radical character or the concentration of an antioxidant substance that provides 50% inhibition percent.

Methods

Simplicia preparation

The whole ginger and cinnamon bark that was still undamaged and fresh was selected, then the ginger and cinnamon bark were cleaned. Cinnamon and ginger bark were washed in running water, and after that, the ginger and cinnamon bark were chopped and dried. The ginger and cinnamon bark was dried in direct sunlight and covered with a black cloth. The dried ginger and cinnamon bark were mashed using a blender, then sieved using a 30/40 mesh sieve.

Making ethanol extract of cinnamon bark and ginger rhizome

Cinnamon (*Cinnamomum verum*) bark and ginger (*Zingiber officinale*) rhizome simplicia that have been sifted, then weighed approximately 100 grams, then put in a closed container, then 1000 mL of 70% ethanol p.a were added as solvent until the simplicia was completely soaked. Maceration was carried out by maceration for 5 x 24 hours at room temperature. Decantation was done up to 5 times. The obtained macerate was collected in one container. The concentration process was carried out using a rotary vacuum evaporator at a temperature of 60 °C.

Phytochemical test

Phenolic identification

Ethanol extract solution of approximately 1 mL was added with 1 mL of iron (III) chloride solution forming a green, red, purple, blue, or black color which means contained phenolic compounds positively in the extract.

Polyphenol identification

The ethanol extract solution of approximately 1 mL was dripped with a mixture of potassium hexacyanoferrate (III) LP and iron (III) chloride solution LP. The presence of polyphenols was indicated by the formation of a blue-to-black color.

Identification of flavonoids

An ethanol extract solution of approximately 2 mL was added with some Zn powder and 2 mL of 2N HCl. Flavonoid compounds would form an orange-to-red color.

Identification of Tannins

The ethanol extract solution was approximately 1 mL added with a 0.5% gelatin solution. A precipitate would form indicating the presence of tannins in the extract.

Identification of saponins

Saponins could be detected by the formation of foam in a hot water. The foam formed would be stable for 30 minutes and would not disappear with the addition of 1 drop of 2N HCl indicating the presence of saponins.

Determination of total phenolic content of ethanol extract combination of cinnamon bark and ginger rhizome (1:2, 1:1, and 2:1)

The ethanol extract combination of cinnamon bark and ginger (1:2, 1:1, and 2:1) was pipetted 1 mL each, then the sample was added with 1.5 mL of Folin Ciocalteau reagent and left for 4-8 minutes, add 1.2 mL 7.5% Na_2CO_3 solution, then homogenized. Distilled water was added to 10 mL and let stand for 88 minutes at room temperature. Measured the absorption at the maximum wavelength of 775 nm.

Determination of the antioxidant activity of ethanol extract combination of cinnamon bark and ginger rhizome (1:2, 1:1, and 2:1)

Antioxidant activity test of ethanol extract combination of cinnamon bark and ginger rhizome was carried out using the DPPH method (1,1-diphenyl-2-picrylhydrazyl) which was made in 5 concentration series, namely 100, 200, 300, 400, and 500 ppm with a ratio of 1:2, 1:1 and 2:1. 3.8 mL of DPPH was taken, then added 0.2 mL of the sample and left in a dark room for 30 minutes. Next, the absorption was measured at a maximum wavelength of 516 nm.

Results and Discussions

Based on the tests carried out qualitatively and quantitatively, the following results were obtained:

Table 1. Results of phytochemical screening of ethanol extract combination of cinnamon bark and ginger rhizome

Secondary Metabolite	Ethanol Extract of Cinnamon Bark Ginger Rhizome			Description
	1:2	1:1	2:1	
Phenolic	+	+	+	The blue or black color was formed
Polyphenol	+	+	+	Blue to black color was formed
Flavonoids	+	+	+	Orange to red color was formed
Tannins	+	+	+	Precipitate was formed
Saponins	+	+	+	Foam was formed

Description:
(+) = contained the compound
(-) = did not contain the compound

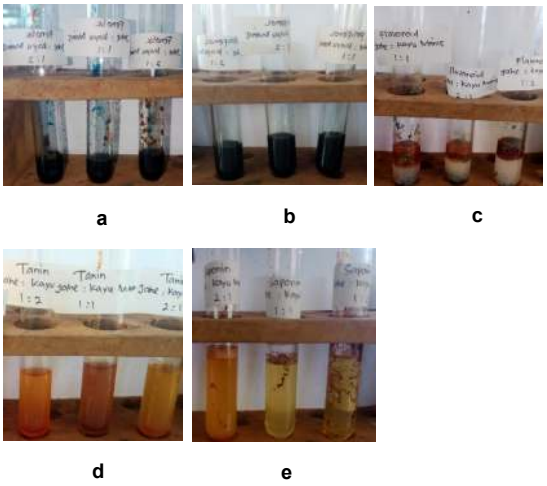

Figure 1. Phytochemical screening a) phenolics, b) polyphenols, c) flavonoids, d) tannins, and e) saponins

Phytochemical screening is a preliminary test that aims to describe the content of secondary metabolites in a natural material. Based on the test results in **Figure 1**, the ethanol extract combination of cinnamon bark and ginger rhizome contained secondary metabolites, including phenolics, polyphenols, flavonoids, tannins, and saponins. After the preliminary test, the total phenolic content and antioxidant activity were measured.

Total phenolic content ethanol extract cinnamon bark and ginger rhizome

Phenolic compounds are compounds in plants with the characteristics of having an aromatic ring containing at least one hydroxyl group. The smaller the IC_{50} value, the higher the antioxidant activity, this is related to phenolic compounds. According to (Khadijah et al., 2017), the phenolic content is directly proportional to the antioxidant activity. Flavonoids, anthocyanins, and tannins belong to the polyphenol group. While phenolic acid is a simple phenol. The presence of phenolic hydrogen is useful for scavenging free radicals and can provide antioxidant activity on some phenolic compounds.

The total phenolic compound can be determined by the Folin-Ciocalteu reagent. Folin-Ciocalteu reagent will react in the presence of phenolic compounds which will form a color change from yellow to blue. The amount of phenol content in the sample solution determines the intensity of the blue color produced. The more the content of phenolic compounds in the sample, the darker the blue color formed (Ismail et al., 2012). In the determination of total phenolics, the standard solution used is gallic acid (GAE). Gallic acid was chosen as the measurement standard because gallic acid is a derivative of hydroxybenzoic acid which is a simple phenolic acid. Gallic acid is also pure and stable (Lee et al., 2013). Based on the measurement of the standard gallic acid curve, a linear regression equation y = 0.0857x + 0.0581 was obtained with a correlation coefficient (r) of 0.9981 which could be seen in **Figure 2.**

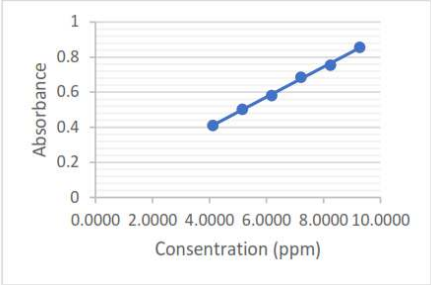

Figure 2. A gallic acid standard curve

Table 2. Total phenolic content of ethanol extract combination of cinnamon bark and ginger rhizome (1:2) concentration of 500 ppm

Replication	Total Phenolic Content (mg GAE/100 g)	Average of Total Phenolic Content (mgGAE/100 g)
1	5.12	
2	5.12	5.14
3	5.18	

Description: GAE = Gallic Acid Equivalent

Table 3. Total phenolic content of ethanol extract combination of cinnamon bark and ginger rhizome (1:1) concentration of 500 ppm

Replication	Total Phenolic Content (mg GAE/100 g)	Average of Total Phenolic Content (mgGAE/100 g)
1	4.69	
2	4.69	4.69
3	4.69	

Description: GAE = Gallic Acid Equivalent

Table 4. Total phenolic content of ethanol extract combination of cinnamon bark and ginger rhizome (2:1) concentration of 500 ppm

Replication	Total Phenolic Content (mg GAE/100 g)	Average of Total Phenolic Content (mgGAE/100 g)
1	5.64	
2	5.63	5.63
3	5.63	

Description: GAE = Gallic Acid Equivalent

Measurement of total phenolic content results of ethanol extract combination of cinnamon bark and ginger rhizome could be seen in **Table 2**, **Table 3,** and **Table 4**. The ethanol extract combination of cinnamon bark and ginger rhizome

with a ratio of 1:2 obtained a total phenolic of 5.14 mgGAE/100 g In a 1:1 ratio, the total phenolic content was 4.69 mgGAE/100 g and for a 2:1 ratio, the total phenolic was 5.63 mgGAE/100 g. The total phenolic content of the cinnamon extract in the study (Antasionasti & Jayanto, 2021) was greater than the ginger rhizome extract studied by Syafitri et al (2018). So that it is comparable to this study, where the total phenolic ratio of 2:1 is greater than the other comparisons. Because the composition of cinnamon extract is more compared to ginger rhizome extract.

Based on the total phenolic test results, it could be indicated that the combination of cinnamon bark extract and ginger rhizome has the potential as an antioxidant.

Antioxidant activity of extract combination of cinnamon bark and ginger rhizome

The phenolic content in the extract combination of cinnamon bark and ginger rhizome was used as the sample for testing the antioxidant activity. The amount of phenolic compounds that have the potential as antioxidants depends on the levels of phenolic compounds in the single extract.

Antioxidant measurements were carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The purpose of quantitative testing was to determine the absorbance of remaining DPPH after adding the extract which then had color degradation from purple to yellow. The DPPH color degradation process was directly proportional to the concentration of the extract. As antioxidants are added, the lone electrons in the DPPH pair with hydrogens from the antioxidants. The reaction between DPPH and neutral H atoms derived from compounds that are antioxidants can be seen in **Figure 3.**

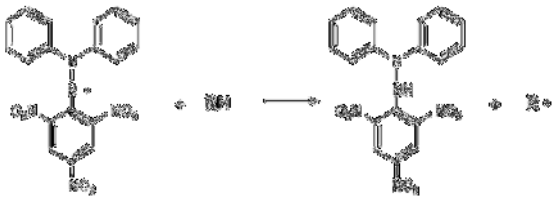

Figure 3. The reaction between DPPH and neutral H atoms is derived from antioxidants (Molyneux, 2004).

From the obtained DPPH absorbance value, it can be determined the percentage value of DPPH radical inhibition (% inhibition). From the value of % antioxidant activity, a linear regression calculation was carried out between % antioxidant activity versus the actual concentration of the test solution so that the 50% inhibitory concentration value (IC_{50}) could be determined. IC_{50} is a concentration that can provide a percentage of free radical scavenging as much as 50%, the smaller the IC_{50} value of a sample, the greater the antioxidant activity (Widyasanti et al., 2016).

Table 5. Antioxidant activity of ethanol extract combination of cinnamon bark and ginger rhizome (1:2)

Replication	% Inhibition	IC_{50} (ppm)	Average of IC_{50} (ppm)
I	21.06 38.02 46.09 53.45 60.04	373.4641	
II	29.13 38.54 43.04 51.78 58.01	373.2754	372.3078
III	29.53 37.35 41.98 49.13 56.82	370.1842	

Table 6. Antioxidant activity of ethanol extract combination of cinnamon bark and ginger rhizome (1:1)

Replication	% Inhibition	IC_{50} (ppm)	Average of IC_{50} (ppm)
I	26.21 38.26 47.25 54.55 61.94	355.4693	
II	26.55 38.26 47.35 54.65 62.04	355.5610	354.3077
III	26.77 38.36 47.41 54.85 62.24	351.8930	

Table 7. Antioxidant activity of ethanol extract combination of cinnamon bark and ginger rhizome (2:1)

Replication	% Inhibition	IC_{50} (ppm)	Average of IC_{50} (ppm)
I	22.18 30.09 50.05 57.94 66.03	344.8032	
II	22.28 30.17 49.67 58.14 66.09	344.2000	344.0863
III	22.32 30.27 49.95 49.13 56.82	343.2548	

Antioxidant activity test results of ethanol extract combination of cinnamon bark and ginger rhizome could be seen in **Tables 5**, **6,** and **7**. The combination with a ratio of 1:2 obtained an IC_{50} value of 372.3078 ppm, at a ratio of 1:1 obtained an IC_{50} value of 354.3077 ppm and a ratio of 2:1 obtained an IC_{50} value of 344.0863 ppm. From the results of the study, there was a linear relationship between the amount of total phenolic content and antioxidant activity in the form of percentage inhibition. Ethanol extract combination of cinnamon bark and ginger rhizome in a ratio of 2:1 contained more total phenolics than the combination ratio of 1:1 and 1:2. This gave a relationship to its antioxidant activity, where the IC_{50} value in a ratio of 2:1 was 344.0863 ppm, this was smaller than the ratio of 1:1 and 1:2. The IC_{50} value obtained ranges from 200-1000 g/mL, so the sample was less active but still had potential as an antioxidant (Yulis & Sari, 2020). The antioxidant activity of the combination of cinnamon and ginger rhizome extracts is contributed by the total phenolic

content in the single extract. The phenolic content of cinnamon is greater than that of ginger. So that the cinnamon composition produces a lower IC50 value than the 1:1 and 1:2 ratios.

Conclusion

Based on the results of the study, it was concluded that the combination of ethanol extract of *Cinnamomum verum* J. Presl and *Zingiber officinale* Roscoe at a ratio of 2:1 was the best combination with a total phenolic content of 5.63 mgGAE/100 g and IC_{50} value was 344.0863 ppm.

References

Antasionasti, I., & Jayanto, I. (2021). Aktivitas antioksidan ekstrak etanol kayu manis (cinnamomum burmani) secara in vitro. *Jurnal Farmasi Udayana*, *10*(1), 38-47.

Arifin, A. S., Yuliana, N. D., & Rafi, M. (2019). Aktivitas antioksidan pada beras berpigmen dan dampaknya terhadap kesehatan. *Pangan*, *28*(1), 11–22.

Aryanta, I. W. R. (2019). Manfaat jahe untuk kesehatan. *E-Jurnal Widya Kesehatan*, *1*(2), 39–43.

Husna, M., Hajrah., & Rijai, L. (2018). Uji aktivitas antioksidan kombinasi ekstrak bunga mawar (rosa damascena mill) dan umbi bengkoang (pachyrizus erosus). *Proceeding of the 8th Mulawarman Pharmaceuticals Conferences* (pp. 63-67). Samarinda: Fakultas Farmasi Universitas Mulawarman.

Ismail, J., Runtuwene, M. R. J., & Fatimah, F. (2012). Penentuan total fenolik dan uji aktivitas antioksidan pada biji dan kulit buah pinang yaki (areca vestiaria giseke). *Jurnal Ilmiah Sains*, *12*(2), 84-88.

Khadijah., Jayali, A. M., Umar, S., & Sasmita, I. (2017). Penentuan total fenolik dan aktivitas antioksidan ekstrak etanolik daun samama (anthocephalus macrophylus) asal Ternate, Maluku Utara. *Jurnal Kimia Mulawarman*, *15*(1), 11-18.

Lee, J. H., Park, K. H., Lee, M. H., Kim, H. T., Seo, W. D., Kim, J. Y., Baek, I. Y., Jang, D. S., & Ha, T. J. (2013). Identification, characterisation, and quantification of phenolic compounds in the antioxidant activity-containing fraction from the seeds of Korean perilla (perilla frutescens) cultivars. *Food Chemistry*, *136*(2), 843–852.

Mahantesh, S. P., Gangawane, A. K., & Patil, C. S. (2012). Free radicals, antioxidants, diseases and phytomedicines in human health: Future perspects. *World Research Journal of Medicinal & Aromatic Plants*, *1*(1), 6–10.

Marianne., Patilaya, P., & Barus, B. T. (2018). Uji aktivitas antioksidan kombinasi ekstrak etanol rimpang temu giring (curcuma heyneana) dan daun pugun tanoh (curanga fel-terrae) menggunakan metode diphenyl picrylhydrazil (DPPH). *Talenta Conference Series: Tropical Medicine* (TM) (pp. 398-404). Medan: Universitas Sumatera Utara.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(2), 211–219.

Nur, S., Sami, F. J., Wilda, R., Awaluddin, A., & Afsari, M. I. A. (2019). Korelasi antara kadar total flavonoid dan fenolik dari ekstrak dan fraksi daun jati putih (gmelina arborea roxb.) terhadap aktivitas antioksidan. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy)*, *5*(1), 33–42.

Sufiana., & Harlia. (2014). Uji aktivitas antioksidan dan sitotoksisitas campuran ekstrak metanol kayu sepang (caesalpinia sappan l.) dan kulit kayu manis (cinnamomum burmannii b.). *Jurnal Kimia Khatulistiwa*, *3*(2), 50–55.

Syafitri, D. M., Levita, J., Mutakin, M., & Diantini, A. (2018). A review: is ginger (zingiber officinale var. roscoe) potential for future phytomedicine? *Indonesian Journal of Applied Sciences*, *8*(1), 1-6.

Wibawa, J. C., Arifin, M. Z., & Herawati, L., (2020). Mekanisme vitamin C menurunkan stres oksidatif setelah aktivitas fisik. *JOSSAE: (Journal of Sport Science and Education)*, *5*(1), 57-63.

Wicaksono, I. B., & Ulfah, M. (2017). Uji aktivitas antioksidan kombinasi ekstrak etanol daun sirsak (annona muricata l.) dan daun jambu biji (psidium guajava l.) dengan metode DPPH (2,2-difenil-1-pikrilhidrazil). *Inovasi Teknik Kimia*, *2*(1), 44–48.

Widyasanti, A., Rohdiana, D., & Ekatama, N. (2016). Aktivitas antioksidan ekstrak teh putih (camellia sinensis) dengan metode DPPH (2,2 difenil-1-pikrilhidrazil). *Journal Fortech*, *1*(1), 1–9.

Wimpy., & Harningsih, T. (2017). Uji aktivitas antioksidan kombinasi ekstrak sarangsemut (myrmecodia pendans) dan ekstrak keladi tikus (typhonium flagelliforme lodd.). *Jurnal Kesehatan Kusuma Husada, 8*(1), 35-41.

Yulis, P. A. R., & Sari, Y. (2020). Aktivitas antioksidan dari limbah kulit pisang muli (musa acuminata linn) dan kulit pisang kepok (musa paradisiaca formatypica). *Al-Kimia*, *8*(2), 189–200.