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RESEARCH **Open Access**

The Flavonoids, Phenolics, and Antioxidant Activity from Ethanol Extract of *Fibraurea tinctoria* Lour

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Abstract
Oxidative stress has long been associated with increased risk for several diseases. Antioxidant is a molecule that inhibits the oxidation of other molecules and delays or prevents oxidative damage. Natural phenolic and flavonoid compounds are plant secondary metabolites directly contributing to antioxidant activity. Fibraurea tinctoria Lour, often called the yellow root, is a plant that has long been known in the traditional medicine of the native tribes of West Kalimantan to treat several diseases, including malaria, jaundice, and diabetes. This study aimed to quantify the total phenolic (TPC) and flavonoid (TFC) contents and antioxidant activity of the ethanol extract of yellow root stems. Yellow root stems were extracted by maceration in 96% ethanol for four days. The total phenolic, flavonoid, and antioxidant activities were measured using the Folin-Ciocalteu, AlCl3, and DPPH methods. Phytochemical screening showed that the ethanol extract of yellow root stems contains phenolics, flavonoids, alkaloids, saponins, tannins, and steroids/triterpenoids. The total phenol and flavonoid contents were 40.2 mg GAE/g extract and 61.2 mg QE/g extract, respectively. The ethanol extract of yellow root stems showed moderate DPPH scavenging activity, with an IC50 of 152.13 ppm. From this study, it can be concluded that this plant could be a potential source of natural antioxidants.

Keywords: Fibraurea tinctoria Lour, Phenolics, Flavonoids, Antioxidant, DPPH.

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1. INTRODUCTION

Free radicals are physiologically active biomolecules generated during metabolic pathways and immune cells. The body requires a certain amount of free radicals to regulate the physiological activity of the cells. However, when the number of free radicals exceeds the physiological range, it causes oxidative stress (Sies, 2020). Oxidative stress interferes with normal cellular processes and causes cell damage, leading to numerous degenerative diseases, including cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases. The body reacts to stress as a defense mechanism to maintain cellular homeostasis. Several endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, can deactivate free radicals and preserve normal cellular activities. However, antioxidant enzymes are occasionally insufficient to combat free radicals and to sustain optimal cellular activities under elevated oxidative stress. Therefore, exogenous antioxidants are necessary (Sadeer et al., 2020).

Natural plant antioxidants and their derivatives have drawn particular attention because of their capacity to scavenge free radicals, exhibit better performance, and are less toxic than synthetic antioxidants. Medicinal plants with high levels of antioxidant constituents have been proposed as a practical therapeutic approach to many diseases. Among the phytoconstituents, phenolics, and flavonoids have attracted considerable interest and have been proven to be effective in counteracting oxidative stress because of their ability to directly contribute to antioxidant action through their hydroxyl groups by donating an electron or a hydrogen atom (Phuyal et al., 2020). In addition, these compounds also promote the synthesis of endogenous antioxidant molecules in cells. Numerous studies have shown that phenolic and flavonoid compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation, or oxygen scavenging in biological systems to prevent the burden of oxidative diseases (Aryal et al., 2019).

One of the medicinal plants that have been widely used in the traditional medicine of the native tribes of Kalimantan was *Fibraurea tinctoria* Lour, or yellow root. The traditional uses of this plant are in the roots and stems. This plant is used by tribes in Kalimantan to treat diabetes, malaria, and jaundice (Noorcahyati et al., 2016). Several studies have been conducted regarding the pharmacological activity of *Fibraurea tinctoria* and have shown that this plant has anti-inflammatory, anti-malarial, anti-microbial, vasodilation, and anti-proliferative in cervical, oral, and liver cancer (Purwaningsih, Maksum, et al., 2023).

Since it was discovered that oxidative stress has been shown to participate in developing various chronic and degenerative diseases, the attention to plants as an essential source of exogenous antioxidants has increased. In addition, the use of natural antioxidants is also increasing, owing to studies indicating possible adverse effects that may be related to the consumption of synthetic antioxidants (Jomova et al., 2023; Martemucci et al., 2022). This study aimed to measure the phenolic and flavonoid content in the ethanol extract of yellow root stems and evaluate the antioxidant activity using the DPPH method. Most phenolic chemicals, including flavonoids and anthocyanins, are responsible for the high antioxidant activity in plants. According to studies, the antioxidant capacity and the levels of phenolic and flavonoid compounds are strongly correlated. The antioxidant activity increases with the concentration of these substances (Muflihah et al., 2021). These findings suggest the importance of assessing the phenolic and flavonoid contents in yellow root stems to find promising natural antioxidants for future use in diverse fields such as medicine, food, and cosmetics.

2. RESEARCH METHOD

a. Plant material

The plants used in this study were collected from Menua Sadap Village in Kapuas Hulu District, West Kalimantan, and identified at the Biology Laboratory, Tanjungpura University, Pontianak, Indonesia.

b. Preparation of ethanol extract from yellow root stems

The stems of the yellow root were washed and dried for two weeks in an aerated manner, crushed using a wood hammer mill, and sifted with a particle size of 40 mesh. The powdered stem (1.00 kg) was extracted by maceration for four days using 96% ethanol, which was replaced daily. The filtrate was evaporated at 50°C until a thick extract was obtained (26.3 g).

c. Phytochemical screening evaluation

Phytochemical screening is used to determine the chemical compounds present in plant extracts. The identification of phytochemical compounds from ethanol extract of yellow root stems, including phenolics, flavonoids, alkaloids, saponins, tannins, and steroids/terpenoids (Farnsworth, 1966; Shaikh & Patil, 2020).

d. Determination of total phenolic contents (TPC)

The total phenolic content was calculated using the Folin-Ciocalteu method. The reaction mixture comprised 50 µL of supernatant and 2.5 mL of 10% Folin–Ciocalteu reagent. After 3 min, 2.0 mL of 7.5% Na_2CO_3 was added, then incubated at 45°C for 15 minutes. Absorbance was measured at 725 nm. Gallic acid was used as a calibration curve. Standard gallic acid in methanol was prepared in concentration of 30, 50, 100, 200, 300, 500, 750 and 1000 µg/mL. The mean of the two measurements was computed and represented as milligrams of gallic acid equivalents (mg GAE/g extract) (Pauliuc et al., 2020).

e. Determination of total flavonoid contents (TFC)

The $AlCl_3$ method was used to determine the total flavonoid content spectrophotometrically. One gram of samples was weighed, put in a volumetric flask measuring 25 mL, calibrated with ethanol, and then homogenized. Two milliliters of the samples were combined with 2 mL of 2% aluminium chloride ($AlCl_3$) ethanol, and absorbance values at 415 nm were recorded after 30 minutes of incubation. A standard curve of quercetin was used to calculate the total flavonoid content. Standard quercetin in ethanol was prepared in concentrations of 5, 10, 15, 20, and 25 µg/mL. The average of three measurements was determined and represented as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) (Moo-Huchin et al., 2015).

f. Antioxidant activity evaluation

Free radical scavenging activity was determined using the DPPH (2,2-diphenyl-1-picrylhidrazyl) method. Five different concentrations of the sample solution in methanol (20, 41, 61, 81, and 102 µg/mL) were added to two milliliters of 0.1 mM DPPH solution. The mixture was vigorously mixed and incubated at room temperature for 30 minutes. The absorbance of the resulting solution was measured at a wavelength of 517 nm. The blank solution was the DPPH solution in methanol. The amount of antioxidants required to reduce the initial DPPH absorbance by 50% was used to express the radical scavenging activity and was expressed in ppm. Plotting the percentage disappearance of DPPH as a function of sample concentration allowed us to establish the IC_{50} value for each sample graphically. The following equation estimated the percent inhibition (Purwaningsih et al., 2020).

$$\%\ Inhibition = \frac{(A0 - A1)}{A0}\ x100\%$$

Where A_0 = absorbance of the control and A_1 = absorbance of the sample.

3. RESULTS AND DISCUSSION

a. Plant determination

The yellow root plants used in this study were obtained from Menua Sadap Village, Kapuas Hulu District, West Kalimantan. The determination was performed at the Biology Laboratory, Tanjungpura University, Pontianak. The determination results showed that the plants used in this study were *Fibraurea tinctoria* Lour from the *Menispermaceae* family.

b. Sample extraction

The extraction revealed an extraction yield was 2.63%. Many variables, such as the type and concentration of solvent, size of the simplicia particles, and extraction period, influence the yield result (Susanty & Bachmid, 2016). A maceration extraction method was employed in this investigation. The foundation of this technique is solid-liquid separation, with water or organic solvents acting as the liquid phase. Methanol, ethanol, water, or a mixture of these solvents is generally used to extract phenolic compounds. Maceration attempts to reduce compound loss or damage during extraction. This method can be used to analyze chemicals that are susceptible to heat. Particle size also significantly affects the extraction outcomes of maceration (Purwaningsih, Fathiah, et al., 2023).

c. Phytochemical screening

Phytochemical screening of the ethanol extract of yellow root stems revealed the presence of alkaloids, flavonoids, phenolics, saponins, tannins, and steroids/triterpenoids (Table 1).

Table 1. The phytochemical screening.

Secondary metabolites	Findings
Alkaloids	+
Flavonoids	+
Phenolics	+
Saponins	+
Tannins	+
Steroids/Triterpenoids	+/+

d. The total phenolic contents (TPC)

The gallic acid standard curve regression equation was y = 0.0011x + 0.0134 with R^2 = 0.997. The total phenolic contents of the ethanol extract of the yellow root stems were 40.2 mg GAE/g extract.

Table 2. The total phenolic contents.

Replication	Abs	Total phenolic contents		Average
		%	mg GAE/g	
1	0.486	3.97	39.7	40.2
2	0.496	4.06	40.6	

The total phenolic contents were determined colorimetrically using the Folin-Ciocalteu reagent based on the reducing power of the phenolic hydroxy groups. The Folin-Ciocalteu

reagent contains sodium molybdate and tungstate. This reagent can react with all types of phenol to form blue molybdenum, whose absorbance can be measured. The stronger the blue color, the higher the phenol content. The absorbance was read at 765 nm (Blainski et al., 2013; Lamuela-Raventós, 2018). Gallic acid, a derivative of hydroxybenzoic acid with a strong antioxidant effect, was used as a standard (Hudz et al., 2019).

e. The total flavonoid contents (TFC)

The quercetin standard curve regression equation was $y = 0.0296x - 0.0088$ with $R^2 = 0.999$. The total flavonoid contents of the ethanol extract of the yellow root stems were 61.2 mg QE/g extract.

Table 3. The total flavonoid contents.

Replication	Abs	Total phenolic contents		Average
		%	mg QE/g	
1	0.509	6.20	62.0	
2	0.503	6.13	61.3	61.2
3	0.494	6.02	60.2	

The principle of the $AlCl_3$ method was the ortho-hydroxyl group on rings A or B of the flavonoid compounds and $AlCl_3$ interact to form a stable complex. When $AlCl_3$ was added, the wavelength shifted toward the visible range, indicated by the appearance of a yellow tint. Sodium acetate is intended to maintain the wavelength in the visible region and provide an acidic environment because quercetin is stable in an acidic environment. Quercetin is a standard solution because it can react with $AlCl_3$ to form complexes (Shraim et al., 2021; Tristantini & Amalia, 2019).

f. Antioxidant activity

The antioxidant activity of ethanol extract of yellow root stems was determined using the DPPH method and expressed as the IC_{50} value. This method is simple, inexpensive, rapid, and effective for thermally unstable chemicals because radical scavenging is measured at room temperature. Some studies also reported that this method has a good correlation for bioactive compounds (phenolics and flavonoids) with a regression factor $R > 0.8$ (Sadeer et al., 2020).

DPPH is a stable radical that appears purple and absorbs at λmax 515-517 nm. The principle of this method is that upon the reaction of the antioxidant with DPPH, the DPPH accepts the hydrogen donor, resulting in the reduction of DPPH to DPPH2, and the solution loses its color from purple to pale yellow. The color change is monitored spectrophotometrically and utilized to determine parameters for antioxidant properties (Angeli et al., 2023).

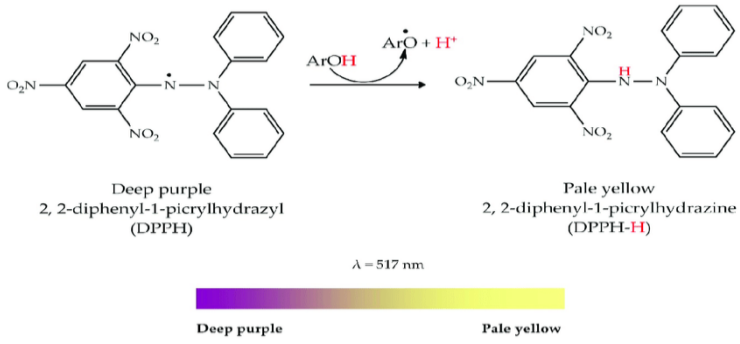

Figure 1. DPPH reaction mechanism (Sadeer et al., 2020)

From the correlation curve between the concentration against % inhibition of the ethanol extract of yellow root stems, the regression equation obtained, y = 0.3259x + 0.42 with R^2 = 0.9954. The calculation results of % inhibition and IC_{50} are presented in Table 4 and the graph of concentration against % inhibition of the ethanol extract of yellow root stems is shown in Figure 2.

Table 4. The antioxidant activity of ethanol extract of yellow root stems.

Concentration (ppm)	Abs	% Inhibition	Average % Inhibition	IC_{50} (ppm)
0	0,853	0,00	0,000	152,13
0	0,853	0,00		
20	0,814	4,57	6,445	
20	0,782	8,32		
41	0,735	13,83	14,710	
41	0,720	15,59		
61	0,671	21,34	20,520	
61	0,685	19,70		
81	0,621	27,20	27,670	
81	0,613	28,14		
102	0,577	32,36	32,415	
102	0,576	32,47		

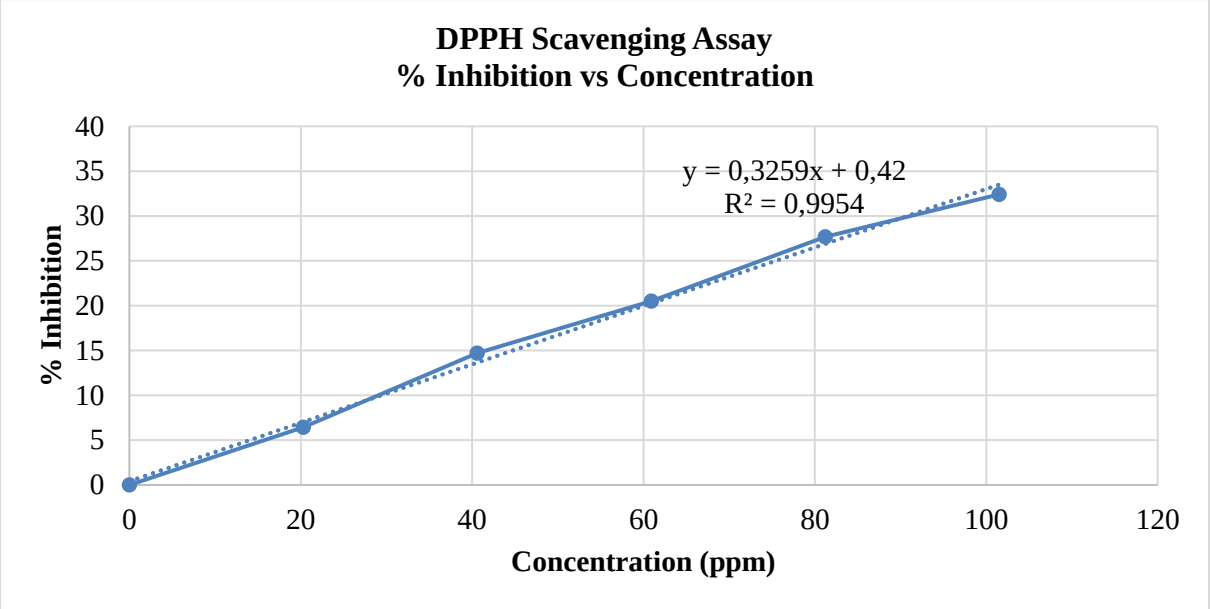

Figure 2. The graph of % inhibition against concentration.

The ethanol extract of yellow root stems showed moderate DPPH scavenging activity, with an IC_{50} value between 101-250 ppm, namely 152.13 ppm. A study of antioxidant activity of *Arcangelisia flava*, *Coscinium blumeanum*, and *Fibraurea tinctoria* from Thailand was published by Keawpradub et al. In their study showed that the stems of *Fibraurea tinctoria* extracted using petroleum ether and boiling water had IC_{50} > 100 µg/mL, whereas chloroform and methanol extract showed an IC_{50} 78.8 and 83.6 µg/mL, respectively (Keawpradub et al., 2005). Based on this study, our results showed a lower antioxidant activity than the chloroform

and methanol extract, but had the same activity as the petroleum ether and boiling water extracts of *Fibraurea tinctoria* from Thailand.

Phenolic compounds and flavonoids are important antioxidants for free radical inactivation because of their ability to donate hydrogen atoms to free radicals. They also have structural characteristics ideal for counteracting free radicals. Several kinds of literature showed that total phenolic and flavonoid content is linearly related to antioxidant capacity (Aryal et al., 2019). The redox properties of phenolic compounds enable them to function as antioxidants. Phenolic compounds reduce or inhibit free radicals by donating hydrogen atoms from their hydroxyl groups. Consequently, DPPH radicals are reduced to a more stable form (Santos-Sánchez et al., 2019). In addition, phenolic compounds can chelate metals, especially iron and copper, to inhibit the formation of metal-catalyzed free radicals (Vuolo et al., 2018). The number and position of the hydroxyl groups are known to influence the activity of phenolic compounds against free radicals. The greater the number of hydroxyl groups in the molecule, the greater the antioxidant activity (Prasonto et al., 2017).

Flavonoids are the most abundant phenolic compounds in various medicinal plants and the third-most bioactive plant compound, which has been reported to have about 10,000 in the literature (Khan et al., 2021). Several studies on antioxidant properties and their relationship to flavonoids have indicated that flavonoids can be used as potential drugs to prevent oxidative stress (Panche et al., 2016). The mechanism of the antioxidant activity of flavonoids, including free radical scavenging and the capacity to chelate metal ions, is influenced by the configuration, substitution, and number of hydroxyl groups in the molecule. The configuration of the B-ring hydroxyl group is the most important determinant because it donates hydrogen and electrons to the hydroxyl, peroxyl, and peroxynitrite radicals and then stabilizes them to produce a relatively stable flavonoid radical (Kumar & Pandey, 2013).

Several studies using natural flavonoids have investigated the relationship between the structure of flavonoids and their antioxidant activities. Typical chemical structures related to the antioxidant activity of flavonoids have been determined, including a hydroxyl group (C in Figure 6), an ortho-dihydroxy in ring B (A in Figure 6), and a C_2-C_3 unsaturated bond combined with a C_4 carbonyl group in the ring C (B in Figure 6), and O-methylation (Banjarnahor & Artanti, 2014).

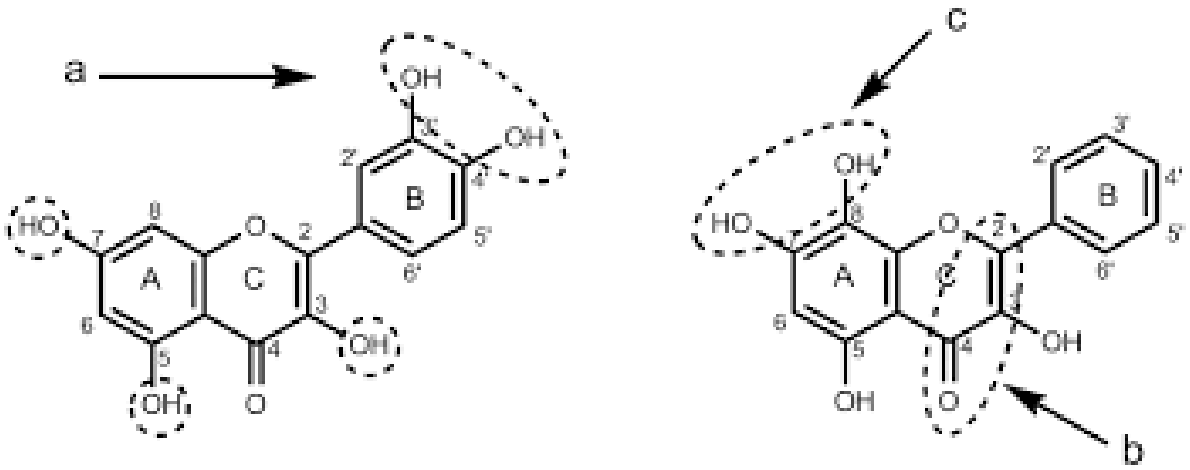

Figure 3. Antioxidant structure-activity relationships (Banjarnahor & Artanti, 2014).

Chelation of trace elements is another mechanism by which flavonoids exert their antioxidant effects. Because flavonoids have chelating capabilities, they can bind metal ions in

the body and stop them from oxidizing. Many flavonoids can chelate metal ions, including Fe^{2+} and Cu^{+}, which are crucial for oxygen metabolism and the production of free radicals (Banjarnahor & Artanti, 2014). The antioxidant activity of flavonoids can also be carried out indirectly, where flavonoids can act as intracellular antioxidants by inhibiting free radical-producing enzymes, such as NADPH oxidase (NOX), lipoxygenase (LO), cyclooxygenase (CO), and xanthine oxidase (XO) (Hernández-Rodríguez et al., 2018).

Considering the many mechanisms of antioxidant action and the limits of each test, research has indicated the necessity of performing multiple types of antioxidant activity measurements. Moreover, *in vitro* antioxidant assays are based on *in vitro* chemical reactions, which are different from actual physiological systems. However, the outcomes of *in vitro* tests for antioxidant activity retain their usefulness in directing investigations involving humans. To ascertain the mechanisms underlying the antioxidant activity of this herbal extract, further research is required to separate and identify the specific phenolic or flavonoid compounds found in these samples.

4. CONCLUSION

The results of the present study suggest that this plant could be a potent source of natural antioxidants because of its phenolic and flavonoid content and its remarkable scavenging effects on DPPH. Although the parameters used in this study were not disease-specific, the quantification of antioxidant properties can serve as a guide for the use of these plants for ROS-related diseases. Further investigations are needed to isolate and identify the phenolic or flavonoid components responsible for the antioxidant activity of these plants, as well as their mechanisms of action, to authenticate their potential use as sources of natural antioxidants, and to validate their traditional uses in several medicinal practices.

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