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Phytochemical Analysis of Bioactive Compounds in Ethanolic Extract of *Sterculia quadrifida* R.Br.

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Abstract. The present study was carried out to analyze the possible active constituents present in the ethanolic extract of stem bark, leaves, and seeds of *Sterculia quadrifida* (Sterculiaceae). *S. quadrifida* is locally known as "Faloak" in East Nusa Tenggara Province and commonly used in traditional medicine to treat hepatitis. Its parts were collected from Kupang City, East Nusa Tenggara province. Chemical compounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Fifteen compounds are identified in stem bark, twenty-one compounds in leaves, and fourteen compounds in seeds that can contribute to the medicinal quality of the plant. The prevailing compounds are Tributyl acetyl citrate (35.66%), 2,6,10,14,18,22-Tetracosahexaene (20.64%) and Hexadecanoic acid (48.84%). A minor component, such as Vitamin E, is also present. Hexadecanoic (synonym - Palmitic acid) acid has been reported to possess antioxidant, antimicrobial, and anti-inflammatory activities.

INTRODUCTION

The genus Sterculia is a member of the family Sterculiaceae, which is widely used in traditional medicine. *Sterculia quadrifida* R.Br. is known as Faloak in the province of East Nusa Tenggara. The plants are flowering from March to June and its fruit emerges from July to October. The bark is used for the treatment of hepatitis/liver dysfunction, restoring stamina, and lower back pain [1,2]. According to an herbalist in Kupang Regency, *S. quadrifida* leaves can be used to treat breast cancer [3]. The Aboriginal people in Australia use *S. quadrifida* leaves to treat sore eyes [4] and consume its seed [5]. This plant has several local names according to the regions, such as 'Faloak' (Kupang), 'Nitaen' or 'Mitaen' (Atambua, Belu Regency), 'Flolo' (Kefamenanu, Timor Tengah Utara Regency), 'Kawarid' (Sumba Tengah Regency), 'Penil' (Alor Regency), 'Ago' (Flores), and 'Klengis' or 'Slengit' (Flores Timur Regency). However, faloak is found abundantly in Timor Tengah Selatan and Kupang Regency [6]. Faloak mostly grows in open areas and coastline [7]. It also has not been cultivated.

Considering the use of faloak bark, leaf, and seed, their chemical constituents' needs to be investigated. Phytochemical screening on *S. quadrifida* bark has been carried out, discovering that it contains flavonoids, phenolics, terpenoids, alkaloids [8], and saponins [9]. However, the analysis of its derived compounds has not been carried out yet. Gas Chromatography-Mass Spectrometry (GC-MS) is a method of separating organic compounds using two compound analysis methods. GC-MS is widely used to analyze non-polar compounds, fatty acids, alkaloids, terpenoids, and lipids [10]. This study aims to obtain information about bioactive components that exist on the bark of *S. quadrifida* stem, leaves, and seeds so that they can be utilized optimally.

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EXPERIMENTAL DETAILS

This research was conducted from July to October 2015. *S. quadrifida* bark was obtained from 3 trees (dbh of 22, 25, and 27 cm), which were taken randomly in the Kupang Regency at 408 m a.s.l. The map of sampling location is shown in Figure 1.

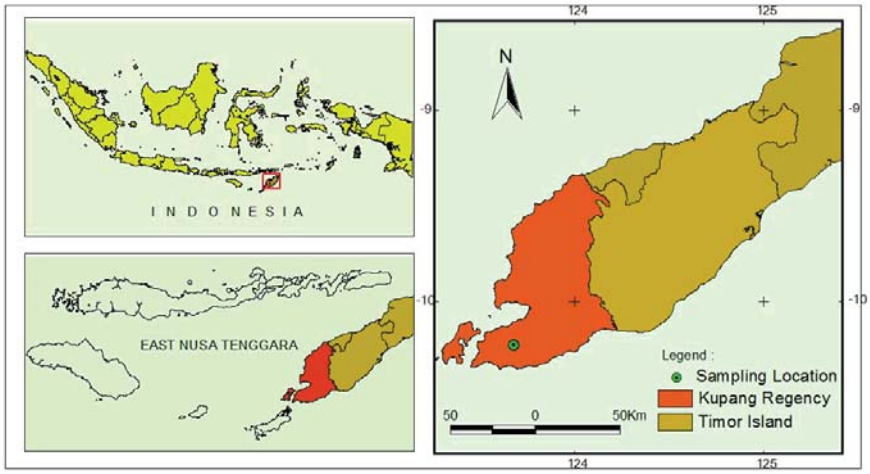

FIGURE 1. Map of sampling location.

Materials collected for extraction are shown in Figure 2. The sampled bark is the bark that has never been peeled (Figure 2a). Bark stripping was done using a sharp machete and without a specific requirement of bark height from the ground. The selected leaves were those with dark green color (Figure 2b), and seeds were taken from dry brown fruits (Figure 2c).

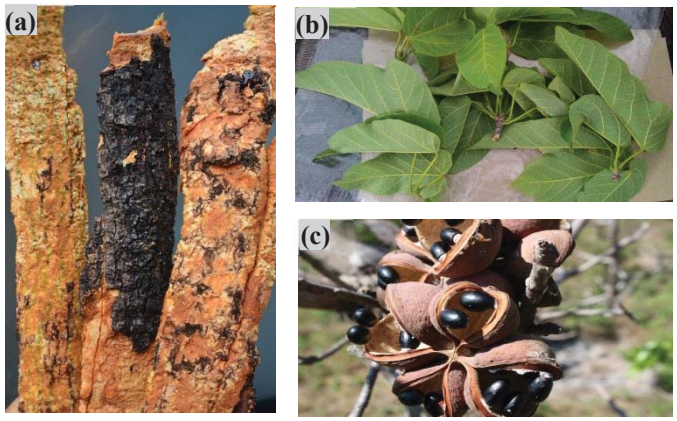

FIGURE 2. Faloak bark (a), leaves (b), fruits and seeds (c).

S. quadrifida bark was dried in an oven at 55 °C for three days, while the leaves and seeds were dried for 24 h. After that, the bark and leaves were turned into powder and sifted with a 40 mesh sieve. The dried seeds were peeled to remove the black outer skin. Fruit flesh was made powder and filtered with a 40 mesh sieve. 50 g of each plant part sample (dried bark, leaves, and seeds powder) was macerated using 95% ethanol (1 : 6) and soaked for 48 h. Ethanol is a semi-polar solvent [11,12] and is widely used for GC-MS analysis [13–17]. The macerates' drying process into extracts required a different time, approximately 45 min for bark and 1 h for seeds and leaves.

The extract of each sample was filtered and dried using a rotary evaporator with a water bath at 50 ºC to obtain the dry extract. Extract of faloak bark, leaf, and seed had different color and texture, as shown in Figure 3. The dry extract of stem bark was reddish-black granules, while the leaf extract was greenish-black and had a paste-like texture. The leaves extract also took a long time to dry, probably due to the leaves' higher water content. The seed extract was brown with a thick creamy texture.

FIGURE 3. Extract of *S. quadrifida* bark, leaf, and seed.

The GC-MS analysis was performed at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Gadjah Mada University. 2.5 g of each extract was subjected to column chromatography and eluted with chloroform. Then, the extract was injected into the GC-MS instrument (Single Quadrupole GCMS-QP2010 SE Shimadzu). Furthermore, the chemical components of stem bark, leaves, and *S. quadrifida* seeds were identified by process conditions, consisting of oven temperature GC 50 ºC, injector temperature 310 ºC, split injection mode, helium carrier gas, interface temperature 305 ºC, ion source temperature 250 ºC, and DB5 MS detectors. The mass spectrogram produced was matched by the instrument automatically into certain compounds based on the similarity of the m/z pattern with mass spectrograms databases in the NIST (National Institute of Standards and Technology) and Wiley library. The percentage of compound components was calculated based on each peak area's ratio to the total peak area of all components in the chromatogram.

RESULTS AND DISCUSSION

The chromatograms of stem bark, leaves, and seeds of *S. quadrifida* confirms the presence of various compounds with different retention times. The GC-MS chromatogram of the ethanolic bark extract of *S. quadrifida* shows 15 peaks indicating the presence of fifteen compounds, as shown in Figure 4. Their retention time, molecular formula, similarity index, and concentration (peak area %) are presented in Table 1. All compounds are detected at 38.425 to 54.869 min. Tributyl acetyl citrate is observed at the retention time of 44.473 min, while the first compound is identified with less retention time (38.425) was Hexadecanoic acid.

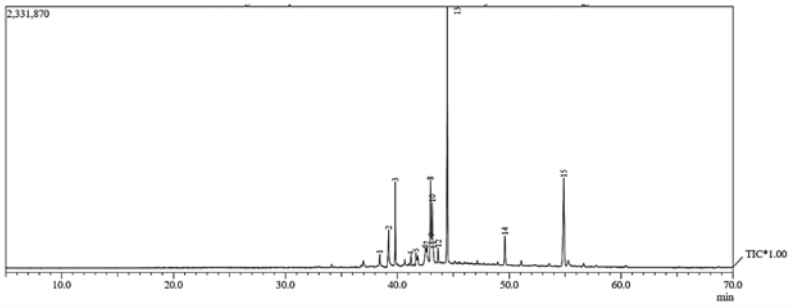

FIGURE 4. GC-MS chromatogram of ethanolic extract of the *S. quadrifida* stem bark

TABLE 1. Compounds identified in the ethanolic stem bark extract of *S. quadrifida*

Number	Retention Time	Name of The Compound	Similarity Index (Si)	Molecular Formula	Peak Area (%)
1	38.425	Hexadecanoic acid, methyl ester	96	$C_{17}H_{34}O_2$	1.2
2	39.231	Hexadecanoic acid	94	$C_{16}H_{32}O_2$	5.29
3	39.819	Hexadecanoic acid, ethyl ester	97	$C_{18}H_{36}O_2$	9.13
4	41.216	ethyl 9-hexadecenoate	89	$C_{18}H_{34}O_2$	0.79
5	41.675	9,12-Hexadecadienoic acid	94	$C_{17}H_{30}O_2$	0.92
6	42.500	9,12-Octadecadienoic acid	86	$C_{18}H_{32}O_2$	2.21
7	42.644	Heptadecene-(8)-Carbonic acid-(1)	93	$C_{18}H_{34}O_2$	1.43
8	42.965	Linoleic acid ethyl ester	95	$C_{20}H_{36}O_2$	9.83
9	43.042	1,Z-5,E-7-Dodecatriene	76	$C_{12}H_{20}$	1.27
10	43.118	Ethyl Oleate	94	$C_{20}H_{38}O_2$	7.69
11	43.217	9-Octadecenoic acid	79	$C_{20}H_{38}O_2$	0.71
12	43.638	Ethyl stearate	96	$C_{20}H_{40}O_2$	1.39
13	44.473	Tributyl acetyl citrate	94	$C_{20}H_{34}O_8$	35.66
14	49.613	Bis(2-ethylhexyl) phthalate	96	$C_{24}H_{38}O_4$	4.11
15	54.869	1,2-Benzenedicarboxylic acid	79	$C_{24}H_{38}O_4$	18.37

GC–MS chromatogram of the 21 peaks of the compounds detected in the ethanolic extract of leaves of *S. quadrifida* is shown in Figure 5. The compounds' retention time, molecular formula, and peak area are listed in Table 2. The compounds in faloak leaf extract were detected at 36.83 minutes to 72.125 min. 2,6,10,14,18,22-Tetracosahexaene is detected at 58.058 minutes and vitamin E at the end of retention time (72.125 min). The most prevalent compounds are 2,6,10,14,18,22-Tetracosahexaene (20.64%), vitamin E (16.79%), Phytol (14.97%), Hexadecanoic acid (9.12%) and 9,12,15-Octadecatrienoic acid, methyl ester (8.4%).

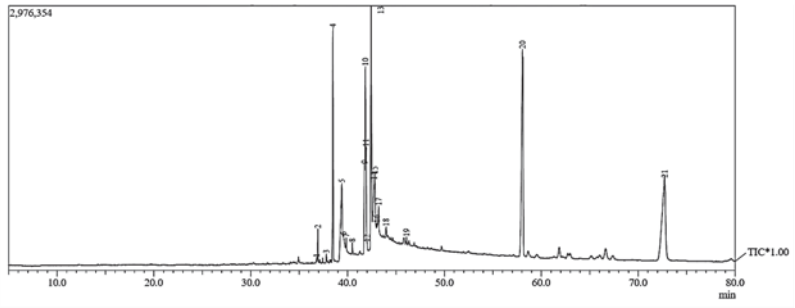

FIGURE 5. GC-MS chromatogram of ethanolic extract of the *S. quadrifida* leaves

TABLE 2. Compounds identified in the ethanolic leaf extract of *S. quadrifida*

Number	Retention Time	Name of The Compound	Similarity Index (SI)	Molecular Formula	Peak Area (%)
1	36.83	2-Pentadecanone	95	$C_{15}H_{36}O$	0.16
2	36.926	Neophytadiene	96	$C_{20}H_{38}$	1.16
3	37.812	Neophytadiene	93	$C_{20}H_{38}$	0.28
4	38.468	Hexadecanoic acid, methyl ester	96	$C_{17}H_{34}O_2$	9.12
5	39.408	Hexadecanoic acid	94	$C_{16}H_{32}O_2$	7.57
6	39.583	Hexadecanoic acid	90	$C_{16}H_{32}O_2$	1.94
7	39.853	Hexadecanoic acid, ethyl ester	97	$C_{18}H_{36}O_2$	0.61
8	40.485	Heptadecanoic acid, methyl ester	96	$C_{18}H_{36}O_2$	0.38
9	41.748	11,14-Octadecadienoic acid	86	$C_{19}H_{34}O_2$	3.63
10	41.84	9,12,15-Octadecatrienoic acid, methyl ester	85	$C_{20}H_{36}O_2$	8.4
11	41.915	Methyl 9-octadecenoate	94	$C_{19}H_{32}O_2$	2.66
12	42.008	13-Octadecenoic acid, methyl ester	88	$C_{19}H_{36}O_2$	0.2
13	42.419	Phytol	95	$C_{20}H_{40}O$	14.97
14	42.75	Cyclohexane	80	$C_{10}H_{16}$	4.76
15	42.813	Heptadecene-(8)-carbonic acid-(1)	92	$C_{18}H_{34}O_2$	3.65
16	43.08	Linoleic acid ethyl ester	85	$C_{20}H_{36}O_2$	1.24
17	43.192	9,12-Octadecadienoic acid	84	$C_{18}H_{32}O_2$	1.27
18	43.977	14,17-Octadecadienoic acid, methyl ester	88	$C_{19}H_{34}O_2$	0.39
19	46.074	Cyclopentane tridecanoic acid, methyl ester	86	$C_{19}H_{36}O_2$	0.18
20	58.058	2,6,10,14,18,22-Tetracosahexaene	95	$C_{30}H_{50}$	20.64
21	72.125	Vitamin E	77	$C_{29}H_{50}O_2$	16.79

The chromatogram of the ethanolic extract of *S. quadrifida* seed shows 14 different compounds (Figure 6). The major phytochemicals include Hexadecanoic acid (48.84%), Heptadecene-(8)-carbonic acid-(1) (24.25), 9,12-Octadecadien-1-ol (8.83%), and 11-Octadecenoic acid, methyl ester, (Z)- (5.18%), as listed in Table 3. The compounds in faloak seed extract are detected at 38.503 to 56.266 min (Table 3). Hexadecanoic acid is identified at a retention time of 40.617 min, and Heptadecene-(8)-carbonic acid- (1) at 43.974 min.

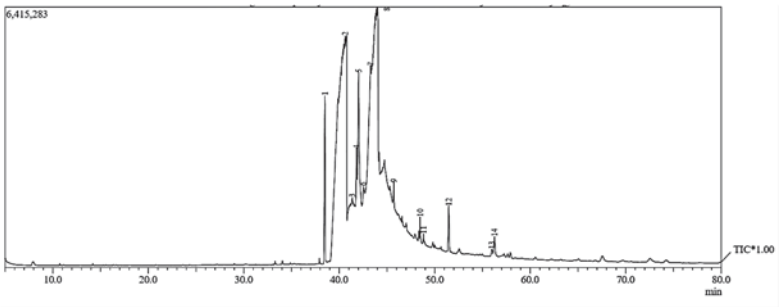

FIGURE 6. GC-MS chromatogram of ethanolic extract of the *S. quadrifida* seeds

TABLE 3. Compounds identified in the ethanolic seed extract of *S. quadrifida*

Number	Retention Time	Name of The Compound	Similarity Index (SI)	Molecular Formula	Peak Area (%)
1	38.503	Hexadecanoic acid, methyl ester	96	$C_{17}H_{34}O_2$	3.55
2	40.617	Hexadecanoic acid	95	$C_{16}H_{32}O_2$	48.84
3	41.365	9,12-Octadecadienoic acid (Z,Z)-	84	$C_{18}H_{32}O_2$	3.08
4	41.817	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	96	$C_{19}H_{34}O_2$	2.21
5	42.034	11-Octadecenoic acid, methyl ester, (Z)-	96	$C_{19}H_{36}O_2$	5.18
6	42.517	Octadecanoic acid, methyl ester	85	$C_{19}H_{38}O_2$	0.7
7	43.292	9,12-Octadecadien-1-ol	85	$C_{18}H_{34}O$	8.83
8	43.974	Heptadecene-(8)-carbonic acid-(1)	93	$C_{18}H_{34}O_2$	24.25
9	45.715	Cyclopentolate	91	$C_{17}H_{25}NO_3$	0.4
10	48.471	2-Propenoic acid, 2-(dimethylamino)ethyl ester 1,3,5-Trisilacyclohexane (CAS)	92 69	$C_7H_{13}NO_2$	0.61
11	48.824	Cyclocarbosilane		$C_3H_{12}SI_3$	0.26
12	51.475	N-2-methyloctadecanoyl pyrrolidine	81	$C_{23}H_{45}NO$	1.16
13	55.697	Piperidine, 1-(1-oxo-9-octadecenyl)	80	$C_{23}H_{43}NO$	0.25
14	56.266	Piperidine, 1-(1-oxo-9-octadecenyl)	92	$C_{23}H_{43}NO$	0.68

The bioactive compounds present in ethanolic extracts of bark, leaves, and seeds of *S. quadrifida* can contribute to the medicinal quality of the plant. The prevailing compounds found in the ethanolic extract of *S. quadrifida* stem bark, leaf, and seed are lipid components. The most prevailing phytochemical compounds in bark extract based on their peak area are Tributyl acetyl citrate (35.66%), Linoleic acid ethyl ester (9.83%), Hexadecanoic acid, ethyl ester (9.13%), Ethyl Oleate (7.69%), and Hexadecanoic acid (5.29%). The most dominant compound in *S. quadrifida* stem bark, Tributyl acetyl citrate, is used as a solvent, flavor ingredient, and in the manufacture of pharmaceutical drugs [18]. Ethyl oleate isolated from *Phyllantus amarus* is also found to have antimicrobial activity [19].

Heptadecene-(8)-carbonic acid-(1) is detected in all extracts. It is also found in the leaf ethanolic extract of *Coleus amboinicus* [20]. It has antimicrobial and antioxidant activity [21]. Hexadecanoic acid and Octadecadienoic acid are fatty acids. Hexadecanoic acid can be found in all extracts examined. Hexadecanoic acid possesses some biological activity such as antioxidants, hypocholesterolemic, nematicide, and pesticide [22]. Phytol and Octadecanoic acid, methyl ester, show anti-inflammatory activities [23,24]. Phytol is a diterpene. Phytol and hexadecanoic acid also present in the leaves extract of *S. urens* [25]. A high percentage of Hexadecanoic acid or Palmitic acid (48.84%) in seed extract of *S. quadrifida* is similar to palmitic acid found in *S. foetida* seed (52%) [26]. Meanwhile, 9,12,15-Octadecatrienoic acid, methyl ester, has anti-inflammatory and antimicrobial activities [27]. Piperidine is an alkaloid and has antimicrobial activity [28] used as a pesticide, CNS-Depressant, and diaphoretic [29]. Vitamin E is found in the leaf extract of *S. quadrififda*. It is commonly found in the leaves of the medicinal plant, such as *Ficus septica*, *Cordoline* sp., *Celocasia argantea*, and *Physalis angulate* [30]. Vitamin E (α-tocopherol) plays a role as an antioxidant [31], reduces diabetic risks [32], and protects the skin from UV irradiation [33]. Other minor compounds identified also possess biological activity, such as Cyclopentolate, which is used as anti-cholinergic in pharmaceutical [30].

The GCMS analysis is the first step towards understanding the nature of active principles in medicinal plants, and this type of study will be helpful for further detailed study. However, individual phytochemical constituent isolation and subjecting it to the biological activity will definitely give fruitful results.

SUMMARY

Faloak bark, leaves, and seeds contain various bioactive compounds and are recommended as a plant of phytopharmaceutical importance. The most prevalent compound in the bark, leaf, and seed extract is Tributyl acetyl citrate (35.66%), 2,6,10,14,18,22-Tetracosahexaene (20.64%), and Hexadecanoic acid (48.84%), respectively. Further research, such as isolation of individual phytochemical constituents and subjecting them to the biological activity, are required.

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