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Full Length Article

Antioxidant Activity and Phytochemical Profile in Sequential Solvent Extract of Faloak (*Sterculia quadrifida*) Leaves and Stem Bark

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Abstract

Faloak (*Sterculia quadrifida* R.Br) is an endemic plant of East Nusa Tenggara Island. This plant was found in the East Nusa Tenggara archipelago, including Timor, Sumba, Flores, Alor and Rote Islands. Traditionally, the community used this plant for a variety of therapeutic purposes. Faloak demonstrate numerous biological activities, primarily attributable to its secondary metabolite compounds. The presence of phenolic compounds and flavonoids renders it a good candidate for the development of a novel natural antioxidant resource. Faloak possesses several biological activities related to its high antioxidant content. However, research on antioxidant, phenolic, and flavonoids content of Faloak leaves and stembark in different solvent remains limited. This study aims to investigate the antioxidant activity of Faloak leaves and stembark the ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (2, 2-diphenyl-1- (2, 4, 6-trinitrophenyl) hydrazyl) radical scavenging assay in different solvents. Measurement of phenolic and flavonoids levels, together with thin layer chromatography (TLC) bioautography, is performed to predict the related antioxidant compounds. The results showed that ethanol, as a polar solvent, produced the highest output, indicating that Faloak mostly comprises a polar compound. Leaves have a higher concentration of phenolic and flavonoid compounds than stembark. Leaves and stembark have significant antioxidant activity. Thin layer chromatography (TLC) combined with bioautography confirmed the presence of polyphenols and flavonoids, which are likely responsible for the antioxidant activity in the Faloak fractions. Bioautography also yielded active compounds with antioxidant activity. The stembark and leaves of Faloak possess antioxidant properties; nevertheless, the leaves demonstrate significant potential as a candidate for diverse pharmacological treatments due to their robust antioxidant activity and sustainability.

Keywords: ABTS; Antioxidant; DPPH; Faloak; Flavonoids; Phenolics

Introduction

Free radicals have one or more unpaired electrons in the outermost orbital, rendering them extremely reactive (Lobo *et al.* 2010). These free radicals include reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS activate signaling pathways to initiate biological processes, whereas oxidative stress and nitrosative stress denote elevated levels of ROS and RNS that inflict damage on DNA, proteins, or lipids

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(Schieber and Chandel 2014). Oxidative stress in the body is a primary contributor to cellular damage and several degenerative diseases, including cancer, cardiovascular diseases, neurodegenerative conditions, inflammation, antiviral and hepatic injury (Phaniendra *et al.* 2015; Nurina *et al.* 2024; Riwu *et al.* 2024). Free radicals can originate from internal or external sources. Endogenous or internal sources are produced by regular metabolism and encompass numerous cell organelles, including mitochondria, fatty acid metabolism, peroxisomes, endoplasmic reticulum, and phagocytic cells. Exogenous sources include tobacco smoke, heavy metals, UV irradiation, air and water pollution, and drugs (Martemucci *et al.* 2022). The prevalence of these events can be reduced by consuming sufficient antioxidants. These substances will impede or prevent the oxidation of other substances, thereby reducing the generation of free radicals (Kasote *et al.* 2015).

An antioxidant is a chemical substance that mitigates the oxidative impact of free radicals by giving an electron to an unpaired free radical. Numerous secondary metabolites found in herbs have demonstrated efficacy as antioxidants and have potential as natural exogenous antioxidants (Sukweenadhi *et al.* 2020). Herbal medicines contain polyphenols, believed to be the primary agents responsible for antioxidants activities. The predominant secondary metabolites in plant are phenolic compounds, which include simple phenols, phenolic acid, coumarins, stilbenes, flavonoids, lignans and tannins (Kumar and Goel 2019). Flavonoids and phenolics are present in several plants and have demonstrated health benefits for humans. These compounds possess antihyperlipidemic, anticancer, cardioprotective, antibacterial and antidiabetic properties (Zeb 2020).

Indonesia has over 7,500 medicinal plants, one of which is Faloak (*Sterculia quadrifida* R.Br). Faloak is a plant that proliferates in East Nusa Tenggara and is extensively used for several medicinal purposes (Nitbani *et al.* 2019). This endemic flora is found in the East Nusa Tenggara islands, including Timor, Sumba, Flores, Alor, and Rote Islands (Siswadi *et al.* 2020). The local names of Faloak include *Faloak* (Kupang), *Komila* (Timor Leste), *Nitaen* (Belu), *Flolo* (North Central Timor), *Kawarid* (Central Sumba), *Penil* (Alor), *Klengis* (East Flores), *Mangiladu* (Gorontalo) (Darojati *et al.* 2022). The community extensively uses several parts of Faloak, including the leaves, stembark, flower and fruits (Fig. 1). Locals have been using Faloak stembark stew empirically to treat numerous diseases, including typhoid fever, hepatitis, malaria, and anticancer (Siswadi *et al.* 2015; Rollando *et al.* 2022). Traditional uses of Faloak are supported by scientific evidence. Water, methanolic and ethanolic extracts of Faloak stembark activity were reported effective against the hepatitis C virus (Sola *et al.* 2018). Ethanolic extracts of Faloak stembark exhibit antiplasmodial activity attributed to their elevated levels of flavonoids, alkaloids and saponins (Tenda *et al.* 2021). The antibacterial, anticancer, antidiabetic, and immunomodulating properties of Faloak extracts have also been reported (Darojati *et al.* 2022). Furthermore, Faloak serves as a natural source of antioxidants to prevent oxidative stress.

Recent scientific studies have demonstrated the antioxidant activity of Faloak, which includes polyphenols as secondary metabolites, including flavonoids, phenolic acids, and tannins, all recognized for their significant antioxidant properties. The Inhibitory Concentration 50 (IC_{50}) of ethanolic extract of Faloak stembark was 14.17 ± 0.55 μg/mL (Dillak *et al.* 2021). Ethyl acetate fraction of the 96% ethanolic extract reportedly had the highest total flavonoids, measuring 4.290 ± 0.029 mg/g, in comparison to the initial ethanolic extract, water fraction, insoluble fraction, and n-hexane fraction (Munawaroh *et al.* 2018). Therefore, it could be categorized as a highly potent antioxidant resource (Saragih and Siswadi 2019). Given the limited availability resources of Faloak stembark, it is essential to investigate other parts of Faloak, with the leaves emerging as a viable option. The leaves have a greater resource availability compared to stembark. Based on the facts provided above, Faloak leaves and stembark showed high potential to be developed as natural antioxidant resources.

Studies on the antioxidant activity, phenolics and flavonoids content of Faloak leaves and stembark in different solvents are still limited. In the prior work, extraction was conducted solely using a single solvent, whereas in this study, we used a different method of extraction by sequential solvent extraction with a gradient solvent (n-hexane, ethyl acetate, and ethanol). Sequential solvent extraction is a popular method for extracting active components from natural sources. This extraction method can separate the secondary metabolite of Faloak based on its polarity by using different solvents with varying polarities (Uthayarasa *et al.* 2010). The objective of this study was to investigate the characteristics of secondary metabolites that have an antioxidant activity in Faloak leaves and stembark, depending on their polarity.

Materials and Methods

Research materials

This study used the stembark and leaves of Faloak. The determination was performed at the Plant Taxonomy Laboratory, Department of Biology, Universitas Padjadjaran, under the code 53/HB/03/2022. Gallic acid, quercetin, ABTS (2,2'-azino-bis-[3-etilbenzotiazolin sulfonic acid) and DPPH (2,2-diphenyl-1- (2,4,6-trinitrophenyl) hydrazyl) were purchased from Sigma Aldrich, Germany, silica gel $G_{60}F_{254}$ TLC plates, $FeCl_3$ 1%, 2-aminoethyl diphenylborinate (NP), PEG 5% and NaOH from Merck, Germany.

Preparation of faloak simplicia

Samples of the stembark and leaves of Faloak were collected from Timor Island, East Nusa Tenggara, Indonesia (latitude -9 13' 60.00" S, longitude 124 55' 59.99" E). The plant was collected in July 2024, with a minimum tree diameter of 30 cm. Fresh leaves and stembark were further processed into dry simplicia powder (Fig. 2).

Preparation of faloak extraction

Extraction was generated by sequential extraction with various solvents according to their polarity. Kinetic maceration at 200 rpm was used in the sequential extraction method of 3 x 1 h for each solvent at room temperature to prevent the loss of active compounds. This study used n-hexane, ethyl acetate, and 80% ethanol as solvents. One hundred grams of simplicia were extracted using 1 L of solvent. The liquid extract was evaporated using a rotary evaporator and water bath to obtain a thick fraction (Sridhar *et al.* 2021).

The yield of each fraction was calculated using the formula presented below:

$$\% \text{ yield} = \frac{\text{weight of thic fraction (g)}}{\text{weight of crude drug powder (g)}} \text{ x } 100\%$$

Total phenolic determination

The total phenolic content assay was conducted using the Folin-Ciocalteu method, in accordance with the Indonesian Herbal Pharmacopoeia (IHP 2017). Gallic acid was used as a standard solution at various concentrations of 30, 40, 50, 70 and 100 ppm. A 1 mL sample or standard was added with 5 mL of 7.5% Folin-Ciocalteu and 4 mL of 1% NaOH solution in a 10 mL volumetric flask. The solution was allowed to stand for one h at room temperature. The absorbance of the standard solution was measured using a UV-Vis spectrophotometer at 765 nm. The total phenolic content in each fraction was calculated as gallic acid equivalent (%GAE). Each sample evaluation was performed in triplicate (IHP 2017).

Total flavonoids determination

The total phenolic content assay was carried out in accordance with the Indonesian Herbal Pharmacopoeia (IHP 2017). Quercetin was used as a standard solution at various concentrations of 25, 50, 75, 90 and 100 ppm. 0.5 mL sample or standard was added with 0.1 mL of 10% $ALCL_3$, 0.1 mL of Na_2CO_3 1 *M*, 1.5 mL ethanol and distilled water up to 10 mL in a volumetric flask. Solutions were allowed to stand for 30 min at room temperature. The absorbance of each standard solution was measured using a UV-Vis spectrophotometer at 415 nm. The total phenolic content in each fraction calculated as quercetin equivalent (%QE). Each sample evaluation was performed in triplicate (IHP 2017).

Antioxidant activity assay with ABTS method

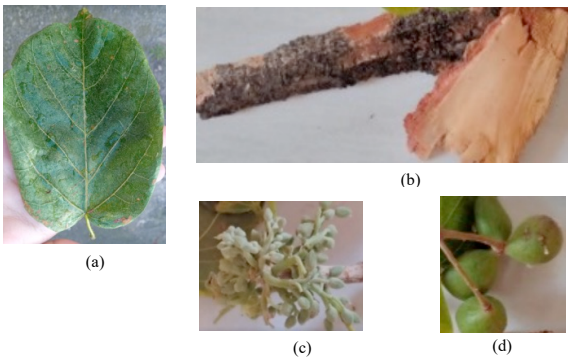

Fig. 1: Morphological characteristics of Faloak. (**a**) Leaves (**b**) Stembark (**c**) Flower (**d**) Fruit

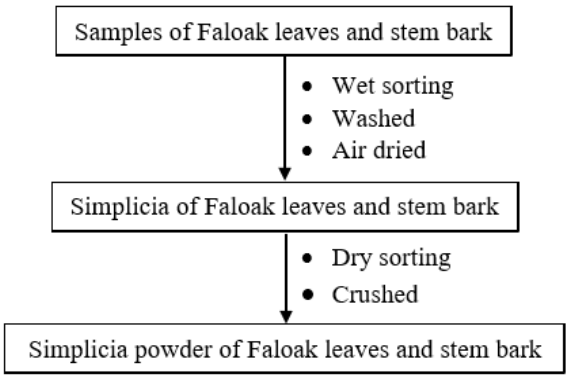

Fig. 2: Preparation of simplicia powder (Tenda *et al.* 2021)

The ABTS antioxidant assay was carried out according to Setiawan *et al.* (2018). ABTS solutions, 7.1 mg ABTS and 3.5 mg potassium persulfate, were dissolved separately in demineralized water. The solutions were mixed and incubated for 12 h in the dark room to form ABTS radicals, which gave a blue-green color. Sample solutions were prepared at different concentration. ABTS solution and samples (1:10 ratio) with varying concentration were pipetted into a 96-well transparent polystyrene microplate, homogenized, and incubated for five min in the dark room. The mixtures were measured at 734 nm with a microplate reader. Each sample evaluation was performed in quadruplicate (Setiawan *et al.* 2018). The free radical scavenging activity was assessed using the following formula:

$$\text{ABTS inhibition activity (\%)} = \left[\frac{(A1 - A0) - (S1 - S0)}{(A1 - A0)}\right] \times 100$$

Where, A1 = absorbance of DPPH solution, A0 = absorbance of blank (ethanol) solution, S1 = absorbance of sample solution and S0 = absorbance of sample blank solution.

Antioxidant activity assay with DPPH method

The DPPH radical scavenging assay was carried out according to Sukweenadhi *et al.* (2020). Sample solutions were prepared at several concentrations. A 400 ppm DPPH solution and samples (1:3 ratio) with varying concentrations were pipetted into a 96-well transparent polystyrene microplate, homogenized, and incubated for 30 min in the dark room. The mixtures were measured at 517 nm with a microplate reader. Each sample evaluation was performed in quadruplicate (Setiawan *et al.* 2018). The free radical scavenging activity was assessed using the following formula:

$$\text{DPPH inhibition activity (\%)} = \left[\frac{(A1 - A0) - (S1 - S0)}{(A1 - A0)}\right] \times 100$$

Where, A1 = absorbance of DPPH solution, A0 = absorbance of blank (ethanol) solution, S1 = absorbance of sample solution and S0 = absorbance of sample blank solution.

TLC-Bioautography of faloak fractions

The TLC-bioautography method was carried out according to Rismawati *et al.* (2018). Faloak leaves and stembark fractions were dissolved in methanol at a solute-to-solvent ratio of 1:10. The sample solutions were spotted on the silica gel TLC plates and subsequently eluted using the selected mobile phases. The plates were later examined under UV254 and 365 nm (Rismawati *et al.* 2018). The plates were further sprayed with derivatizing reagents, including $FeCl_3$, NP/PEG and ABTS, to detect phenolic, flavonoids and antioxidant properties (Fig. 3).

Statistical analysis

All analyses were performed in triplicate for phenol and flavonoid analysis, and in quadruplicate for the antioxidant assay. The data are shown as mean ± standard deviation for the replication. Linear regression was applied to determine Inhibitory Concentration 50 (IC_{50}) of the sample.

Results

The 80% ethanolic extract had the maximum yield in both leaves (12.27%) and stembark (8.13%). This result indicated that the predominant metabolite of Faloak leaves and stembark were extracted using a polar solvent. Semipolar metabolites were distributed in ethyl acetate extract and non-polar metabolite in n-hexane extract.

Gallic acid and quercetin are phenolic and flavonoids compounds that are typically used as standards for determining total phenolic and flavonoids content. Chemical reactions between Folin-Ciocalteau and phenolic compounds provide a blue molybdenum-tungsten complex, allowing for absorbance measurement through visible spectrophotometry. The addition of 7.5% Na_2CO_3 as a weak base is intended to create an alkaline environment. The reaction occurs solely in alkaline settings as complex

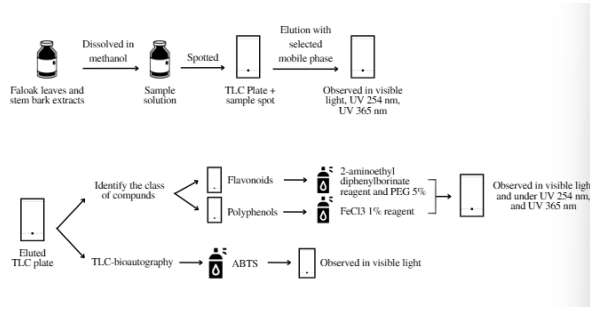

Fig. 3: TLC-bioautography scheme of faloak (This scheme is made by author)

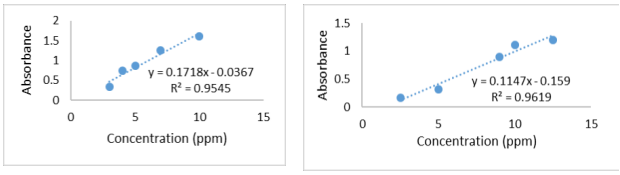

Fig. 4: Standard curve for (**a**) gallic acid (phenolic) and (**b**) quercetin (flavonoid)

formation requires the proton dissociation of phenolic compounds. Increased concentrations of phenolic compounds will result in a darker blue in the solution (Hasnaeni *et al.* 2019). Total phenolic content of the Faloak fraction was calculated using the calibration curve of gallic acid ($y = 0.1718x - 0.0367$, $R^2 = 0.9545$), seen in Fig. 4, while the total flavonoid content was calculated using the calibration curve of quercetin ($y = 0.1147x - 0.159$, $R^2 = 0.9619$).

The ethanolic extract of Faloak stembark and leaves had the highest phenolic content, measured as % GAE, followed by the ethyl acetate. In this study, the ethanolic extract of Faloak leaves and stembark exhibited a higher phenolic content compared to the ethyl acetate extract. The measurement of flavonoid content indicated that the ethanolic fraction of leaves possessed the highest flavonoid level, followed by the ethyl acetate fraction of leaves, the ethyl acetate fraction of stembark and the ethanolic fraction of stembark. These results indicated that the phenolic and flavonoids contents may serve as the active antioxidants in Faloak leaves and stembark.

The antioxidant activity of n-hexane, ethyl acetate, and 80% ethanolic fractions of Faloak leaves and stembark was initially tested qualitatively as a preliminary test. The n-hexane fractions of leaves and stembark showed negative antioxidant activities, as evidenced by the absence of any noticeable color change in the solutions; therefore, they were eliminated from further testing. Conversely, the ethyl acetate and the 80% ethanolic fraction of Faloak leaves and stembark showed positive results, as evidenced by the disappearance of bluish-green color in the ABTS assay and the transition from yellow to colorless in the DPPH assay. The antioxidant activity of each fraction was subsequently evaluated using a microplate reader. The result

was reported as IC_{50} values, indicating the concentration of the extracts required to neutralize 50% of ABTS and DPPH radicals. A lower IC_{50} indicates a higher antioxidant activity of the sample. The findings indicated that the ethyl acetate fraction of Faloak stembark (IC_{50} 48.49) exhibited robust antioxidant activity, followed by the ethanolic fraction of Faloak leaves (IC_{50} 91.82) and stembark (IC_{50} 67.19) with significant antioxidant activities. The ethyl acetate fraction of Faloak leaves (IC_{50} 233.88), on the other hand, displayed the lowest antioxidant activity (Table 3). The DPPH antioxidant assay (Table 4) showed that the 80% ethanolic fraction of Faloak leaves (IC_{50} 58.06) and stembark had a higher activity than the ethyl acetate extract (IC_{50} 21.18). The ethyl acetate leaves extract showed the lowest antioxidant activity and this result is similar to the ABTS assay. The stembark had a higher antioxidant activity in both the ABTS and DPPH methods compared to the leaves.

The leaves and stembark extract were individually applied to analytical TLC plates and eluted using selected mobile phases. Upon elution, the plates were sprayed with specific spot-visualizing reagents: 1% $FeCl_3$ for the detection of polyphenols, NP/PEG for flavonoids, and ABTS for bioautography to identify the presence of antioxidant compounds.

A positive result for polyphenols was indicated by a dark green or bluish-black color in visible light, while a positive result for flavonoids was indicated by a light blue, green, yellow, orange fluorescence or an increase in intensity under UV 365 nm. A positive result of antioxidants was indicated by a pale blue to white-colored spot on a turquoise background in visible light (Spangenberg *et al.* 2011; Nile and Park 2015). The results were summarized in Table 5. Based on results (Fig. 5), the area exhibiting positive antioxidant activity also yielded favorable results to $FeCl_3$ and NP/PEG. Thus, the active compounds in the ethyl acetate and 80% ethanolic fraction of Faloak leaves and stembark, showing white color spot in ABTS and giving a positive color of spot to $FeCl_3$ and NP/PEG, were likely polyphenols and flavonoids.

Table 1: The yield of faloak extracts using various solvents

Plant Parts	Solvent	Yield (%)
Leaves	n-Hexane	1.70 ± 0.05
	Ethyl acetate	1.60 ± 0.30
	80% ethanol	12.27 ± 0.54
Stembark	n-Hexane	0.35 ± 0.02
	Ethyl acetate	0.59 ± 0.08
	80% ethanol	8.13 ± 0.58

Note: Data are presented as mean ± standard deviation (n = 3)

Table 2: Total phenolic and flavonoids content of faloak extracts

Plant Parts	Solvent	Total phenolic content (% GAE)	Total flavonoids content (% QE)
Leaves	Ethyl acetate	2.54 ± 0.01	2.05 ± 0.08
	80% ethanol	3.85 ± 0.02	2.23 ± 0.05
Stembark	Ethyl acetate	2.69 ± 0.03	1.64 ± 0.07
	80% ethanol	4.50 ± 0.01	1.23 ± 0.05

Note: Data are presented as mean ± standard deviation (n = 3); GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent

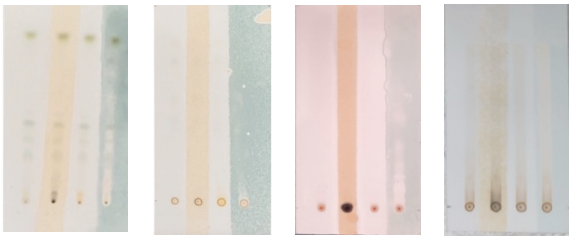

Fig. 5: TLC-Bioautography results for leaves and stembark of faloak

Note: TLC System (SP: stationary phase; MP: mobile phase); Left to right are visible, $FeCl_3$ 1%, NP/PEG, ABTS

(a) Ethyl acetate fraction of faloak leaves. SP: silica gel GF 254; MP: chloroform : ethyl acetate : methanol (5:5:1)

(b) Ethanolic fraction of faloak leaves. SP: silica gel GF 254; MP: chloroform ethyl : acetate : formic acid (3:3:0.5)

(c) Ethyl acetate fraction of faloak stembark. SP: silica gel GF 254; MP: chloroform : methanol (9:1)

(d) Ethanolic fraction of faloak stembark. SP: Silica gel GF 254; MP: 2-propanol : chloroform : glacial acetic acid (5:1:0.5)

Discussion

The extraction yields of Faloak leaves and stembark (Table 1). Extraction yield is a parameter that determines the quality of an extract, representing the ratio of the weight of the resulting fractions to the original weight of simplicia (Monagas *et al.* 2022). A higher yield value indicates the production of more fractions, signifying a better fractionation process (Dhanani *et al.* 2013). The principle "like dissolves like" was applied in selecting solvents (Rasul 2018). In the sequential solvent-extraction procedures, n-hexane, ethyl acetate, and 80% ethanol served as a nonpolar, semipolar and polar solvent, respectively. Among the three solvents, 80% ethanol produced the highest yield of fraction in both leaves and stembark. This demonstrated that the predominant secondary metabolites found in Faloak leaves and stembark are polar compounds. This result indicates that ethanolic 80% is the best solvent to get the optimum extract (Uthayarasa *et al.* 2010). This research used kinetic maceration as an extraction method. Kinetic maceration is a conventional extraction that uses kinetics to improve extraction efficacy. It is simple, recognized as an energy-saving procedure, suitable for thermolabile compounds, cost-effective and scalable (Rasul 2018; Gori *et al.* 2021).

Quantification of phenolic and flavonoids was determined to both extract of leaves and stembark. The ethanolic extract in both leaves and stembark showed the highest level, especially in phenolic level. Phenolic compounds have hydrophilic properties and demonstrate substantial solubility in polar solvents, indicating that the highest phenolic level was founded in ethanolic extract (Rodrigues *et al.* 2022). In other studies, leaves contain a higher concentration of polyphenol compounds than other parts and these compounds are typically found in polar solvents. Phenolics and flavonoids are secondary

metabolites found in the plant. Phenolic compounds are bioactive secondary metabolites that have a significant role in a wide range of therapeutic effects (Mamari 2021). The term "phenol" refers to a phenyl ring containing one or more hydroxyl substituents. Phenolic is a natural compound that has a benzene ring with one or more hydroxyl groups, along with functional derivates, such as esters, methyl esters and glycoside (Tsao 2010). Polyphenol is a natural compound characterized by the presence of at least two phenyl rings, each containing one or more hydroxyl substituents (Lattanzio 2013). It includes a diverse category of bioactive phytochemicals, encompassing flavonoids, stilbenes, phenolic acid and lignans (Fraga *et al.* 2019). They are natural compounds synthesized in numerous plants that possess high antioxidant capacity (Dias *et al.* 2021). The compounds are also linked to several therapeutic effects, such as obesity, diabetes, cardiovascular disease, hyperlipidemia, cytotoxicity and neurodegenerative diseases in humans (Fraga *et al.* 2019; Rana *et al.* 2022).

The ethanolic extract of leaves contained slightly higher flavonoids level compared to ethyl acetate extract. The previous study showed the identical result that the polar solvent can enhance the amount of Phenolic and flavonoids in Faloak extraction (Purwantiningsih *et al*. 2024). Flavonoid compounds have high solubility in polar and semipolar solvents, *e.g*., ethanol, methanol, ethyl acetate, n-butanol and water (Hikmawanti *et al.* 2021).

Flavonoids and their conjugates constitute a broad category of natural chemicals, with over 8,000 distinct flavonoids identified. Each part of a plant might have varying types and levels of flavonoids. Flavonoids constitute a primary category of polyphenols characterized by a pair of aromatic rings interconnected by a three-carbon chain inside a heterocyclic structure. The chemical structure of flavonoid compounds is based on C6C3C6 skeleton (Lattanzio 2013). The major classes include flavones, isoflavones, flavonols, anthocyanidins, flavanones, flavanols, chalcones and aurones. Flavonoids may either bind or not bind with sugar moieties (Chaves *et al.* 2020). Therefore, measuring phenolic and flavonoid levels is crucial for predicting the antioxidant potential of the fraction. Additionally, the level of phenolic and flavonoids vary across various plant parts (Table 2).

Numerous studies indicated the free radical scavenging action of phenolic and flavonoids. The maximum scavenging activity seemingly necessitated the presence of the 3-OH group linked to the 2.3-double bond and adjacent to the 4-carbonyl in ring C. The other mechanism involves the orthodiphenolic structure in B ring, as the monophenolic ring is ineffective as a hydrogen donor (Rice-Evans *et al.* 1996). In in-vivo studies, phenolic and flavonoids have significant antioxidant activity. Numerous compounds, including flavonols, flavones, hydroxycinnamic acids, hydroxybenzoic acids, coumestans, anthocyanins and diferuloylmethanes, have demonstrated antioxidant activity in animal models. Recent study reveals that phenolic compounds help protect the body against many health ailments (Martins *et al.* 2016). The result showed that stembark had a higher phenolic content than the leaves. Both the leaves and stembark also contained flavonoids compounds, with a higher concentration in the leaves. Martati *et al.* (2023) and Dean (2024) identified phenolic compounds in stembark of Faloak, *e.g*., vanillin, apocynin, methyl cinnamate, scopoletin, L-pipevolinic acid, arecoline, δ-valerolactam, 3.4 dihydroxybenzaldehyde, 4-hydroxybenzaldehyde, epicathechin, rutin and various fatty acid (Martati *et al*. 2023; Dean 2024).

Table 3: Antioxidant activity of faloak fractions by the ABTS assay method

Plant parts	Solvent	Concentration (ppm)	Inhibition (%)	IC_{50} (ppm)
Leaves	Ethyl acetate	50	15.53 ± 1.96	233.88
		100	19.64 ± 0.93	
		200	52.02 ± 0.47	
		400	78.19 ± 2.02	
	80% ethanol	25	19.21 ± 1.99	91.82
		50	40.87 ± 0.95	
		100	50.46 ± 2.15	
		150	72.93 ± 0.92	
Stembark	Ethyl acetate	10	12.84 ± 0.88	48.49
		25	34.58 ± 1.66	
		50	54.13 ± 0.49	
		100	90.96 ± 0.57	
	80% ethanol	10	14.21 ± 0.69	67.19
		27.5	20.97 ± 1.30	
		55	56.69 ± 1.33	
		100	80.09 ± 2.51	

Note: Data are presented as mean ± standard deviation (n = 4); IC_{50} is the concentration required to inhibit 50% of free radicals

Table 4: Antioxidant activity of faloak fractions by the DPPH assay method

Plant parts	Solvent	Concentration (ppm)	Inhibition (%)	IC_{50} (ppm)
Leaves	Ethyl acetate	200	34.75 ± 0.30	387.44
		400	49.07 ± 0.93	
		600	68.15 ± 0.65	
		800	88.93 ± 0.43	
	80% ethanol	25	26.74 ± 0.71	58.06
		50	46.74 ± 1.53	
		100	83.37 ± 2.40	
		150	94.17 ± 0.23	
Stembark	Ethyl acetate	10	8.06 ± 0.24	26.37
		20	41.86 ± 0.31	
		30	58.26 ± 0.62	
		40	79.42 ± 0.62	
	80% ethanol	10	19.74 ± 1.89	21.18
		20	51.22 ± 0.91	
		30	73.01 ± 0.68	
		40	92.84 ± 0.18	

Note: Data are presented as mean ± standard deviation (n = 4); IC_{50} is the concentration required to inhibit 50% of free radicals

Table 5: TLC screening and bioautography of faloak fractions

Plant Parts	Solvent	Results		
		Polyphenol	Flavonoids	Antioxidant
Leaves	Ethyl acetate	+	+	+
	80% ethanol	+	+	+
Stembark	Ethyl acetate	+	+	+
	80% ethanol	+	+	+

Note: (+) positive

Content of catechins in the *S. quadrifida* stembark infusion was prepared at a concentration of 7.786% w/w using the HPLC method. Catechin is a polyphenol compound comprised of two aromatic rings and several hydroxyl groups. The presence of o-hydroxyl in the B ring and hydroxyl group in the A and C rings play a crucial role in its antioxidant activity (Riwu *et al.* 2023). Scopoletin, often referred to as 6-methoxy-7 hydroxycoumarin, is a coumarin group distributed in many plants (Cakir *et al.* 2022). Scopoletin possesses a scavenging function that mitigates damage caused by superoxide anions (Shaw *et al.* 2003). Its antioxidant mechanism is associated with ROS scavenging activity through hydrogen atom transfer (HAT) (Antika *et al.* 2022).

According to several previous studies, ABTS and DPPH (are among the three most prevalent assay for antioxidant evaluation, alongside FRAP (ferric-reducing antioxidant power (Ilyasov *et al.* 2020). The quantitative assessment of antioxidant activity was determined using the ABTS and DPPH assay methods to estimate the IC_{50} value. The IC_{50} values represent the concentration of extracts required to eliminate 50% of radicals (Olszowy-Tomczyk *et al.* 2021). ABTS radical cation can be produced and absorb at 734 nm in water (Dorsey *et al.* 2017). The addition of an antioxidant to the ABTS solution resulted in a reduction in absorption due to the termination of the ABTS radical cation (Liang and Kitts 2014). The ABTS assay results showed that the ethanolic and ethyl acetate fractions of the stembark had strong antioxidants in both methods, but the ethyl acetate fraction of the leaves showed much less antioxidant activity compared to others.

DPPH is considered as a widely used method to measure antioxidant activity due to its accuracy, ease of use, and cost-effectiveness. This calorimetric method can assess antioxidant activity using the stable synthetic radical DPPH. As an antioxidant compound reacts with DPPH (purple), its color changes from violet to yellow. DPPH contains an unpaired electron originating from the nitrogen atom. As a hydrogen-donating compound reacts with DPPH, it converts DPPH (purple) into DPPH-H (yellow) (Hawash *et al.* 2022). The result showed that ethanolic and ethyl acetate fractions of Faloak stembark had an exceptionally strong antioxidant activity, followed by the ethanolic fraction of Faloak leaves, which demonstrated strong antioxidant activities. In contrast, the ethyl acetate fraction of Faloak leaves displayed a very weak antioxidant activity (Table 4), Consistent outcomes were observed in both the ABTS and DPPH assays, conforming that the ethanolic and ethyl acetate fractions of stembark exhibited very strong antioxidants in both methods, while the ethyl acetate fraction of leaves exhibited markedly weaker antioxidant activity compared to others. In a previous study, Faloak stembark infusion generated moderate antioxidant activity with an IC_{50} value 51.5 μg/mL (Riwu *et al.* 2023). The research findings indicated that sequential solvent extraction could enhance the antioxidant efficacy of Faloak. Sequential solvent extraction is the process of separating secondary metabolites of plants according to their solubility in a solvent (Abubakar and Haque 2020). This method increases the selectivity of targeted compounds, improves the extraction yield and reduces waste. The antioxidant activity increased due to the elevated concentration of active compounds in the fractional extract. The concentration of antioxidant active compounds can increase the efficacy of natural antioxidants.

Thin layer chromatography is a method that offers insights into profiling, fingerprinting, and both qualitative and quantitative assessment of phytoconstituents (Gaurav *et al.* 2023). Bio-autography has advanced swiftly in the discovery of novel antioxidant substances from plants. This approach offers rapidity, cost-effectiveness, and enhanced bioassay-directed fractionation of bioactive chemicals (Suleimana *et al.* 2009). TLC bio-autography has been extensively used to assess antibacterial, antifungal, anticancer, antioxidant, and other enzymatic activities. There are three types of TLC bio-autography: agar diffusion, direct bio-autography and agar overlay bio-autography (Wang *et al.* 2021). This research used direct bio-autography by spraying the radical solution to TLC plate. The result can be directly obtained by the color changes of the TLC spot. The antioxidant bio-autography assay using the ABTS method revealed that the white spot indicates positive antioxidant activity (Huang *et al.* 2017). The results indicated that compounds exhibiting antioxidant activity are phenolic and flavonoid compounds.

Conclusion

The 80% ethanolic extract of Faloak stembark had significantly superior antioxidant activity compared to others. The stembark exhibited a higher amount of polyphenols than the leaves, although the leaves contained a greater quantity of flavonoids compounds. The ethanolic extract of Faloak leaves also showed high antioxidant activity and have potential as a new candidate for various pharmacological treatments due to their robust antioxidant activity and commendable sustainability.

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Author Contributions

FS, KK, RB, ATP, KA prepared and planned the experimental design. FS, SV, JR, SBL, PVD, LO and SAF carried out the experiment. FS, KK and ATP conducted the statistical data analysis. FS, KK, ATP and KA wrote the article.

Conflicts of Interest

No conflict of interest.

Data Availability

Data may be solicited from the corresponding author for justifiable scientific purposes.

Ethics Approval

In this research we didn't use the animal, cell or human, so we didn't need ethical approval for this research.

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