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Phytochemical Screening and Total Phenolic Compounds of Red Ginger (*Zingiber officinale*) and Secang Wood (*Caesalpinia sappan*) As Preliminary Test of Antiarthritis

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Abstract: 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 the secondary metabolite qualitatively and total phenolic compunds on red ginger and secang wood with Folin-Ciocalteu method. Total phenolic compounds are defined as the Gallic Acid Equivalent (GAE). The results showed that the total phenolic compunds of red ginger and sappan wood were 21.90 mg GAE/g extract and 27.65 mg GAE/g extract, respectively. The phenolic compounds in red ginger and secang wood have the potential as antiarthritic.

Keywords: phytochemical, total phenolic content, red ginger, secang wood

Abstrak: *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 senyawa metabolit sekunder dan kadar fenolik total pada jahe merah dan kayu secang dengan metode Folin-Ciocalteu. Kadar fenolik total dinyatakan dalam Gallic Acid Equivalent (GAE). Hasil penelitian menunjukkan bahwa kadar fenolik total jahe merah dan kayu secang masing-masing sebesar 21,90 mg GAE/g ekstrak dan 27,65 mg GAE/g ekstrak. Senyawa fenolik yang terkandung pada jahe merah dan kayu secang ini berpotensi sebagai antiarthritis.*

Kata kunci: *fitokimia, kadar fenolik total, jahe merah, kayu secang*

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 is widely found in plants. Phenolic compounds in nature have a variety of structures such as flavonoids, phenols, polyphenols (lignins, melanins, tannins) (Tahir *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. Therefore, 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 (Ridhuan 2015).

Secang (*Caesalpinia sappan*) is a plant belonging to the Caesalpiniaceae family. Secang is one of the plants used as a natural pigment. The part of the secang plant that often used is secang wood. 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). Brazilin is the main component in secang wood which plays an important role into pharmacological effect. 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. Inflammation is triggered by oxidative stress which induces oxidative tissue damage and also cause by excessive cytokines production. One of the pro-inflammatory cytokines that play an important role is Tumor Necrosis Factor- α (Fitriyah *et al.* 2020). 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.

MATERIALS AND METHODS

Tools and Materials

Tools used in this research are glassware, vortex, micropipette, rotatory evaporator (Buchi R-300), freeze dryer (Alpha 1-2 LDplus-Martin Christ), and UV-Vis spectrophotometer (Shimadzu UV-1800 UV Vis).

Materials that used in this research are rhizome of red ginger (*Zingiber officinale*) and secang wood (*Caesalpinia sappan*) obtained from Wonokromo DTC market Surabaya, ethanol 96%, distilled water, gallic acid (Merck), sodium carbonate, Folin-Ciocalteu reagent, ferric chloride, chloric acid, magnesium, methanol, sulfuric acid, ammonia, acetic acid anhydride, Mayer reagent, Wagner reagent, and Dragendorff reagent.

Sample Preparation

Secang wood and red ginger obtained from the Wonokromo DTC market, Surabaya, East Java. Next, the samples were cleaned and cut into small pieces then grinded to powder.

Extraction

Extraction of red ginger and secang wood was performed using maceration method using ethanol 98% 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 was then evaporated using a rotatory evaporator to obtain an ethanolic extract of red ginger and secang wood.

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 Dragondorff reagent. The other one tube is a blank. The positive result is white, brown, and orange in the sample (Prahasti & Hidajati 2019).

Flavonoid

1 mg ethanol extract diluted in 3 mL 96% ethanol, 2 drops chloric acid and small amount of magnesium were added. Appearance of red, yellow, orange, or blue colour indicated the presence of flavonoids (Gowri & Vasantha 2010).

Phenolic

To 1 mg of ethanol extract, 5 drops methanol and 3 drops Ferric chloride 5% were added. Formation of blue or green indicated the presence of phenols (Gowri & Vasantha 2010).

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 (Prahasti & Hidajati 2019).

Steroids

Acetic acid anhydride 2 mL was added to 1 mg ethanol extract then 2 mL sulfuric acid was added. The positive result is blue or green in sample (Prahasti & Hidajati 2019).

Tannins

1 mg sample diluted in 10 mL of distilled water then heated. Filtrate obtained was added 2-3 drops ferric chloride 1%. Formation of blue or green indicated the presence of tannin (Prahasti & Hidajati 2019).

Terpenoids

2 mL chloroform and 1 mL concentrated sulfuric acid were added to 1 mg sample. Appearance of reddish-brown indicated the presence of terpenoid (Gowri & Vasantha 2010).

Determination of total phenolic compounds

Total phenolic content was determined quantitatively using Folin-Ciocalteu reagent with gallic acid as the standard. Determination of total phenolic content was performed using UV-Vis spectrophotometer.

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 (Andriani & Murtiswi 2018).

Determination standard 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-Ciocelteau reagent for 3 minutes in a test tube. Each tube was added to 1.2 mL of 7.5% sodium carbonate and vortexed for 3 seconds. After 30 minutes, the absorbance was measured at 769 nm. The standart curve was made between concentration (ppm) and absorbances (Andriani & Murtiswi 2018).

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 (Andriani & Murtiswi, 2018).

RESULTS AND DISCUSSIONS

Extraction of Red Ginger and Secang Wood

Extraction is the first step in purification and isolation of bioactive compounds in plant material (Čujić *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 is a solvent that is effective for extracting secondary metabolites and has good penetration ability on the hydrophilic and lipophilic site so that it penetrates the cell membranes and enters the cell to interact with secondary metabolites (Andriani & Murtiswi 2018).

Red ginger and secang wood extracts are brownish yellow and red, respectively. The yield of the extract obtained was calculated. Yield determination aims to determine the extract content 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%

Phytochemical Screening

Phytochemical are the medicinally active compounds founds in part of plants such as roots, leaves, seed, 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 screening 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 screening 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.

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. The principle of the Folin-Ciocalteu method is the reduction of phosphomolybdates in alkaline conditions by the hydroxyl group 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 complexes. The blue color will get darker in line with the concentration of phenolic ions formed. The greater

Table 2. Phytochemical screening ethanol extract of red ginger

Test	Observation	Results
Alkaloids	Orange precipitate with Dragendorff reagent, white color with Mayer reagent	positive
Flavonoids	Formed a yellow color	positive
Phenolics	Formed a greenish blue color	positive
Saponins	No stable foam is formed	negative
Steroids	Formed a greenish blue color	positive
Tannins	Formed a bluish green colour	positive
Terpenoids	Formed a brownish red color	positive

Table 3. Phytochemical screening ethanol extract of secang wood

Tests	Observation	Results
Alkaloids	Orange precipitate with Dragendorff reagent, no precipitate is formed with Mayer and Wagner reagent	positive
Flavonoids	Forming an orange color	positive
Phenolics	Forming a dark blue color	positive
Saponins	No stable foam is formed	negative
Steroids	Discoloration	negative
Tannins	Formed a bluish green colour	positive
Terpenoids	Formed a brownish red color	positive

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.

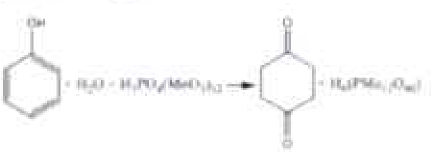

Figure 1. The Reaction between phenolic with Folin-Ciocalteu (Khadijah *et al.* 2017)

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 (Khadijah *et al.* 2017). Gallic acid reacts with the Folin-Ciocalteu reagent to produce a yellow solution which indicates the presence of phenolics, then Na_2CO_3 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 Figure 2.

Linear relationship was obtained when a graph was plotted for concentration vs absorbance. The concentration range 10-50 ppm with a correlation

Table 4. The absorbance of gallic acid measured with Folin-Ciocalteu reagent

Concentration (ppm)	Absorbance
10	0.221
20	0.471
30	0.561
40	0.649
50	0.818

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.

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 mg GAE / g of extract and the total phenolic compounds of the ethanol extract were 27.65 mg GAE / 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. Siregar & Verena (2019) and Koleangan *et al.* (2021) reported that total phenolic compounds in red ginger and secang wood are 21.58 mg GAE/g extract and 28.58 mg GAE/g extract. The amounts of phenolic

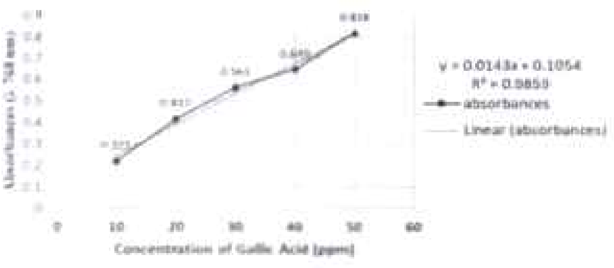

Figure 2. Standard curve of gallic acid measured with Folin-Ciocalteu method

Table 5. Total Phenolic content of red ginger and secang wood extract determined with Folin-Ciocalteu method

Extract	Absorbance	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

compounds are depending by several factors such as location, harvest time, and plant age.

The presence of phenolic compounds in red ginger and secang wood plays an important role in inhibit inflammation by mechanism free radical scavenging and inhibition cyclooxygenase enzymes. Phenolic compounds can catch free radicals that cause the occurrence tissue damage that will trigger the biosynthesis of arachidonic acid to inflammatory mediators, namely prostaglandin (Verseny & Nugroho 2011). Prostaglandins are inflammatory mediators associated with joint damage leading to inflamed joints in rheumatoid arthritis.

CONCLUSIONS

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. Determination of phenolic compounds using the Folin-Ciocalteu method, the results showed that the total phenolic compounds of red ginger and secang wood were 21.90 mg GAE/g extract and 27.65 mg GAE/g extract, respectively. The presence of phenolic compounds in red ginger and secang wood can inhibit joint inflammation in rheumatoid arthritis.

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