

Phytochemical Screening and Total Phenolic Compounds Assay of Red Ginger (*Zingiber officinale*) and Secang Wood (*Caesalpinia sappan*) as Antiarthritic

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Abstract

Arthritis rheumatoid adalah penyakit auto-immune yang menyebabkan terjadinya inflamasi kronis pada persendian yang ditandai dengan adanya kelebihan produksi sitokin proinflamasi seperti TNF- α dan IL-1. Senyawa fenolik terutama antosianidin dan elagitanin memiliki aktivitas penghambatan TNF- α yang menginduksi ekspresi siklooksigenase-2 penyebab inflamasi. Skrining fitokimia menunjukkan bahwa ekstrak etanol jahe merah memiliki kandungan senyawa alkaloid, fenolik, flavonoid, tannin, terpenoid, dan steroid sedangkan ekstrak etanol kayu secang memiliki kandungan senyawa alkaloid, fenolik, flavonoid, tannin, dan terpenoid. Penelitian ini bertujuan untuk mengetahui kadar fenolik total pada jahe merah dan kayu secang dengan metode Folin-Ciocalteu. Kadar fenolik total dinyatakan dalam *Gallic Acid Equivalent* (GAE). Pengujian keduanya menggunakan spektrofotometer UV-Vis. Kadar fenolik total yang didapatkan pada ekstrak etanol jahe merah sebesar 21,90 mgGAE/g ekstrak dan kadar fenolik total ekstrak etanol kayu secang sebesar 27,65 mgGAE/g ekstrak. Senyawa fenolik yang terkandung pada jahe merah dan kayu secang ini berpotensi sebagai antiarthritic

Kata kunci: fitokimia, fenolik, jahe merah, kayu secang

Rheumatoid arthritis is an auto-immune disease that causes chronic inflammation occurs of the joints. Rheumatoid arthritis is characterized by the the overproduction of proinflammation cytokines such as TNF- α and IL-1. The phenolic compounds mainly anthocyanin and elagitanin have TNF- α inhibition activity that induces cyclooxygenase-2 expressions that cause inflammation. Phytochemical screening showed that ethanol extract of red ginger contains alkaloids, phenolics, flavonoids, tannins, terpenoid, and steroids while ethanol extract of secang wood contains alkaloids, phenolics, flavonoids, tannins, and terpenoid. This research aims to identify total phenolic compounds on red ginger and secang with Folin-Ciocalteu method. Total phenolic compounds are defined as the Gallic Acid Equivalent (GAE). Both tests using UV-Vis spectrophotometer. Total phenolic compounds in red ginger ethanol extracts is 21,90 mg GAE/g extract and total phenolic secang wood ethanol extract is 27,65 mg GAE/g extract. The phenolic compounds in red ginger and secang wood have the potential as antiarthritic

Keywords: phytochemical, phenolic, red ginger, secang wood

1. INTRODUCTION

Rheumatoid arthritis is an auto-immune disease that causes chronic inflammation occurs of the joints. Rheumatoid arthritis is characterized by the overproduction of proinflammation cytokines such as TNF- α and IL-1 (Wang et al., 2011) . According to WHO, the incidence of rheumatoid arthritis in 2016 reached 20% of the world's population, while the number of arthritis sufferers in Indonesia reached 67,4% for independent elderly (Riskesdas, 2018). Bioactive compounds such as phenols have an important role in human health, due to their pharmacological activity (Chandrasekara & Shahidi, 2018).

Phenol is a secondary metabolic compound that are widely found in plants. Phenolic compounds in nature have a variety of structures such as flavonoids, phenols, polyphenols (lignins, melanins, tannins) (Tahir, Muflihunna, et al., 2017). Phenolic compounds in plants have several pharmacological effects such as antioxidant, anti-inflammatory, anti-proliferative, antimutagenic, antimicrobial, anti-carcinogenic, and prevention of heart disease. So that, phenolic compounds have the potential to be used as a natural active ingredient in pharmaceutical industries.

Red ginger (*Zingiber officinale*) is a type of rhizome that is often used as traditional medicine. Secondary metabolite compounds of red ginger especially from the flavonoid, phenol, and terpenoid groups (Azkiya et al., 2017). Red ginger contains many phenolic compounds such as gingerol and shogaol (Susanti & Panunggal, 2015). The gingerol and shogaol are found as [6]-gingerol and [6]-shogaol which provide pharmacological effects such as anti-inflammatory and antioxidants (Riduan, 2015).

Secang wood (*Caesalpinia sappan*) is a plant belonging to the Caesalpiniaceae family. Secondary metabolite compounds in secang wood are flavonoids and phenols such as O-methylsappanol, protosappanin A, protosappanin B, protosappanin E, brazilin, and others (Batubara et al., 2010). Secang wood showed pharmacological effects such as anti-hyperglycemic, anti-hypercholesterolemic, antihepatotoxic, anti-inflammatory, and sedative activity (Jung et al., 2015).

Phenolic compounds in red ginger and secang wood have the potential to be anti-inflammatory as an alternative treatment for rheumatoid arthritis. Phenolic compounds such as brazilin in secang and gingerol in red ginger have TNF- α inhibitory activity which induces the expression of cyclooxygenase-2 which causes inflammation (Lansky & Newman, 2007). Based on this background, a preliminary test of total phenolic compounds of red ginger and secang wood was carried out before testing the pharmacological effects of anti-arthritis.

2. RESEARCH METHODS

Tools

Tools used in this research are glassware, vortex, micropipette, rotatory evaporator, freeze dryer, and UV-Vis spectrophotometer.

Materials

Materials that used in this research are rhizome of red ginger (*Zingiber officinale*), secang wood (*Caesalpinia sappan*), ethanol 96%, distilled water, gallic acid, sodium carbonate, Folin-Ciocalteu reagent, FeCl₃, HCl, magnesium, Methanol, H₂SO₄ ammonia, acetic acid anhydride, Mayer reagent, Wagner Reagent, and Dragendorff reagent.

Procedures

A. Extraction

Extraction of red ginger and secang wood using maceration method with ethanol as solvent. In this process, 500 grams of powdered red ginger and secang wood are placed in a covered container with ethanol solvent. The maceration was carried out for 3 days and occasionally stirred. The filtrate obtained then evaporated using a rotatory evaporator to obtain an ethanolic extract of red ginger and secang wood.

B. Phytochemical screening

The ethanol extract of red ginger rhizome and secang wood were subjected to different chemical tests to detect bioactive compounds such as alkaloids, flavonoids, phenolic, steroid, saponins, tannins, and terpenoids.

Alkaloid

1 ml chloroform and 1 ml ammonia were added to 1 mg sample then heated and shaken. The solution was filtered and the filtrate was divided into four test tubes. Each test tube was identified by Mayer, Wagner, and Dragendorff reagent. The other one tube is a blanco. The positive result is white, brown, and orange in the sample.

Flavonoid

1 mg ethanol extract diluted in 3 ml ethanol 96%, 2 drops HCl and small amount of Mg were added. Appearance of red, yellow, orange, or blue colour indicated the presence of flavonoids.

Phenolic

To 1 mg of ethanol extract, 5 drops methanol and 3 drops FeCl₃ 5% were added. Formation of blue, green, black, violets, or red indicated the presence of phenols.

Saponins

In a test tube, extract was mixed with 5 ml of distilled water and shake. Formation of stable foam indicated the presence of saponins.

Steroids

Acetic acid anhydride 2 ml was added to 1 mg ethanol extract then 2 ml H₂SO₄ was added. The positive result is blue or green in sample.

Tannins

1 mg sample diluted in 10 ml of distilled water then heated. Filtrate obtained was added 2-3 drops FeCl₃ 1%. Formation of blue or green indicated the presence of tannin.

Terpenoids

2 ml chloroform and 3 ml concentrated H₂SO₄ were added to 1 mg sample. Appearance of brownish-red indicated the presence of terpenoid.

C. Determination of total phenolic compounds

Total phenolic compound was determined quantitatively using Folin-Ciocalteu reagent with gallic acid as the standard.

1. Determination of maximum wavelength

Determination of maximum wavelength using gallic acid 50 ppm as standard which has been added with 1,5 ml Folin-Ciocalteu and mixed for 3 minutes, followed by addition 1,2 ml of sodium carbonate 7,5%. The absorbance was measured at wavelength of 500-900 nm

2. Determination curve of gallic acid

Determination of the standard curve of gallic acid was carried out by mixing 0.5 ml of 10, 20, 30, 40, and 50 ppm gallic acid solution with 10% Folin-Ciocalteu reagent for 3 minutes in a test tube. Each tube was added to 1.2 ml of 7.5% Na₂CO₃ and vortexed for 3 seconds. After 30 minutes, the absorbance was measured at 769 nm. The calibration curve was made between concentration (ppm) and absorbances.

3. Determination of total phenolic compounds of red ginger extract and secang wood extract

1 mg ethanol extract of red ginger and secang wood, respectively dissolved to 10 ml with a mixture of ethanol and distilled water (1:1). Then 0,5 ml of each ethanol extract was mixed with the same reagent, as performed to determine calibration curve. After 30 minutes, the absorbance was measured at 769 nm.

3. RESULTS AND DISCUSSION

A. Extraction of red ginger and secang wood

Extraction is the first step in purification and isolation of bioactive compounds in plant material (Ćujić et al., 2016) Extraction of red ginger and secang wood using maceration method with ethanol 96%. The advantages of maceration method is easy and does not require high temperatures which are likely to damage chemical compounds that have several bioactivities (Ramadhan et al., 2020). Ethanol 96% is a solvent that has effectiveness to extracting secondary metabolites and has good penetration ability on the hydrophilic and lipophilic side so that it penetrates the cell membranes and enters the cell to interact with secondary metabolites (Andriani & Murtiswi, 2018).

The extract obtained was calculated the yield. Yield determination aims to determine the content of secondary metabolites carried by the solvent during maceration. The yield of red ginger rhizome extracts in ethanol was 4,10% while the yield of secang wood extracts in ethanol was 9,28%. Data of extract yields from each simplicia is presented in table 1.

Table 1. Weight of Simplicia and Extract Yields

Extract	Simplicia's weight (g)	Extract's weight (g)	Yield (%)
Red ginger	500	20,53	4,10%
Secang wood	500	46,39	9,28%

B. Phytochemical screening

Phytochemical are the medicinally active compounds founds in part of plants such as roots, leaves, ginger, barks, rhizome, and other (Bandiola, 2018). Phytochemical screening of ethanolic extract of red ginger and secang wood was conducted qualitatively to find out the class of compounds found in a plant (Supomo et al., 2019). Phytochemical test results showed that the ethanol extract of red ginger contains Phytochemical test results showed that the ethanol extract of red ginger contains alkaloids, flavonoids, phenolics, tannins, terpenoids, and steroids, while the ethanol extract of secang wood contains alkaloids, flavonoids, phenolics, tannins, and terpenoids. The results of the phytochemical test of the ethanol extract of red ginger and secang wood are presented in table 2 and table 3.

Both the extracts showed the presence of phenolic.

Table 2. Phytochemical screening ethanol extract of red ginger

Uji	Hasil	Kesimpulan
Alkaloids	Orange precipitate with Dragendorff reagent, white color with Mayer reagent	+ alkaloids
Flavonoids	Formed a yellow color	+ flavonoids
Phenolics	Formed a greenish color	+ phenols
Saponins	No stable foam is formed	- saponins
Steroids	Formed a greenish color	+ steroids
Tannins	Formed a turquoise colour	+ tannins
Terpenoids	Formed a brownish-red color	+ terpenoids

Keterangan: (+) indicates present, (-) not detected

Table 3. Phytochemical screening ethanol extract of secang wood

Uji	Hasil	Kesimpulan
Alkaloids	Orange precipitate with Dragendorff reagent, no precipitate is formed with Mayer and Wagner reagent	+ alkaloids
Flavonoids	Forming an orange color	+ flavonoids
Phenolics	Forming a black color	+ phenols
Saponins	No stable foam is formed	- saponins
Steroids	No discoloration	- steroids
Tannins	Formed a turquoise colour	+ tannins
Terpenoids	Formed a brownish-red color	+ terpenoids

Keterangan: (+) indicates present, (-) not detected

C. Determination of total phenolic compounds

Total phenolic compounds in samples were analyzed quantitatively using Folin-Ciocalteu method. This method is commonly used because simple and easy to do. Folin-Ciocalteu reagent contains phosphomolybdic/phosphotungstic acid complexes.

The principle of the Folin-Ciocalteu method is the oxidation of phenolic hydroxyl groups and reduction of phosphomolybdates in alkaline conditions by the aromatic nuclei of phenol compounds to form a molybdenum tungsten complex (Senet et al., 2018) The reaction occurs in alkaline conditions so that sodium carbonate is needed to make alkaline conditions. Alkaline conditions convert phenolic compounds into phenolic ions due to proton dissociation. The phenolic-hydroxyl group reacts with the Folin-Ciocalteu reagent to form blue

phosphomolybdates-phosphotungstate. The blue color will get darker in line with the concentration of phenolic ions formed. The greater the phenolic concentration, the more phenolic ions will reduce heteropoly acids so as to produce a darker blue color (Muchsin et al., 2016) The reaction is presented in figure 1.

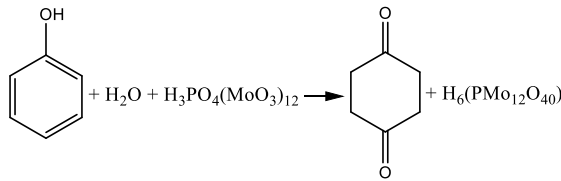


Figure 1. The Reaction between phenolic with Folin-Ciocalteu (Ikram et al., 2017)

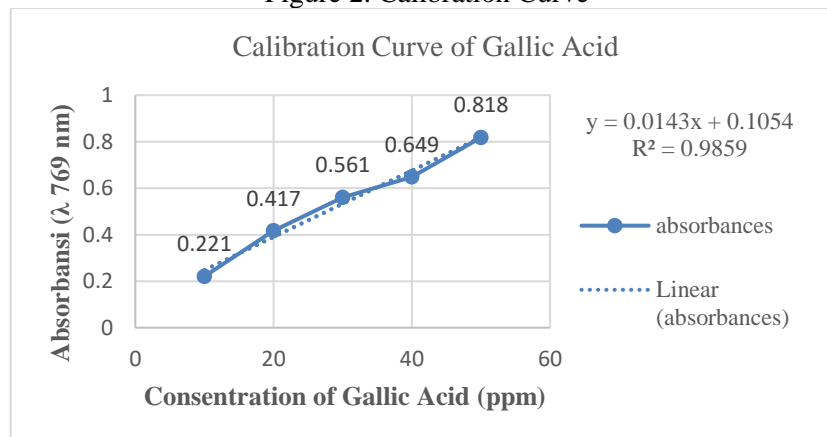
Gallic acid is a derivative of hydroxybenzoic which is a natural and stable phenolic. Gallic acid also a standard solution used to determination of total phenolic compounds (Ikram et al., 2017) Gallic acid reacts with the Folin-Ciocalteu reagent to produce a yellow solution which indicates the presence of phenolics, then Na₂CO₃ is added as an alkaline condition.

Standard gallic acid curves were made with various concentrations of 10, 20, 30, 40, and 50 ppm. The absorbance measurement using a UV-Vis spectrophotometer was carried out at 769 nm. The standard absorbance value of gallic acid can be seen in table 4 and the calibration curve can be seen in fig 2.

Table 4. the absorbance of gallic acid

Konsentrasi (ppm)	Absorbansi
10	0,221
20	0,471
30	0,561
40	0,649
50	0,818

Figure 2. Calibration Curve



Linear relationship was obtained when a graph was plotted for concentration vs absorbance. The concentration range 10-50 ppm with a correlation coefficient value $R^2 = 0,9850$ and the linear regression equation was $y = 0,0143x + 0,1054$. Total Phenolic Compounds (TPC) with the Folin Ciocalteu method refers to GAE. Gallic Acid Equivalent (GAE) is a reference for measuring the amount of phenolic compounds present in a material (Phuyal et al., 2020) Total phenolic compounds were expressed as gram of gallic acid equivalent/ g of ethanolic extract. The results of the measurement of total phenolic compounds are presented in table 5.

Table 5. Determination Total Phenolic Compounds

Extract	Absorbances (y)	Concentration (mg/L)	Total phenolic compounds (mg GAE/g extract)
Red ginger	0.7318	0,0438	21,90
Secang wood	0.8962	0,0553	27,65

Based on the results of the research that has been done, the total phenolic compounds of the ethanol extract of red ginger were 21.90 mgGAE / g of extract and the total phenolic compounds of the ethanol extract were 27.65 mgGAE / g of extract. It means that for every gram of red ginger ethanol extract, there is phenolic compounds equivalent to 21.90 mg of gallic acid and every gram of ethanol extract of secang wood has phenolic which is equivalent to 27.65 mg of gallic acid. The presence of phenolic compounds in red ginger and secang wood indicates that the ethanol extract of red ginger and secang wood has potential as anti-arthritis.

4. CONCLUSIONS

Determination of phenolic compounds using the Folin-Ciocalteu method, the total phenolic compounds of red ginger (*Zingiber officinale*) extract was 21.90 mgGAE / g extract and the total phenolic compounds of secang wood ethanol extract (*Caesalpinia sappan*) was 27.65 mgGAE / g extract. Thus, the phenolic compounds in red ginger and secang wood have the potential to be anti-arthritis.

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