IDENTIFICATION OF CHEMICAL COMPOUNDS AND ASSAY OF ANTIOXIDANT ACTIVITY OF LEAF EXTRACT OF DAUN BA'BALIK ANGIN (ALPHITONIA EXCELSA) DOWNSTREAM LEFT CANTUNG REGION FROM VARIOUS LEVELS OF FRACTIONS

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Abstract

Babalik angin (*Alphitonia excelsa*) leaves, one of the plants which are used by people everyday for bath soap and antioxidants can protect the skin from various cell demage due to UV radiation, anti-aging and protesction from reactive oxygen species.

Purpose: to determine the chemical compounds content of the leaves of babalik angin (*Alphitonia excelsa*) as well as to determine the antioxidant activity of extracts of bacan angin (*Alphitonia excelsa*) leaves from various levels of fraction

Method: In this study using the maceration extraction method using 96% ethanol as a solvent. Soaking was done three times for 24 hours, the results of the thick extract were carried out with a rotary evaporator the tested for secondary metebolites with color reagents and quantitatively with 2,2-difenil-1-pikrilhidrazil

Results: The results of the secondary metabolite test found in Babalik leaves. Wind includes flavonoids, alkaloids, terpenoids and saponins. On IC50. Score wind leaf extract with N-hexane fraction obtained IC50 results of 64,997 mg/ml, Ethyl acetate has an IC50 value of 89.303 mg/ml, Methanol has an IC50 value 5,671 mg/ml, from the antioxidant fraction of Babalik Angin leaf extract with an IC50 value of 50-100 mg/ml is said to have a strong antioxidant activity value.

Conclusion: Compound research using this fraction level has strong antioxidant activity

Keywords: Antioxidant, Wind-Turning Ba's leaves (*Alphitonia Excelsa*), DPPH (2,2 diphenyl-1- picrihydrazil) phytochemical screening.

1. Introduction

In developing countries, one of them is Indonesia which has the largest tropical forest in the world which has potential as a medicinal plant. Of the approximately 40,000 thousand types of medicinal plants that have been known in the world, there are 30,000 of them allegedly located in Indonesia. However, there are only 1,200 types of plants that have been used as raw materials for herbal medicines (Nugroho and Ningsih., 2017).

Islands in Indonesia that have abundant natural resources, one of which is the island of Kalimantan, precisely in the province of South Kalimantan, in the Kotabaru area, Cantung

village, downstream left, Kelumpung Hulu, which has Ba'balik wind plants that can grow in forest areas, shrubs, and steep slopes., et al., 2016). This plant is usually used by people for bathing because it can produce foam to remove dirt and dust. This Ba'balik leaf has a distinctive mint smell. How to use the leaves of Ba'balik Angin, namely by washing the leaves and then placing them in a container that already contains water and then rubbing it until it produces foam. It turns out that from Australian research according to (Tarannum Naz, 2013) there are efficacy of the Ba'balik Angin plant (Alphitonia Excelsa) as an antiseptic on the skin and contains secondary metabolites in the plant. The secondary metabolites of Ba'balik Angin leaf include flavonoids, saponins, and tannins where these compounds have medicinal effects. The part in the treatment of strong positive young leaves contains saponins because the characteristic of saponins (sapo) is to produce foam.

Antioxidants function to scavenge free radicals. Free radicals are molecules with unpaired electrons in their outer orbitals, so they are very reactive and then cause a chain reaction that, if anything happens in the body, can continue to be damaged.

In this study, the antioxidant activity was examined using the DPPH method (2.2 *diphenyl-1-picrihydrazil*) spectrophotometrically. The dpph method was chosen because it is simple, easy, fast and sensitive and only requires a small sample. The parameter used for the dpph free radical scavenging test is IC50, which is the concentration of the extract or test fraction needed to capture DPPH radicals as much as 50%.

2. Material and Methdos

The materials used in this study were 96% ethanol reagent Ba'balik Leaves, aquadest, FeCl3, Mayer and Dragendorff, concentrated HCL, Methanol, N-Butanol, Lieberman Burchard reagent, N-Hexane, Acetic Acid, Ethyl acetate, DPPH (2 ,2 diphenyl-1-picrihydrazil), quercetin.

The tools used include scissors, jars, measuring cups, evaporation cups, beakers, erlenmayer, hotplates, filter paper, measuring flasks, dropper pipettes, measuring pipettes, tube racks, test tubes, stirring rods, horn spoons, spectrophotometry UV-Vis, analytical balance.

The method used in this study is a descriptive observational study that qualitatively describes the content of secondary metabolites and quantitatively examines antioxidant activity

Sample Preparation

The sample used is the Ba'balik Angin Leaf (*Alphitonia Excelsa*) obtained in the city area Kotabaru Cantung kiri hilir,kelumpung hulu and conducted research at the Pharmaceutical Technology Laboratory, Department of Pharmacy, University of Sari Mulia Banjarmasin.

Fractionation

Weigh the 10 grams of the leaf extract, add 50 ml of aquadest ad until homogeneous, add 50 ml of n-hexane and stir until the gas runs out. Let stand for 30-60 minutes, separate the water extract and the n-hexane extract. Water extract is put into a separating funnel, add 3 times strike until clear. For ethyl acetate and methanol the work is the same

Identification of Secondary Metabolites

Flavonoid Test

Flavonoid testing will be carried out by taking 1-2 ml of extract and adding one gram of Mg powder and concentrated HCL solution. If it produces a red-orange color it contains positive flavonoids (Noviyanti and Linda, 2020).

Alkaloid Test.

The alkaloid test will be carried out by taking 1-2 ml of extract which has been dissolved with 5 ml of HCI2N. The solution obtained was divided into 2 test tubes. Extraction of the leaves of Ba'balik Angin was added with 3 drops of Mayer and Dragendrof reagents. A positive result is the presence of alkaloids when a white precipitate is formed with Mayer's reagent and Orange with Dragendrof's reagent (Fadhly, ddk, 2015).

Terpenoid Test

Terpenoid testing will be carried out by taking 1-2 ml of leaf extract which has been dissolved with n-hexane. Then, put a little into a test tube which then add 1 ml of glacial acetic acid, 1 ml of concentrated H2SO4. If there is a reddish brown ring formed at the boundary of the two solvents, it indicates the presence of terpenoids (Fajriaty, 2018).

Saponin Test

A total of 1 gram of leaf extract was put into a test tube, added 10 mL of hot water, then shaken vigorously for 10 seconds containing saponins to form a foam as high as 1-10 cm for not less than 10 minutes and with the addition of 1 drop of 2 N HCL, the foam did not disappear. (Muthmainnah, 2017).

Preparation of DPPH solution.

Weighed 1.9 mg of DPPH powder and then dissolved with ethanol p.a then put into a 50 ml volumetric flask then add ethanol p.a until the flask mark is shaken so that the solution homogeneous. Obtained 0.1 mM DPPH solution initial/parent concentration (Mardiah *et al*., 2017).

Determination of the maximum wavelength of DPPH.

4 ml of DPPH solution was taken to calculate the wavelength. Added 4 ml of methanol p.a then vortexed for 1 minute then leave in a dark place for 1 hour. After that, the solution is run with UV-Vis spectrophotometry and obtained the wavelength (Mardiah *et al.,* 2017).

Determination of operating time.

1 ml of 0.1 MM DPPH solution was taken and put in a test tube. Then 4 ml of standard 40 . quercetin was added ppm, then vortexed for 1 minute until homogeneous. Solution run by spectrophotometry Uv-vis at wavelength a predetermined maximum time interval of 5 minutes for 60 minutes (Mardiah et al., 2017).

Preparation of quercetin comparison solution.

Weighed 10 mg of quercetin powder, then dissolved with 10 ml methanol p.a. Put into a 10 ml volumetric flask until the mark limit and shaken until homogeneous then test the concentration series of 20 ppm, 30 ppm, 40 ppm, and 50 ppm then put into the flask measure each, add 10 methanol to the last mark let stand for 1 hour then tested with spectrophotometry Uv-vis.

Determination of the IC50 value of the quercetin comparison solution.

Take 1 ml of 0.4 mM DPPH solution. Then added at 4 ml of each standard solution of quercetin. Leave it for 60 minutes mix the solution in a dark place during operation time. Next, read the absorbance of each solution using spectrophotometry UV-vis at wavelengths maximum DPPH that has been determined (Mardiah *et al*., 2017).

3. Result and Discusion

This research was conducted to determine the secondary metabolite content of Ba'balik Angin Leaf Extract (Alphitonia Excelsa) and to test the antioxidant activity of various levels of fractions. The simplest one is made to facilitate extraction by maceration method with 96% ethanol solvent, the choice of 96% ethanol solvent because it has a broad absorption capacity so that all compounds can be pulled out of the simplicia at a temperature of 65°C in a rotary evaporator. Fractionation is the separation between liquids, in which the fractionation will be carried out in stages based on the level of polarity from non-polar, semi-polar and polar. Antioxidants are compounds that protect cells from the harmful effects of free radicals. Free radicals are molecules that have no stability in the atom against the outermost orbit by having one or more unpaired electrons. dpph method serves to measure single electrons such as hydrogen transfer activity as well as to measure free radical inhibitory activity. spectrophotometry UV-Vis is a tool that can be used to measure the absorption spectrum of plant substances in very dilute solutions using a blank comparator on a solvent. The phytochemical identification of secondary metabolites tested by the test tube method included alkaloids, flavonoids, saponins, and terpenoids.

Table 4. 2 Screening Test Results of Bebalik Angin Leaf Extract

Compound	Reagent	Result
Alkaloids	Mayer's reagent	Positif
	Dragendorft's reagent	Positif
Flavonoids	Concentrated Mg and HCl powder	Positif
Terpenoids	1 mL glacial acetic acid + 1 mL H_2SO_4 solution	Positif
Saponins	10 mL of water and 1 drop of 2 N . HCl	Positif

1. Determination of Wavelength 2.2 Diphenyl 1-1 Picryhydrazil (DPPH)

The maximum wavelength of DPPH measurement is at a wavelength of 518 nm. According to the theory of (Molynex, 2004) the maximum wavelength of dpph measurement is 450-550.

2. The results of the measurement of DPPH absorption (2.2 Diphenyl 1-1 Pikrihidrazil)

The measurement test of the blank solution was carried out three times, and then the average was calculated. The results of the measurement of the blank DPPH solution (2.2 Diphenyl 1-1 Pikrihydrazil).

Table 4. 3 DPPH . blanko solutions

sample	Absorption (518)	Average
Blanko	0,414	
	0,416	
	0,416	0,415

3. Operating time

The time used to react DPPH with an antioxidant compound required constant time, the constant time in this study was at 55 minutes

4. Determination of IC50 value of quercetin comparison solution

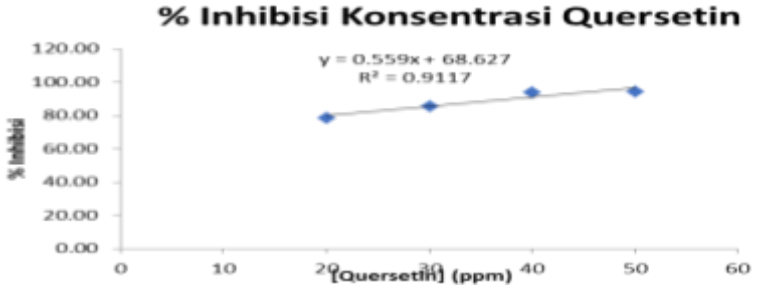
Table 4. 4 Results of IC50 determination of quercetin comparison solution

Sample	Concentration (ppm)	% inhibition	IC_{50}
	20	78,55	

Quercetin	30	85,78	20,466 ppm
	40	93,98	
	50	94,46	

Quercetin Inhibition Curve

Figure 4. 3 IC50 quercetin determination of the IC50 value of the quercetin comparison solution



(Data source: processed data)

5. Determination of IC50 value from fraction level

Table 4. 5 Determination of the IC50 value of the N-Hexane . fraction

Sample	Concentration (ppm)	% Inhibition	IC_{50}
Fraksi N-heksan	30	30,60	64,997 ppm
	40	30,84	
	50	44,09	

N-Hexane extract inhibition curve

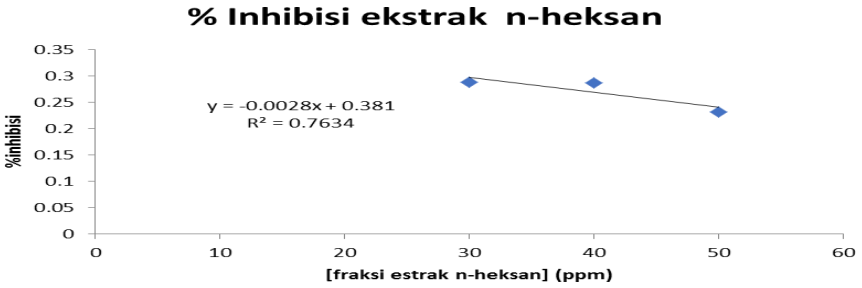


Figure 4. 4 the results of the determination of the IC50 value of the N-hexane ekstrak extract fraction

(Data source: processed data)

Table 4. 6 Determination of the IC50 value of the Ethyl acetate

Sample	Concentration (ppm)	% Inhibition	IC_{50}
Ethyl acetate fraction	30	-59,17%	89,303 ppm
	40	39,27%	

	50	46,02%

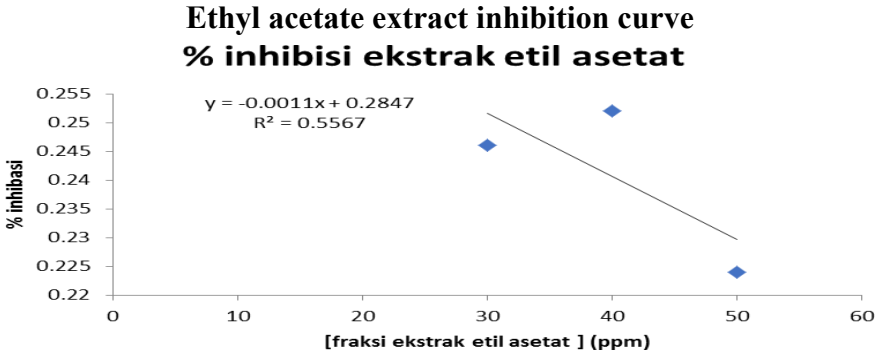


Figure 4. 5 the results of determining the IC50 value of the ethyl acetate extract fraction

Sampel	Konsentrasi (ppm)	% Inhibisi	IC_{50}
Fraksi Metanol	30	47,22%	52,671 ppm
	40	54,93%	
	50	58,07%	

Table 4. 7 Determination of the IC50 value of the Methanol fraction

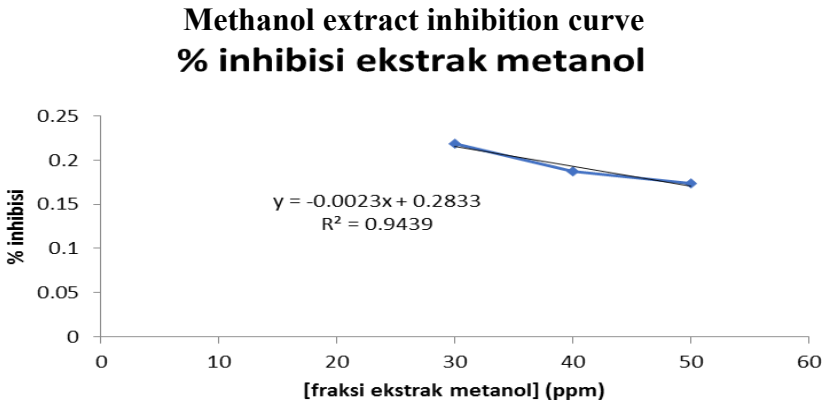


Figure 4. 6 the results of the determination of the IC50 value of the methanol extract fraction (Data source: processed data)

In the alkaloid test using Mayer's reagent, the result is a white precipitate if the positive result contains alkaloids, a white precipitate will be formed with Mayer's reagent (Fadhly, ddk,

2015). Mayer's reagent contains potassium iodide and mercury chloride (potassium tetraiodomercurate(II), the formation of a white precipitate in Mayer's reagent is estimated that nitrogen in the alkaloids will react with metal ions K+ from potassium tetraiodomercurate(II) to form a potassium-alkaloid complex which settle (Ergina, 2014). Using Dragendroff's reagent, orange results are obtained, if the positive result contains alkaloids, an orange precipitate will be formed with Dragendroff's reagent (Fadhly, et al, 2015)

The color changes to orange because the alkaloids have a tertiary amine group RN, this group can react similarly to ammonia (NH3) and act as a base that can react with acids to form ammonia salts, this happens depending on the nature of the tertiary amine alkaloids will produce an intensive color. which has a yellow to orange (orange) color to brownish red after being sprayed with dragendroff. Secondary amines will produce colors that are less intense or not visible light.

There is a flavonoid test using Mg and HCL powder to get a red-orange color, if it produces a red-orange color it contains positive flavonoids (Noviyanti and Linda, 2020). The purpose of adding Mg and HCl is to reduce the benzopyron core contained in the flavonoid structure so that red or orange flavilium salts are formed (Ergina, 2014).

In the terpenoid test using glacial acetic acid and concentrated H2SO4 solution, the results obtained are a reddish brown ring is formed on the border of the two solvents. If there is a reddish brown ring formed on the border of the two solvents, it indicates the presence of terpenoids while on steroids there is no blue or green ring so it is not present. steroid compound, whereas if a blue or green ring is formed, it indicates the presence of a group of steroid compounds (Fajriaty, 2018). The terpenoid test reaction is the condensation or release of H2O and incorporation with carbocations. This reaction begins with the acetylation of the hydroxyl group using acetic anhydride. The acetyl group which is a good leaving group will be released, so that a double bond is formed. Furthermore, the hydrogen group and its electrons are released, causing the double bond to move. These compounds undergo resonance acting as electrophiles or carbocations. The attack of the carbocation causes electrophilic addition, followed by the release of hydrogen. Then the hydrogen group and its electrons are removed, as a result, the compound undergoes a conjugate extension which shows the appearance of a brownish ring (Nugrahani, 2016).

In the saponin test, which is using hot water and cooled then shaken vigorously for 10 seconds to form a foam as high as 1-10 cm. , the foam does not disappear (Muthmainnah, 2017). The appearance of foam indicates the presence of glycosides which have the ability to form foam in water which is hydrolyzed into glucose and other compounds (Nugrahani, 2016).

Based on the results of linear regression analysis between the relationship between quercetin concentration and the percentage of inhibition obtained, the equation Y = 0.559x + 68.627 with a coefficient of relation (r) 0.9117. The IC50 value for the comparison of quercetin was obtained by entering the value of 50 inhibitory activity in the regression equation. The IC50 value of quercetin obtained is -20,466 g/ml. The results showed that the comparison sample of quercetin had a very strong activity, namely 20.466 g/ml. According to (Lung, 2015) the level of strength of the intensity of antioxidant activity is very strong, including the IC50 value range of less than 50 g/ml, which means that the comparison test for quercetin is in accordance with the theory.

Determination of the IC50 value of the leaf extract of the N-hexane fraction obtained 64,997 mg/ml, the IC50 value of the leaf extract of the leaf extract of the ethyl acetate fraction was obtained 89.303 mg/ml, the IC50 value of the leaf extract of the methanol fraction was obtained at the IC50 value of 52,671 mg/ml. So from the results obtained the IC50 value in the N-hexane, Ethyl acetate, Methanol fractions. can be categorized as having strong antioxidant activity because the IC50 value is in the range of 50-100 mg/ml

4. Conclusion

Based on the results of the study, it can be concluded that the results of the screening test of chemical compound profiles in the leaves of Babalik Angin contain flavonoids, alkaloids, terpenoids and saponins. Quantitative testing of antioxidant activity levels showed clear results that the N-hexane fraction of wind leaf extract had strong antioxidant activity with an IC50 value of 64,997 g/ml, the ethyl acetate fraction of wind leaf extract had strong antioxidant activity with an IC50 value of 89,303 g/ml. , , The methanol fraction of wind leaf extract has a strong antioxidant activity with an IC50 value of 52.671 g/ml. Of the three leaf extracts, the fraction of N-hexane, Ethyl acetate, Methanol has a strong value for the antioxidant activity test because the IC50 value is 50-100 mg/ml, which means that the antioxidant activity is strong.

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Declaration of Interest Statement

The authors declare that they have no conflict of interest

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