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Research Article

Antioxidant Activity of *n*-hexane and Etil Acetate Fractions of Bangkal (*Nauclea subdita* (Korth.) Steud.) Leaves

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Abstract

Bangkal (*Nauclea subdita* (Korth.) Steud.) is a tropical plant belonging to the Rubiaceae family, commonly found in South Kalimantan. This plant is one of the plants that has efficacy as a medicinal plant. This study aimed to quantitatively identify secondary metabolites and antioxidant activity in the *n*-hexane and ethyl acetate fractions of *N. subdita* leaves. The method of identification of secondary metabolites using the test tube. Antioxidant activity using the DPPH method based on IC_{50} value. The results of identifying secondary metabolites in the *n*-hexane fraction of *N. subdita* leaves contain alkaloids, flavonoids, steroids, and phenolic compounds, while the ethyl acetate fraction of *N. subdita* leaves contain alkaloids, flavonoids, steroids, tannins, saponins, and phenolics. The results of the antioxidant activity test of the *n*-hexane fraction and the ethyl acetate fraction of the leaves of *N. subdita* showed IC_{50} values of 229.61178±3.65919 and 54.54296±0.02236 ppm, respectively. Based on the IC_{50} value, the *n*-hexane fraction of *N. subdita* leaves had weak antioxidant activity, and the ethyl acetate fraction of *N. subdita* leaves had strong antioxidant activity.

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INTRODUCTION

Medicinal plants can be defined as plants that are part, all parts, and or parts of plant exudates that can be used as drugs, medicinal ingredients, or medicinal ingredients[1]. Medicinal plants are known to have efficacy because they contain secondary metabolite compounds, such as phenolic compounds, alkaloids, flavonoids, and terpenoids. These secondary metabolites are believed to have efficacy as a medicine for a disease which can also be used to maintain a healthy body[2].
Antioxidants have essential functions in the health of the body, including inhibiting and neutralizing the oxidation reactions that involve free radicals[3]. Antioxidants are substances that function as free radical inhibitors; generally, antioxidants work by inhibiting the formation of radicals that have the potential to carry out autoxidation[4]. Antioxidants are commonly found in various parts of plants, such as roots, stems, leaves, flowers, fruits, skins, and seeds[5]. One of them is found in the bangkal (*Nauclea subdita* (Korth.) Steud)[6].
Nauclea subdita is a tropical plant belonging to the Rubiaceae family, which is generally found in swamps, lowlands, watersheds and rivers, and mountain forests[6]. People typically use this plant for such treatments by applying the leaves to boils for tumors, and the boiled water of the leaves is used to treat diarrhea and toothache. This plant also contains antioxidants and steroids to help the growth of cells in the skin, so many people from Banjar Tribe use it as a basic powder ingredient and call it *pupur bangkal*[7]. This is supported by research conducted by Wardhani and Akhyar[8], stating that phytochemical screening was carried out on extracts from the stem bark of the *N. subdita*, which contain compounds belonging to the alkaloids, flavonoids, saponins, and polyphenols, and quinones. Research on the leaves of *N. subdita* will contribute to developing the utilization of this plant, and the leaves are abundantly available and easy to harvest and process.
Previous research on the antioxidant activity of leaf and stem bark extracts of *N. subdita* has IC_{50} values of 79.62 ppm and

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307.1496 ppm[8]. This activity needs to be investigated further by researching to identify the content of secondary metabolites and the antioxidant activity of the *n*-hexane and ethyl acetate fractions of *N. subdita* leaves as a basis for obtaining the active compounds responsible for their antioxidant activity.

MATERIALS AND METHODS

Materials

The tools used in this study included a blender (Panasonic), separating funnel (Schott Duran), fume hood (Local), refrigerator, oven (Finco), pro pipette, analytical balance (Pioneer), water bath (Memmert), UV lamp with wavelengths of 254 and 366 nm, and UV-Vis Spectrophotometer (Perkin Elmer). The materials used in this study included distilled water, Mayer's reagent, Dragendorff's reagent, Mg powder, concentrated HCl, Liebermann-Burchard reagent, 1% gelatin, 5% $FeCl_3$, chloroform, 10% H_2SO_4, DPPH, quercetin, thin-layer chromatography (TLC) plate silica gel GF_{254}, methanol p.a., *n*-hexane p.a., ethyl acetate p.a., and leaves of *N. subdita*.

Methods

Determination

The leaves of *N. Subdita* were taken from the Banua Banjarbaru Botanical Garden, Jalan Dharma Praja I, Trikora, Cempaka, South Kalimantan Provincial Government Office Area, Banjarbaru, South Kalimantan, Indonesia. Plant determination was carried out at the Technical Implementation Unit of the Regional Research and Development Agency of the Banua Banjarbaru Botanical Garden, South Kalimantan with voucher number of 050/03-LIT/KRB. Plant samples in stems, leaves, roots, and fruit were made into a herbarium, and further determination was carried out.

Sample extraction

The leaves of *N. Subdita* were collected, processed into simplicia, and ground into powder. The powder weighed as much as 300 g, was then put into a macerator, then added methanol solvent up to 1 cm above the surface of the powder while measuring the volume of the inserted solvent. After all the powder was submerged, the leaves were stirred gently, then covered using aluminum foil. Extraction was carried out for 7 x 24 hours by changing the solvent every 24 hours and stirring every 8 hours or three times per 24 hours of the extraction process. Stirring and maceration were performed to increase the extraction process's effectiveness. The maceration results were then filtered and evaporated to obtain a thick extract. The thick extract was stored in a tightly closed container[9].

Fractionation

Fractionation was performed using a separating funnel with the liquid-liquid method. Fractionation was carried out using *n*-hexane and ethyl acetate as solvents. The extract from the maceration was taken, weighed 10 g, then suspended with 25 mL of distilled water. The extract suspension was then put into a separating funnel, then added with *n*-hexane into the separating funnel. The separating funnel was shaken slowly, then allowed to stand until two layers formed: the water and solvent. The *n*-hexane layer was removed, and the water layer was put back into a separatory funnel to be refracted using *n*-hexane solvent until it was wholly marked with the mixed solvent becoming clear. The same method was repeated for fractionation using ethyl acetate. The obtained liquid fraction was then evaporated using a water bath at a temperature of 50°C to obtain a thick fraction with constant weight, and the results were expressed in percent yield[10].

Identification of secondary metabolites

Identifying secondary metabolites of *n*-hexane and ethyl acetate fractions of *N. Subdita* leaves was used to determine the content of alkaloids, flavonoids, steroids, tannins, saponins, and phenolics[11].

Alkaloids: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were put into different test tubes, then 5 drops of Mayer's reagent were added to the first test tube of each fraction, and 5 drops of Dragendorff's reagent were added into the second test tube of each fraction.

Flavonoids: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were put into different test tubes, then 1 mg of Mg powder was added to each test tube, then 5 drops of concentrated HCl were added.

Steroids: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were put into different test tubes, then 10 drops of Liebermann-Burchard reagent were added.

Tannins: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were put into different test tubes, then 5 drops of 1% gelatin solution were added.

Saponins: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were diluted with 10 mL of distilled water in different test tubes, then shaken vigorously for 10 seconds.

Phenolics: About 1 mL of *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves were put into different test tubes, then 5 drops of 1% $FeCl_3$ were added.

Thin-layer chromatography

The methanol extract, *n*-hexane, and ethyl acetate fraction of *N. Subdita* leaves were dissolved using methanol : chloroform (1 : 1) v/v. The TLC plate was prepared, then activated in an oven at 105°C for 15 minutes. Each extract and fraction was spotted on the TLC plate, then eluted using *n*-hexane : ethyl acetate eluent in a ratio of 8 : 2, 5 : 5, and 2 : 8 v/v. The TLC plate was then observed under UV light at 254 and 366 nm, and each spot's Rf value was calculated.

Qualitative antioxidant activity

The TLC plate eluted and observed before was sprayed using 0.1 mM DPPH and allowed to dry. TLC spots with antioxidant activity change color to yellow on a purple background[12], then the Rf value for each stain that appears on the TLC plate that has been sprayed was calculated.

Quantitative antioxidant activity

DPPH solution: As much as 4 g of DPPH powder was weighed and dissolved with methanol p.a using a 25 mL volumetric flask, added with methanol p.a to the limit mark, then shaken until homogeneous to obtain a 0.4 mM DPPH solution. The solution was stored in a dark glass bottle and away from light.

Determination of maximum wavelength: About 1 mL of 0.4 mM DPPH solution was added with 4 mL of methanol p.a, then vortex for 1 minute. The solution was incubated in a dark room for 30 minutes. Determination of the maximum absorption wavelength of the DPPH solution was carried out using a UV-Vis spectrophotometer at a wavelength (λ) of 450-550 nm.

Determination of operating time: About 1 mL of 0.4 mM DPPH solution was put in a test tube with aluminum foil, then added with 4 mL of quercetin solution. The mixture was vortex for a minute, then the absorbance was read using a UV-Vis spectrophotometer every 2 minutes for 40 minutes, at the maximum wavelength of DPPH.

Determination of IC_{50} of quercetin: About 5 mg of quercetin was dissolved with methanol p.a, then put into a 50 mL volumetric flask, added methanol p.a to the limit mark, then shaken until homogeneous to obtain 100 ppm mother liquor. The grade series solution is prepared with a concentration of 1, 2, 4, 8, 10, and 12 ppm of 100 ppm quercetin mother liquor using a 10 mL volumetric flask. Each of the concentration series solutions was taken as much as 4 mL and then put into a test tube, added with 1 mL of 0.4 mM DPPH solution, vortex for a minute, then left in a dark room according to the operating time that has been obtained. After that, the absorbance of each solution was read using a UV-Vis spectrophotometer at the maximum wavelength of DPPH received.

Determination of IC_{50} of n-hexane and the ethyl acetate fractions of N. subdita leaves: As much as 25 mg of *n*-hexane thick fraction was dissolved with methanol p.a, put into a 50 mL volumetric flask, added methanol p.a to the limit mark, then shaken until homogeneous to obtain 500 ppm mother liquor. The 500 ppm solution was then made into a series of solutions with a concentration of 50, 100, 150, 200, 250, 300, and 350 ppm using a 10 mL volumetric flask. For ethyl acetate fraction, as much as 5 mg of n-hexane thick fraction was dissolved with methanol p.a, put into a 50 mL volumetric flask, added methanol p.a to the limit mark, then shaken until homogeneous to obtain 100 ppm mother liquor. The 100 ppm solution was then made into a series of solutions with a concentration of 10, 20, 30, 40, 50, 60, and 70 ppm using a 10 mL volumetric flask. As much as 4 mL from each concentration series solution was taken into a test tube, then 1 mL of 0.4 mM DPPH solution was added, vortex each tube for a minute, then allowing it to stand according to the operating time obtained. After that, the absorbance of each concentration was read using a UV-Vis spectrophotometer at the maximum wavelength of DPPH received.

Data analysis

The antioxidant activity of the *n*-hexane and ethyl acetate fractions of *N. Subdita* leaves in inhibiting DPPH radicals could be seen from the calculation results of the % inhibition value of the compounds tested. Determination of the IC_{50} value was accomplished by making a linear regression between the concentration of the test solution (*x*-axis) and the percentage of inhibition (*y*-axis), as shown in **Equation 1**[13].

$$IC50: \frac{(50-a)}{b} \quad [1]$$

RESULTS AND DISCUSSION

Extraction

Extraction was carried out using the maceration method and methanol as a solvent. The methanol extract of *N. subdita* leaves obtained was 49.13 g from 300 g of simplicia powder, with a percentage yield of 19.86%. Extraction separates a substance from its mixture by dividing it between two immiscible solvents to take the solute from one solvent to another[14]. Extracts obtained were dry, viscous, or liquid preparations made by extracting vegetable or animal simplicia according to a suitable method, outside the influence of direct sunlight[15].

Fractionation

Fractionation is carried out after obtaining the methanol extract of *N. subdita* leaves. The solvents used are *n*-hexane and ethyl acetate because they have different levels of polarity, *n*-hexane solvent has non-polar properties, and ethyl acetate solvent has semi-polar[16]. The results of the % yield of the fraction of *N. subdita* leaves obtained in this study are 10.8% for the *n*-hexane and 9.4% for the ethyl acetate fraction. The percentage yield shows that the *n*-hexane fraction is more in number than the ethyl acetate fraction. The difference in the percentage yield of the *n*-hexane and ethyl acetate fractions is caused by the difference in the number of compounds attracted to each solvent according to the level of polarity. Fractionation is separating compounds based on their level of polarity[17]. The principle of separation in the fractionation process is based on the difference in polarity level and a specific gravity between the two fractions[18].

Identification of secondary metabolites

Secondary metabolites are identified using the tube method to determine the secondary metabolites contained in the *N. subdita* leaves resulting from a metabolic process. The compounds tested included alkaloids, flavonoids, steroids, tannins, saponins, and phenolics. The results from the *n*-hexane and the ethyl acetate fractions of *N. subdita* leaves are shown in **Table I**. The identification test in the *n*-hexane fraction is positive for alkaloids, flavonoids, steroids, and phenolics, while the tannins and saponins are negative. Meanwhile, the test of ethyl acetate fraction is positive for alkaloids, flavonoids, steroids, tannins, saponins, and phenolics. Saponin compounds are active compounds that are polar. Therefore, the ethyl acetate fraction has positive results for tannins and saponins because the ethyl acetate solvent has semi-polar properties, which can also attract polar compounds[19]. Tannin compounds can be extracted using semi-polar solvents such as ethyl acetate[20]. Therefore, the test results for tannins and saponins in the *n*-hexane fraction, a non-polar solvent, are negative.

Table I. Secondary metabolites of the n-hexane and the ethyl acetate fractions of *N. subdita* leaves

Secondary metabolites	Sample	
	n-hexane fraction	Ethyl acetate fraction
Alkaloids	+	+
Flavonoids	+	+
Steroids	+	+
Tannins	-	+
Saponins	-	+
Phenolics	+	+

Note: (+): Present; (-): Absent

Thin-layer chromatography

A test of TLC is carried out to ensure that the fractionation process that has been carried out has separated the mixture of compounds in the extract entirely based on the level of polarity. The samples tested using the TLC method are methanol extract, *n*-hexane, and ethyl acetate fractions of *N. subdita* leaves. The results of these readings obtained that the TLC plate is fluorescent, and the spots are dark in UV light at 254 nm, while at UV light at 366 nm, the TLC plate is dark in color, and the spots are fluoresced[21]. The TLC test results are the chromatograms observed under UV light at 254 and 366 nm, and the Rf values are calculated as presented in **Table II**.

The appearance of the TLC stain under a UV lamp at 254 nm is a dark stain on the fluorescent plate. This happens because of the interaction between UV light and the fluorescence indicator on the scale[22]. The appearance of the TLC stain under a 366 nm UV lamp produces a dark-colored plate because the plate will not fluoresce under the light of that wavelength, while the resulting stain will fluoresce. This fluorescent stain occurs because of the interaction between UV light and stains with a chromophore group bound by auxochrome[23].

Table II. Rf from TLC of methanol extract, *n*-hexane, and ethyl acetate fractions of *N. subdita* leaves at UV light 254 and 366 nm

Mobile phase (*n*-hexane : ethyl acetate)	Sample	UV Wavelength (nm)	
		254	366
8 : 2	Methanol extract	1). 0.44	1). 0.08
		2). 0.6	2). 0.28
		3). 1.0	3). 0.44
			4). 0.62
	n-hexane fraction	1). 0.1	1). 0.08
		2). 0.18	2). 0.5
		3). 0.28	3). 0.68
		4). 0.44	4). 0.86
		5). 0.52	
		6). 0.64	
		7). 1.0	
	Ethyl acetate fraction	1). 0.24	1). 0.06
		2). 0.4	2). 0.26
		3). 0.6	3). 0.42
		4). 0.98	4). 0.62
5 : 5	Methanol extract	1). 0.12	1). 0.06
		2). 0.8	2). 0.14
		3). 0.88	3). 0.9
		4). 0.99	4). 0.98
	n-hexane fraction	1). 0.1	1). 0.05
		2). 0.16	2). 0.16
		3). 0.76	3). 0.46
		4). 0.86	4). 0.82
		5). 0.96	
	Ethyl acetate fraction	1). 0.06	1). 0.04
		2). 0.12	2). 0.16
		3). 0.18	3). 0.22
		4). 0.8	4). 0.38
		5). 0.9	5). 0.46
		6). 0.98	6). 0.82
			7). 0.9
			8). 0.98
2 : 8	Methanol extract	1). 0.06	1). 0.22
		2). 0.42	2). 0.54
		3). 0.96	
	n-hexane fraction	1). 0.26	1). 0.2
		2). 0.48	2). 0.56
		3). 0.92	3). 0.74
	Ethyl acetate fraction	1). 0.08	1). 0.22
		2). 0.18	2). 0.5
		3). 0.26	3). 0.58
		4). 0.98	4). 0.78
			5). 0.98

Qualitative antioxidant activity

A qualitative test of antioxidant activity is carried out using TLC. This test is carried out by spraying using a DPPH reagent. If the sample has the potential as an antioxidant, the stain on TLC will change color to pale yellow on a purple background[12]. Antioxidant compounds will donate hydrogen atoms to DPPH radicals to form a yellow color[26]. The results of the qualitative antioxidant test are shown in **Table III**.

Table III. Rf from TLC of methanol extract, *n*-hexane, and ethyl acetate fractions of *N. subdita* leaves by DPPH

Mobile phase (*n*-hexane : ethyl acetate)	Sample	Rf value
8 : 2	Methanol extract	1). 0.53
	n-hexane fraction	1). 0.69
		2). 0.86
		3). 0.94
	Ethyl acetate fraction	1). 0.1
		2). 0.52
5 : 5	Methanol extract	1). 0.18
	n-hexane fraction	1). 0.17
	Ethyl acetate fraction	1). 0.18
		2). 0.32
2 : 8	Methanol extract	1). 0.1
		2). 0.3
	n-hexane fraction	1). 0.08
	Ethyl acetate fraction	1). 0.12
		2). 0.27
		3). 0.46
		4). 0.6

Quantitative antioxidant activity

Determination of the maximum wavelength of DPPH is carried out to determine the wavelength with complete sensitivity. The purpose of determining the maximum wavelength is that the absorbance obtained is maximal, the difference in absorbance of each concentration series will be more significant because the sensitivity is higher, and the shape of the curve obtained will be linear[24]. This is indicated by the maximum or most significant absorbance[25]. The DPPH wavelength range is 515-520 nm[26]. The results obtained are at a wavelength of 515.30 nm, as shown in **Figure 1**.

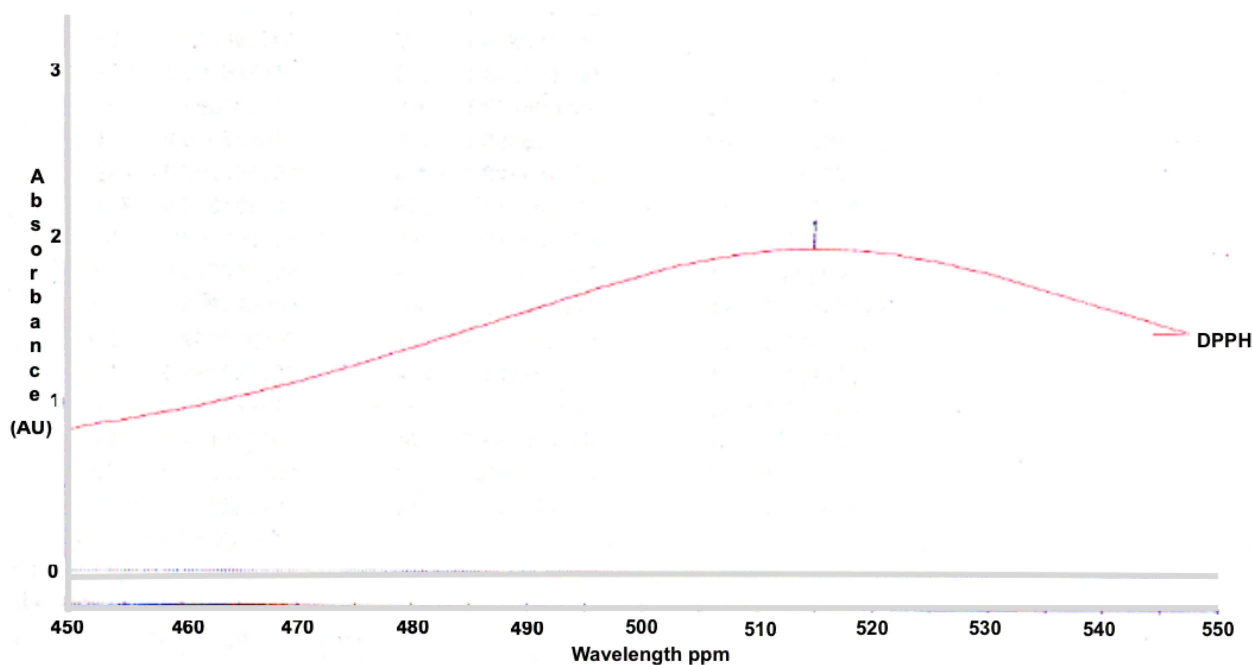

Figure 1. The maximum wavelength of DPPH.

Operational time is the measurement time taken when the solution absorbs a stable absorption light. The operating time determines the most appropriate time for the test solution to reduce DPPH radicals. In addition, the operating time also

shows that the reaction between the test solution and DPPH has stabilized, which can be indicated by the absence of a decrease in absorbance[27]. The results obtained in the stable reaction started from the 24th minute to the 40th minute (**Figure 2**) with a difference in absorbance value of 0.0006. This shows that the operating time obtained in this determination has different values but produces absorbance values that are uniform with each other, so it shows that DPPH has reacted stably with a standard solution of quercetin[28].

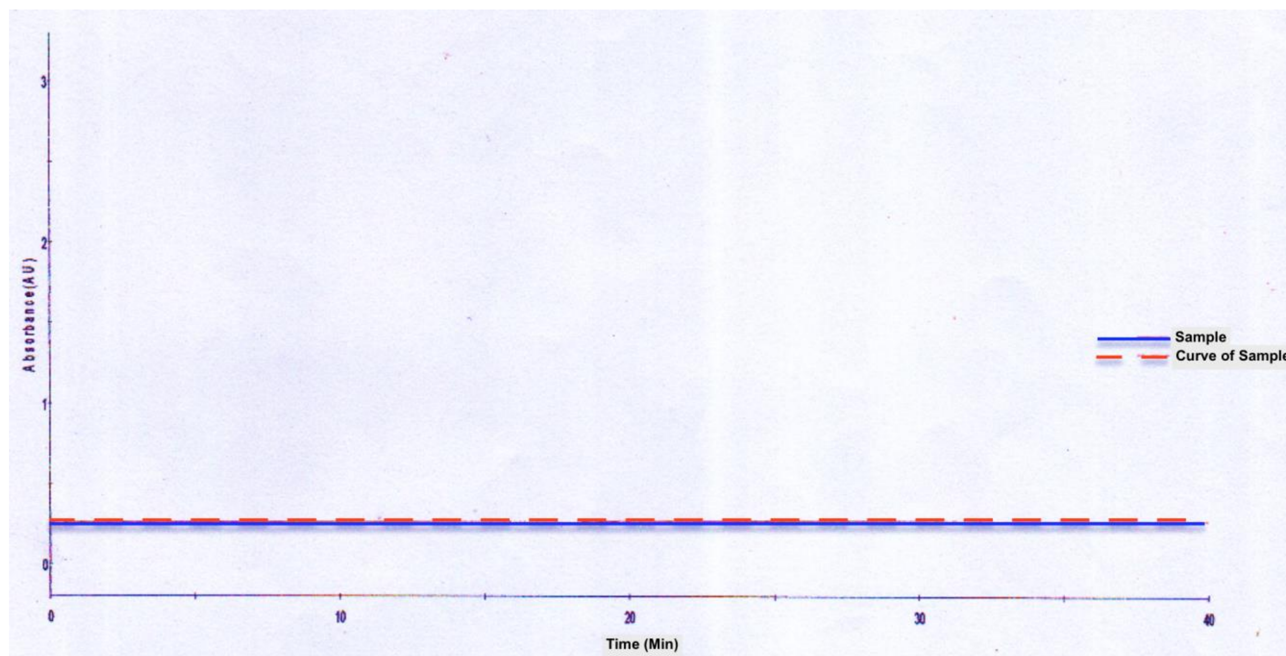

Figure 2. The determination of operating time.

Quercetin is the main flavonoid compound in the flavonol group and can be easily found in plants. In addition, quercetin is chosen because it has been shown to have potent antioxidant activity[29]. Quercetin will donate its proton to DPPH and become a radical compound. The unpaired electrons generated in the chemical structure of quercetin are localized into the aromatic system so that the quercetin revolutionary compound is relatively less reactive and has low energy[30]. Compared with other positive controls, ascorbic acid or gallic acid, quercetin can better stabilize DPPH radicals[31]. The antioxidant activity of the samples is tested by comparing their antioxidant activity with a positive control or comparison. The comparison or positive control used is quercetin in determining the IC_{50} value.

The results of the linear regression equation between the concentration of quercetin and the percentage of inhibition are $y = 5.9683x - 2.1812$ with a correlation coefficient (r) of 0.995 (**Figure 3**). The range of values for the r is 0.98, so the resulting data is by the provisions. The antioxidant activity value of the quercetin comparison solution is shown in **Table IV**. Comparing quercetin's antioxidant activity results obtained an average IC_{50} of 8.74306 ± 0.09144 ppm. The IC_{50} value obtained is included in the strong category with an IC_{50} value range of <50 ppm[26].

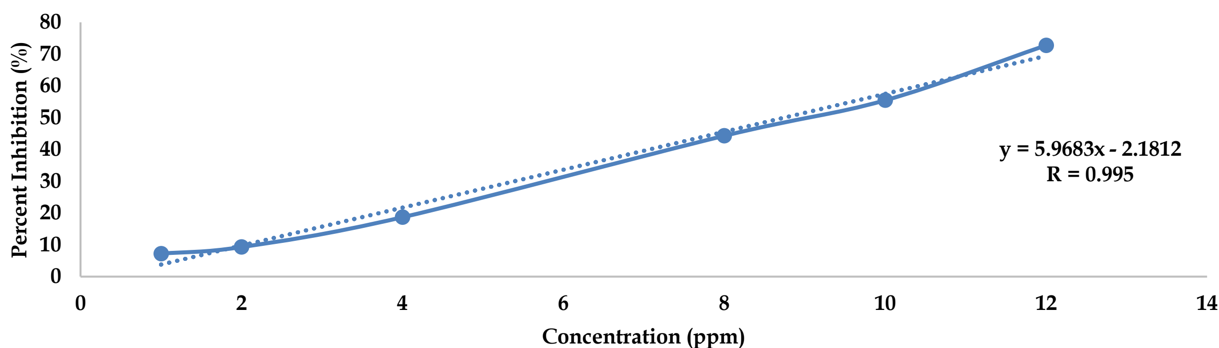

Figure 3. Linear regression equation of quercetin.

Table IV. The antioxidant activity of the quercetin

Concentration (ppm)	$\bar{x}$ Inhibition (%)	SD	RSD (%)	$\bar{x}$ IC_{50} ± SD (ppm)	RSD (%)
1	7.225	0.00269	0.156	8.74306 ± 0.09144	1.04571
2	9.300	0.00708	0.419		
4	18.672	0.00201	0.133		
8	44.302	0.00466	0.450		
10	55.476	0.02412	2.910		
12	72.765	0.00970	1.914		

Determination of the IC_{50} value of the *N. subdita* leaves *n*-hexane fraction is carried out by making test solutions in a series of concentrations of 50, 100, 150, 200, 250, 300, and 350 ppm, which are taken from the results of the dilution of the standard solution of 500 ppm. The regression equation (**Figure 4**) was obtained from the relationship between the *n*-hexane fraction concentration series of *N. subdita* leaves with the percent inhibition of $y = 0.1494x + 15.696$ with the r of 0.962. The IC_{50} value is inversely proportional to antioxidant activity, so the lower the concentration that can reduce DPPH radicals by 50%, the stronger the antioxidant activity[32]. The results of the antioxidant activity of the *n*-hexane fraction of *N. subdita* leaves obtained an average IC_{50} of 229.61178 ± 3.65919 ppm (**Table V**). The IC_{50} value obtained is included in the weak category, with an IC_{50} value range of >200 ppm[26].

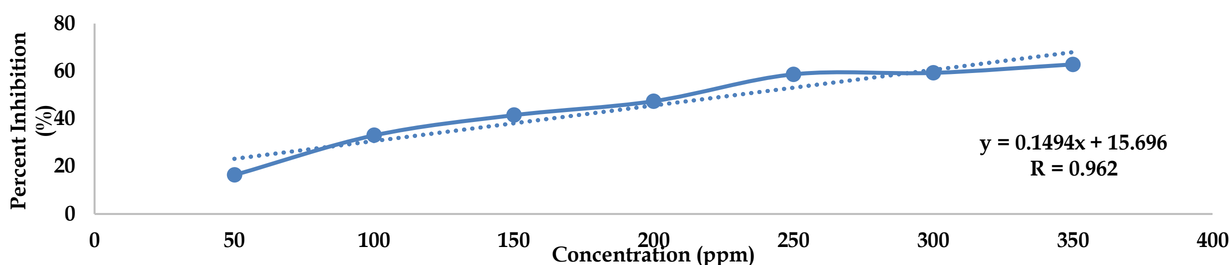

Figure 4. Linear regression equation of *n*-hexane fraction of *N. subdita* leaves.

Table V. The antioxidant activity of the *n*-hexane fraction of *N. subdita* leaves

Concentration (ppm)	$\bar{x}$ Inhibition (%)	SD	RSD (%)	$\bar{x}$ IC_{50} ± SD (ppm)	RSD (%)
50	16.356	0.00059	0.045	229.61178 ± 3.65919	1.59383
100	33.009	0.00050	0.048		
150	41.506	0.00035	0.038		
200	47.387	0.00010	0.012		
250	58.675	0.00059	0.092		
300	59.280	0.00036	0.057		
350	62.851	0.00052	0.090		

Determination of the IC_{50} value of the ethyl acetate fraction of *N. subdita* leaves was carried out by making a 100 ppm standard solution. Then a series of concentrations of 10, 20, 30, 40, 50, 60, and 70 ppm are made. The linear regression equation is obtained from the relationship between concentration and the percentage of inhibition of the ethyl acetate fraction of *N. subdita* leaves (**Figure 5**) of $y = 0.7529x + 8.9346$ with the r of 0.994. The results of the antioxidant activity of the ethyl acetate fraction of *N. subdita* leaves obtained an average IC_{50} of 54.54296 ± 0.02236 ppm, as shown in **Table VI**. The IC_{50} value obtained is included in the strong category, which has an IC_{50} value range of 50-100 ppm[26].

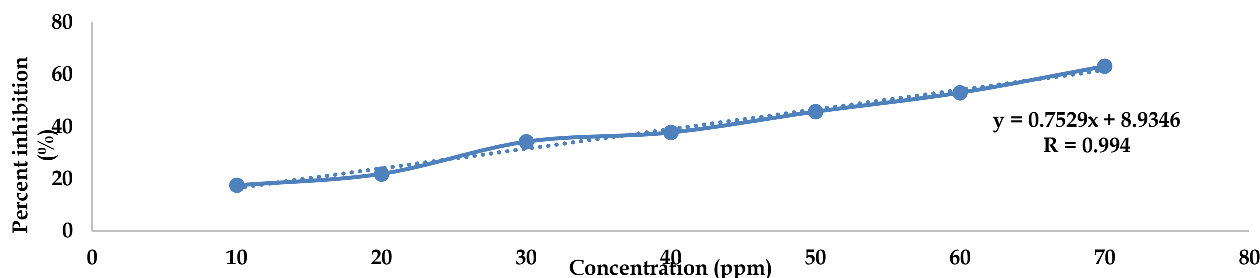

Figure 5. Linear regression equation of ethyl acetate fraction of *N. subdita* leaves.

Table VI. The antioxidant activity of the ethyl acetate fraction of *N. subdita* leaves

Concentration (ppm)	$\bar{x}$ Inhibition (%)	SD	RSD (%)	$\bar{x}$ IC_{50} ± SD (ppm)	RSD (%)
10	17.498	0.00029	0.022	54.54296 ± 0.02236	0.0499
20	21.901	0.00021	0.017		
30	34.235	0.00015	0.015		
40	37.768	0.00023	0.024		
50	45.739	0.00030	0.035		
60	53.028	0.00012	0.016		
70	63.182	0.00042	0.072		

CONCLUSION

Identification test of secondary metabolites in the *n*-hexane fraction of *N. subdita* leaves contain alkaloids, flavonoids, steroids, and phenolics, while the ethyl acetate fraction contains alkaloids, flavonoids, steroids, tannins, saponins, and phenolics. The *n*-hexane fraction of *N. subdita* leaves has weak antioxidant activity, and the ethyl acetate fraction has strong antioxidant activity.

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AUTHORS' CONTRIBUTION

Consept: **A.A., A.M., S.S**.; Design: **A.M., A.A., S.S**; Supervision: **S.S.**; Data collection and processing: **A.A., A.M., S.S.**; Resources: **S.S.**; Materials: **A.A., A.M., S.S.**; Analysis and interpretation: **A.A., A.M., S.S.**; Literature search: **A.A., A.M., S.S.**; Writing: **A.M., A.A., S.S.**; Critical review: **A.A., S.S**.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this manuscript.

REFERENCES

1. Marrelli M. Medicinal Plants, Plants. 2021;10(7):1355. doi:10.3390/plants10071355

2. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babaghith AO, et al. Flavonoids a Bioactive Compound from Medicinal Plants and Its Therapeutic Applications. Biomed Res Int. 2022;2022:5445291. doi:10.1155/2022/5445291

3. Zehiroglu C, Sarikaya SBO. The importance of antioxidants and place in today's scientific and technological studies. J Food Sci Technol. 2019;56(11):4757-74. doi:10.1007/s13197-019-03952-x

4. Flieger J, Flieger W, Baj J, Maciejewski R. Antioxidants: Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. Materials. 2021;14(15):4135. doi:10.3390/ma14154135

5. Manessis G, Kalogianni AI, Lazou T, Moschovas M, Bossis I, Gelasakis AI. Plant-Derived Natural Antioxidants in Meat and Meat Products. Antioxidants. 2020;9(12):1215. doi:10.3390/antiox9121215

6. Asmiyarti NI, Wibowo MA. Uji Aktivitas Antioksidan Metode DPPH dan Uji Sitotoksik Metode BSLT pada Ekstrak Metanol Daun Bongkal (Nuaclea subdita (Korth) Steud). J Kimia Khatulistiwa. 2014;3(1):58-62.

7. Aisiah S. Potensi Tumbuhan Bangkal (Nauclea Orientalis) untuk Pengendalian Bakteri Aeromonas hydrophila. Fish Sci. 2016;2(4):166-77. doi:10.20527/fs.v2i4.1172

8. Wardhani RRAAK, Akhyar O. Analisis Skrining Fitokimia, Aktivitas Antioksidan dan Antibakteri Propionibacterium acnes Ekstrak Etanol Kulit Batang dan Daun Tanaman Bangkal (Nuclea subdita). J Ilmiah Berkala Sains Terapan Kimia. 2018;12(2):64-75. doi:10.20527/jstk.v12i2.4956

9. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020;12(1):1-10. doi:10.4103/jpbs.jpbs_175_19

10. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018;13:20. doi:10.1186/s13020-018-0177-x

11. Dubale S, Kebebe D, Zeynudin A, Abdissa N, Suleman S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol. 2023;15:51-62. doi:10.2147/jep.s379805

12. Muthia R, Saputri R, Verawati SA. Uji Aktivitas Antioksidan Ekstrak Etanol Kulit Buah Mundar (Garcinia forbesii King.) Menggunakan Metode DPPH (2,2-diphenyl-1-picrylhydrazil). J Pharmascience. 2019;6(1):74-82. doi:10.20527/jps.v6i1.6079

13. Itam A, Wati MS, Agustin V, Sabri N, Jumanah RA, Efdi M. Comparative Study of Phytochemical, Antioxidant, and Cytotoxic Activities and Phenolic Content of Syzygium aqueum (Burm. f. Alston f.) Extracts Growing in West Sumatera Indonesia. ScientificWorldJournal. 2021;2021:5537597. doi:10.1155/2021/5537597

14. Abdelmohsen UR, Sayed AM, Elmaidomy AH. Natural Products' Extraction and Isolation-Between Conventional and Modern Techniques. Front Nat Prod. 2022;1:873808. doi:10.3389/fntpr.2022.873808

15. Silaban H. The Effect of Various Concentrations of Ethanol Extract of the Leaves of Paederia foetida L. on the Growth of Escherichia Coli Bacteria. J Drug Deliv Ther. 2021;11(6):61-7. doi:10.22270/jddt.v11i6.5037

16. Thavamoney N, Sivanadian L, Tee LH, Khoo HE, Prasad KN, Kong KW. Extraction and recovery of phytochemical components and antioxidative properties in fruit parts of Dacryodes rostrata influenced by different solvents. J Food Sci Technol. 2018;55(7):2523-32. doi:10.1007/s13197-018-3170-6

17. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lighfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 2017;6(4):42. doi:10.3390/plants6040042

18. Friesen JB, McAlpine JB, Chen SN, Pauli GF. Countercurrent Separation of Natural Products: An Update. J Nat Prod. 2015;78(7):1765-96. doi:10.1021/np501065h

19. Hayat J, Akodad M, Moumen A, Baghour M, Skalli A, Ezrari S, et al. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. Heliyon. 2020;6(11):e05609. doi:10.1016/j.heliyon.2020.e05609

20. Autor E, Cornejo A, Bimbela F, Maisterra M, Gandia LM, Martínez-Merino V. Extraction of Phenolic Compounds from Populus Salicaceae Bark. Biomolecules. 2022;12(4):539. doi:10.3390/biom12040539

21. Syarifah AL, Retnowati R, Soebiantoro. Characterization of Secondary Metabolites Profile of Flavonoid from Salam Leaves (Eugenia polyantha) Using TLC and UVSpectrophotometry. Pharm Sci Res. 2019;6(3):155-63. doi:10.7454/psr.v6i3.4219

22. Hancu G, Simon B, Kelemen H, Rusu A, Mircia E, Gyeresi A. Thin layer chromatographic analysis of Beta-lactam antibiotics. Adv Pharm Bull. 2013;3(2):367-71. doi:10.5681/apb.2013.059

23. Sherma J, Rabel F. A Review of Thin Layer Chromatography Methods for Determination of Authenticity of Foods and Dietary Supplements. J Liq Chromatogr Relat Technol. 2018;41(10):645-57. doi:10.1080/10826076.2018.1505637

24. Pratiwi RA, Nandiyanto ABD. How to Read and Interpret UV-Vis Spectrophotometric Results in Determining the Structure of Chemical Compounds. Indones J Educ Res Technol. 2022;2(1):1-20. doi:10.17509/ijert.v2i1.35171

25. Sari AK, Ayati R. Penentuan Aktivitas Antioksidan Ekstrak Etanol Daun Jeruk Purut (Citrus hystrix D. C) dengan Metode DPPH (1,1-diphenyl-2- picrylhydrazyl). J Curr Pharm Sci. 2018;1(2):69-74.

26. Molyneux P. The Use of The Stable Free Radical Diphenylpicryl-Hydrazyl (DPPH) for Estimating Antioxidant Activity. Sonklanakarin j Sci Technol. 2004;26(2):211-9.

27. Shahidi F, Zhong Y. Measurement of antioxidant activity. J Funct Foods. 2015;18(B):757-81. doi:10.1016/j.jff.2015.01.047

28. Wolosiak R, Drużyńska B, Derewiaka D, Piecyk M, Majewska E, Ciecierska M, et al. Verification of the Conditions for Determination of Antioxidant Activity by ABTS and DPPH Assays-A Practical Approach. Molecules. 2021;27(1):50. doi:10.3390/molecules27010050

29. Adawiyah R, Rizki MI. Aktivitas Antioksidan Ekstrak Etanol Akar Kalakai (Stenochlaena palustris Bedd) Asal Kalimantan Tengah. J Pharmascience. 2018;5(1):71-7.10.20527/jps.v5i1.5788

30. Li Z, Moalin M, Zhang M, Vervoort L, Hursel E, Mommers A, et al. The Flow of the Redox Energy in Quercetin during Its Antioxidant Activity in Water. Int J Mol Sci. 2020;21(17):6015. doi:10.3390/ijms21176015

31. Maesaroh K, Kurnia D, Anshori JA. Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC Terhadap Asam Askorbat, Asam Galat dan Kuersetin. Chim Nat Acta. 2018;6(2):93-100. doi:10.24198/cna.v6.n2.19049

32. Rizki MI, Nurlely, Fadlilaturrahmah, Ma'shumah. Aktivitas Antioksidan Ekstrak Etanol Daun Cempedak (Artocarpus integer), Nangka (Artocarpus heterophyllus), dan Tarap (Artocarpus odoratissimus) Asal Kalimantan Selatan. J Curr Pharm Sci. 2021;4(2):367-72.