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## Determination of Total Flavonoid Content, Antioxidant and Antibacterial Activities of Yellow Root (*Fibraurea tinctoria* L.)

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**Abstract.** Yellow root (*F. tinctoria*), an endemic plant of Kalimantan, is extensively used in traditional medicine for its alkaloid compounds, the major bioactive compound in the Menispermaceae family. Despite this, research on the biological properties of its flavonoid compounds is scarce. This study examines the correlation between the flavonoid content of yellow root and its antibacterial and antioxidant activities. The yellow root extract and its fractions, known to contain flavonoids, were analyzed for total flavonoid content using a colorimetric method with a 2% AlCl<sub>3</sub> reagent. Antioxidant activity was measured using the DPPH test, and antibacterial activity against *S. aureus* and *E. coli* was evaluated using the well diffusion method. The result showed that methanol fraction has the highest total flavonoid content with 52.23 ± 0.00781 mg QE/g extract. Extract methanol, fraction methanol and ethyl acetate of yellow root exhibited weak antioxidant activity with IC<sub>50</sub> values of 472.5; 390.6; and 369.3 mg/L, respectively. The antibacterial activity of the three samples against *S. aureus* at concentration of 50 µg/µL showed a weak-strong category. The research highlights the potential of yellow root can be utilized in developing the implementation of natural products for traditional medicine.

### Introduction

Yellow root (*F. tinctoria*), which belongs to the Menispermaceae family, is widely found in Indonesia and is cultivated on the islands of Kalimantan, Sumatra, and Sulawesi. Traditionally, the yellow root is used by the ethnic people of Kalimantan, such as the Dayak, Banjar and Kutai to treat several diseases such as malaria, hepatitis and diabetes (Rinaldi, *et al.*, 2017; Suratno, *et al.*, 2019). Pharmacological studies have shown that yellow root exhibited antibacterial, antimalarial, antioxidant, antidiabetic, anti-inflammatory, and immunostimulant (Hapsari, *et al.*, 2019; Maryani, *et al.*, 2020; Diliarosta, *et al.*, 2021).

It is believed that the biological activity of yellow root correlates to the presence of alkaloids, flavonoids, saponins, and terpenoids (Maryani, *et al.*, 2013; Supomo, *et al.*, 2020). Additionally, the yellow root extract was found

to be non-toxic to the liver and kidneys in rats, suggesting its safety for use in traditional medicine (Rachmawati & Ulfa, 2018). The predominant chemical constituent of yellow root is berberine alkaloids and it has antibacterial activity (Wahyuni & Marpaung, 2020). Besides alkaloids, the yellow root also contains flavonoids. Based on the literature, the total flavonoid content of yellow root extract from Banjar, Bangka Belitung and Jember was 1.67%, 0.31%, and 5.61% respectively (Wahyudi, *et al.*, 2016; Sari, *et al.*, 2018).

This paper discusses the relationship between total flavonoid content with their antioxidant and antibacterial activities of the extracts and fractions of yellow root (*F. tinctoria*) from West Kalimantan, which has been traditionally and widely used by the local tribe as the herbal medicine for generations. The research aims to identify the potential of flavonoids by examining their antioxidant and antibacterial activity against *S. aureus* and *E. coli*.

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## Experimental

### Material and Methods

The equipment used in this study were autoclave (All America), laboratory glassware (Pyrex and Iwaki), laminar flow (Biobase), rotary evaporator (RE100-Pro D-Lab), shaker incubator (Sartorius Certomat IS), sigmat digital vernier caliper and spectrophotometer UV-Vis (Shimadzu 1280) were used during experiments.

The materials used in this study were yellow root that was collected from Sanggau, West Kalimantan, 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  (w/v), 0,12 M  $\text{CH}_3\text{COONa}$ , quercetin (Sigma Aldrich), 2,2-diphenyl-1-picryl hydrazyl (DPPH), methanol, ethyl acetate, peptone, *n*-hexane, yeast extract, NaCl, and tetracycline were utilized for the study.

### Procedures

#### Extraction and Fractionation (Widiyastuti, *et al.*, 2022)

The yellow root was cleaned and powdered to obtain 185 g. It was then macerated with methanol for 3x24 hours. The methanol extract was concentrated using a vacuum rotary evaporator to yield a crude extract, which was then separated into fractions using *n*-hexane and ethyl acetate.

#### Phytochemical Tests (Sudira, *et al.*, 2019)

The phytochemical screening was applied for alkaloids, flavonoids, terpenoids, and tannins on crude extract, fractions of *n*-hexane, ethyl acetate, and methanol. Each sample was alternately added by reagents of Mayer, Wagner, and Dragendroff for alkaloids, 10% NaOH for flavonoids, the Liebermann-Burchard reagent for terpenoids and steroids, and 5%  $\text{FeCl}_3$  for tannins.

#### Determination of total flavonoid content (Aminah, *et al.*, 2017)

*Determination of the maximum wavelength of quercetin.* 1 mL of quercetin (20 ppm) was dropped by 1 mL of 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  w/v and 1 mL of 0.12 M  $\text{CH}_3\text{COONa}$ . The mixture was measured for a maximum wavelength using a UV-Vis spectrophotometer.

*Plot of a quercetin curve.* Various concentrations of 4, 8, 12, 16, and 20 ppm of quercetin were prepared. 1 mL of each concentration was dropped by 1 mL of 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  and 1 mL of 0.12 M  $\text{CH}_3\text{COONa}$ . The mixture was incubated for 0-6 minutes and measured with the UV-Vis spectrophotometer at a maximum wavelength ( $\lambda$  max) which has been obtained previously.

*Determination of total flavonoid content.* The crude extract and each fraction except for *n*-hexane were prepared to have a concentration of 200 ppm. 1 mL of each

sample was added by 1 mL of 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  and 1 mL of 0.12 M  $\text{CH}_3\text{COONa}$ , then all samples were incubated for 0-6 minutes and measured with the UV-Vis spectrophotometer. Each experiment was carried out for three replications.

#### Antioxidant Activity (Suena, *et al.*, 2021)

*Determination of the maximum wavelength of DPPH.* A solution of 40 ppm of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared and its maximum wavelength was measured by the UV-Vis spectrophotometer.

*Antioxidant activity.* The quercetin was prepared for concentrations 2, 4, 6, 8, and 10 ppm. Each extract and fraction were also made for 100; 200; 300; 400; and 500 ppm. 1 mL of each sample including each quercetin concentration was added with 2 mL of 40 ppm DPPH and the mixtures were incubated for 30 minutes. Finally, the absorbances were measured at 517 nm with a UV-Vis spectrophotometer to obtain the  $\text{IC}_{50}$  values.

#### Antibacterial Activity with a Well Diffusion Method

*Preparation of Nutrient Agar (NA) as a solid media* (Fathollahi, A., & Coupe, S.J., 2021). NA media was made by mixing 0.5 g peptone, 0.2 g yeast extract, 0.5 g sodium chloride (NaCl), and 2 g of agar in 100 mL of distilled water in the Erlenmeyer. Next, the media was heated on a hot plate and then sterilized by autoclaving at 121°C for 15 minutes. After cooling, 25 mL of sterile media was transferred into a petri dish and left in the laminar chamber until it was solid.

*Preparation for bacterial suspension.* The bacterial suspension was prepared following to the procedure of Wang, *et al.*, (2020), with a few modifications. Each *E. coli* and *S. aureus* was lightly scratched from solid media (stock) then transferred into the erlenmeyer containing sterile Nutrient Broth (NB) liquid media. The cultured broths were incubated at 37°C and shaken at 180 rpm for 16 hours in rotary shaker until the bacterial suspension became turbid.

## Result and Discussion

### Phytochemical Test

Table 1 revealed the masses of crude extract and each fraction after maceration and partition. The methanol fraction was a major component which indicated that yellow roots contain mostly polar compounds. Phytochemical screening (Table 2) of yellow root extract and fractions showed the presence of alkaloids, flavonoids, and terpenoids.

The main constituent of secondary metabolites of the yellow root is alkaloids based on positive results for Wagner, Mayer, as well as Dragendroff tests. In addition to alkaloids, flavonoids were also detected in extract and fractions (except for *n*-hexane fraction) by giving a positive result with 10% NaOH reagent indicating the presence of chalcone, flavanones, flavonols, and proanthocyanins (Geissman, 1962).

**Table 1.** Yield of Extract and Fractions of Yellow Root.

Samples	Weight (g)	Yield (%)
Methanol Extract	12.9	6.98
Methanol Fraction	6.3	48.8
Ethyl Acetate Fraction	3.7	28.7
<i>n</i> -Hexane Fraction	1.3	10.1

**Table 2.** Phytochemical Test.

Samples	Alkaloid			Flavonoid	Terpenoid	Tannin
	Mayer	Wagner	Dragendroff	NaOH 10%	Liebermann-Burchard	FeCl <sub>3</sub> 5%
Methanol Extract	+	+	+	+	+	-
Methanol Fraction	+	+	+	+	+	-
Ethyl Acetate Fraction	+	+	+	+	+	-
<i>n</i> -Hexane Fraction	-	-	-	-	+	-

### Total Flavonoid Contents

The maximum wavelength of quercetin was 424 nm. The methanol fraction, showing a positive result for flavonoids, has the highest total flavonoid content (Table 3), indicating that flavonoid glycosides are the main component in the yellow root. Several studies have indicated that the Menispermaceae family contains flavonoid glycosides such as kaempferol 3-O- $\beta$ -D-glucopyranoside, kaempferol 3-O- $\beta$ -D-glucurono pyranoside, and C-glycosyl isoflavonoids (Reynaud, *et al.*, 2005; Hao, D.C., & Yang, L., 2016; Devkota, *et al.*, 2017).

**Table 3.** Total Flavonoid Content of Each Samples.

Samples	Total Flavonoid Contents (mg QE/g extract)
Methanol Extract	32,8 $\pm$ 0,00058
Methanol Fraction	52,2 $\pm$ 0,00781
Ethyl Acetate Fraction	30,6 $\pm$ 0,00404

Total flavonoid content obtained from the methanol extract of yellow root in this study was 3.28% which was greater than previously reported by Sari, *et al.*, (2018), that was 1.66%. Some factors that can be attributed to differences in results include yellow root species and the environment where the yellow root grows.

### Antioxidant Activity

Table 4 shows that the crude extract and fractions of methanol and ethyl acetate exhibited weak antioxidant activity, attributed to antagonistic interactions among active compounds in the extract or fractions which did not

contribute effectively to enhancing antioxidant properties (Rikantara, *et al.*, 2022).

**Table 4.** Antioxidant Activity (Zamzani & Triadisti, 2021).

Samples	IC <sub>50</sub> (ppm)	Category
Quercetin	7.401	Very Strong
Methanol Extract	472.5	Very Weak
Methanol Fraction	390.6	Very Weak
Ethyl Acetate Fraction	369.3	Very Weak

Except for positive control, although the ethyl acetate fraction has the lowest total flavonoid contents (Table 3), its antioxidant activity was higher than the extract and fraction methanol. This indicates the presence of flavonoids with antioxidant potential, though in relatively low quantities and have been separated from glycosidic flavonoids (polar). The glycosidic flavonoids may decrease antioxidant activity due to the inhibition of electron delocalization of radicals into the aromatic ring of the flavonoids (Qomaliyah, *et al.*, 2023).

### Antibacterial Activity

The methanol extract of yellow root demonstrated weak antibacterial activity ( $\leq 7$  mm) against *S. aureus* and *E. coli*, due to the synergistic interaction of all bioactive compounds in the methanol extract. Meanwhile, the methanol and ethyl acetate fractions were effective only against *S. aureus* (Table 5). This is consistent with the study by Galappathie, *et al.*, (2014), that the antibacterial activity of yellow root is more effective against *S. aureus* bacteria than *E. coli*.

The methanol fraction exhibits the highest antibacterial activity against *S. aureus*, categorized as strong ( $\geq 8$  mm), due to its high content of glycosidic flavonoids, as indicated in Table 3. Glycosidic flavonoids are effective against bacteria by disrupting the permeability of the cell membrane in gram-positive bacteria, in contrast to gram-negative bacteria (Tagousop, *et al.*, 2018). The antibacterial activity of yellow root also demonstrates a bacteriostatic effect, as observed by bacterial growth inhibition on the agar media from the clear zone area after inoculation with a loop.

**Table 5.** Antibacterial Activity of Yellow Roots at 50  $\mu\text{g}/\mu\text{L}$ .

Samples	Diameter of Inhibition Zone (mm)	
	<i>S. aureus</i>	<i>E. coli</i>
Methanol Extract	6,403 $\pm$ 0,112	3,013 $\pm$ 0,375
Methanol Fraction	8,727 $\pm$ 0,084	-
Ethyl Acetate Fraction	6,600 $\pm$ 0,131	-

## Conclusion

Yellow root is more effective as an antibacterial agent than as an antioxidant, due to its flavonoid glycosides content, which exhibits synergistic effects. This is demonstrated by its ability to inhibit the growth of *S. aureus*, even though its antioxidant activity is relatively weak. Therefore, yellow root has potential as an alternative or additional treatment for bacterial infections.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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