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Antioxidant Activity of Lotion Etanol Extract of Akway Bark (Drymis Piperita Hook F.) By Abts Methods

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ABSTRACT

Akway contains saponins, tannins, flavonoids, alkaloids, quinones, and terpenoids. Akway bark has high antioxidants; antioxidant testing with the DPPH method on away bark gives an IC50 value of 9.68. The purpose of this research is to test the antioxidant activity of akway bark extract lotion preparation with the ABTS method. The study was conducted with the extraction stage using the maceration method with 96% ethanol solvent, then making lotion preparations with three lotion preparation formulations with different concentrations of akway bark extract (1.5, 2.0, and 2.5%). The test results showed that Formula I, II, and III had IC50 values of 71.41 ppm, 45.07 ppm, and 16.50 ppm, respectively, while the TEAC values were 0.143, 0.166, and 0.194 mM, respectively. This study concludes that Formula I, II, and III lotion preparations show the ability to counteract 50% of ABTS free radicals and show the highest antioxidant capacity in Formula III.

KEYWORD: Akway bark, lotion, antioxidant, ABTS

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I. INTRODUCTION

The human body has various mechanisms to support its physiological functions. One such mechanism is through the release of free radicals or reactive oxygen species that can be inhaled or consumed. To reduce or prevent damage from free radical stress, the body can produce antioxidant defense mechanisms. These defense mechanisms include free radical scavenging, metal chelating, and various enzyme activities to neutralize reactive species as soon as they are formed. Consumption or use of ingredients that contain antioxidants can also maintain antioxidant levels in the body, one of which is akway wood. . The balance between free radicals and antioxidants in the body affects health. The lack of adequate antioxidant intake from food consumption causes this. One source of antioxidants that come from outside the body can be obtained from plants. Akway wood (Drymis Sp) has a high antioxidant content. Simplisia of Akway plant bark contains alkaloids, saponins, and tannins, which are pretty high, and ethanol extract of akway bark contains alkaloid compounds, saponins, triterpenoids, flavonoids, and tannins. (Tethool & Purwaningsih, 2019) .

Previous research conducted by Pratiwi et al. (2022) regarding the anti-inflammatory test of the ethyl acetate fraction of akway bark in male rats found that the ethyl acetate fraction of akway bark had an anti-inflammatory activity with a percent anti-inflammatory power of 48.47%, while the percent pain inhibition of the ethyl acetate fraction of akway bark was 33.02%. Research by Pratiwi et al. (2024) shows that a lotion preparation formula from Akway bark extract can protect against sunlight with an SPF value of 16.00. Research conducted by Cepeda et al. (2019) regarding the results of testing the DPPH free radical ability of akway bark extracts at a concentration of 100 µg/mL of methanol, ethanol, and etilasetat extracts, respectively, amounted to 79.00; 62.00; 18.96%. The ability to counteract free radicals increased to 90.00, 89.35, and 73.44%. Similarly, research by Lestari and Syafii (2019) found that akway extract with E100 solvent has powerful antioxidant properties with an IC50 value of 9.68, while E75 extract with a concentration of 500 ppm has sunscreen activity with the highest SPF value of 16.95. In another study also conducted by Apriliani (2018), extracts and fractions of away bark have the highest

antioxidant activity content with IC50 values of 9.93 ppm and 9.07 ppm.

ABTS is an organic compound that, under normal conditions, is colorless, but when oxidized with potassium persulfate ($K_2S_2O_8$), it produces ABTS-+ cation radicals, which are blue-green. The ABTS method is one of the methods used to determine the antioxidant ability (Halliwell & Gutteridge, 2007).

II. RESEARCH METHODS

A. Tools

The tools used in this research are analytical scales, glass jars, stirring rods, filter paper, beakers, measuring cups, blenders, aluminum foil, cipro bottles, glass funnels, mesh no. 50, water bath, rotary evaporator, desiccators, drop pipettes, sudips, spatulas, mortars and stemper, porcelain cups, lotion containers, watch glass, UV spectrophotometry.

B. Materials

The materials used were akway bark, stearic acid, distilled water, ethyl acetate, DMDM hydantoin, cherry blossom fragrance, propylene glycol, phenoxyethanol, cetyl alcohol, 96% ethanol (technical), ethanol p.a.

C. Extract Preparation

Samples of akway bark were taken from Manokwari, West Papua. Samples were washed thoroughly with running water, drained, and aerated. Then dried using an oven with a temperature of 50ºC. The dried simplisia was then pulverized into powder. The process of making simplisia is that the akway wood sample is sorted, washed with running clean water, then drained, then the sample is cut with a chopper until it becomes a minor part, then dried for ± 10 days until the sample is dry. The dried samples were then dry sorted and pulverized using a blender and sieved using mesh 60.

The process of making extracts of akway bark (Drimys piperita Hook f.) is that the dry simplisia of 500 g of akway bark is extracted using 96% ethanol with maceration techniques for 1 × 24 hours. Then, the marinade is stored in a glass container and tightly closed to prevent evaporation during soaking. Then, the remaceration process was carried out twice. In the maceration process, stirring is carried out once every day, namely 3 times during the soaking process. After going through the maceration process for 3×24 hours, the marinade was then filtered to obtain liquid extract; from the results of maceration, the liquid extract was evaporated using a rotary evaporator (55°C) and thickened with a water bath (50°C).

D. Lotion Making

Lotions were made according to the formulation listed in Table 1. The oil phase ingredients (stearic acid, cetyl alcohol, liquid paraffin, and *phenoxyethanol*) were placed in a glass beaker. The aqueous phase (triethanolamine, propylene glycol, DMDM *hydantoin*, and distilled *water*) was put in a glass beaker. The oil and water phases were heated separately in a water bath at 70-75ºC. After everything was melted, the oil phase was poured slowly into the heated mortar while stirring, followed by pouring the water phase while still mixing until a *lotion mass was* formed. Then, add akway bark extract and fragrance to the mixture while stirring until homogeneous and put in a container.

Table 1. Formulation of Akway Bark Ethanol Extract Lotion Formulation

Ingredients	***Formula (%) (b/v)***			***Ingredient Function***
	FI	***FII***	***FIII***	
Ethanol extract from akway bark	*1.5*	*2.0*	*2.5*	Active substance
Cetyl alcohol	*1*	*1*	*1*	Emulsifiers
Stearic acid	*10*	*10*	*10*	Emulsifiers
Propylene glycol	*5*	*5*	*5*	*Humectant*
Triethanolamine	*1*	*1*	*1*	Thickener
Paraffin liquid	*7*	*7*	*7*	*Emolient*
phenoxyethanol	*0,5*	*0,5*	*0,5*	Preservatives
DMDM hydantoin	*0,05*	*0,5*	*0,5*	Preservatives
Cherry blossom fragrance	*6 drops*	*6 drops*	*6 drops*	*Fragrance*
Aquades	*Ad 100*	*Ad 100*	*Ad 100*	Carrier

Description:

F I: Formulation of lotion with 1.5% concentration of ethanol extract of akway bark

F II: Formulation of lotion with a concentration of ethanol extract of akway bark 2.0%

F III: Lotion formulation with 2.5% concentration of ethanol extract of akway bark

E. ABTS Assay

ABTS solution was prepared by mixing 5 ml of 7 mM ABTS stock solution and 5 ml of 2.45 mM potassium persulfate solution; the mixture was incubated for 12-16 hours. ABTS solution was added with 70% ethanol until an absorbance value of 0.7 ± 0.02 was obtained at a wavelength of 734 nm. 0.1 ml of ethanol extract lotion of akway bark was mixed with 0.9 ml of ABTS solution. The mixture was incubated in a dark room for 6 minutes; then, the absorbance was measured at a wavelength of 734 nm with a UV-Vis spectrophotometer. Formulas 1, 2, and 3 (50, 100, 150, 200, and 250 µg/mL) or Trolox standards (3, 5, 8, 12, 15, 17, and 20 µg/mL) were added to diluted ABTS-+ solution, and the absorbance reading was taken 6 min after mixing using the spectrophotometer. Results are presented as the ability of phenols to scavenge 50% of free radical ABTS-+ (IC50) and TEAC (Trolox equivalent antioxidant capacity). Parameters IC50 (µM) and TEAC (µM) were determined with a relative uncertainty of less than five percent. All determinations were carried out in triplicate.

III. RESULTS AND DISCUSSION

A. Extraction Result

The percentage inhibition value can indicate the amount of a compound that can inhibit the oxidation process of ABTS free radicals. The greater the percentage inhibition value, the better the antioxidant activity of a compound. The percentage inhibition value is directly proportional to the concentration of a sample tested, so the more the concentration of a sample tested increases, the greater the percentage inhibition value obtained. Meanwhile, the absorbance of the sample is inversely proportional to the concentration of a sample tested, so the more the concentration of a sample tested increases, the lower the absorbance value. The inhibition value of the akway bark extract lotion preparation formula can be seen in the following Table 2:

Table 2. Results of the calculation of %inhibition of Formulations 1, 2, and 3 of Akway Bark Extract Lotion Preparations

Formula	Concentration (µg/mL)	% Inhibition
FI	50	47.42
	100	53.19
	150	57.75
	200	63.22
	250	66.87
FII	50	51.98
	100	55.93
	150	58.66
	200	61.40
	250	66.87
FIII	50	59.27
	100	63.83
	150	66.57
	200	69.00
	250	72.64

Based on the calculation of % inhibition, the increase in concentration causes an increase in the % inhibition value of each formula; at a concentration of 50 ppm, formula II and formula III produce % inhibition values above 50%, while for formula I, the % inhibition value is below 50%. Based on the results in Table 2, it shows that akway bark extract contained in this lotion preparation formula can inhibit free radicals, where the free radicals used in this study are 2'-Azinobis(3-Ethylbenzothiazoline-6-Sulphonic Acid (ABTS). Compounds that show higher percent inhibition can be said to have better antioxidant capacity in counteracting free radicals and reducing oxidative damage (Rice-Evans & Miller, 1996).

After getting the % inhibition value, the IC50 and TEAC (Trolox equivalent antioxidant capacity) calculations were then carried out; the calculation results can be seen in Table 3 below:

Table 3. Antioxidant properties of lotion formulas (1,2 and 3) of akway bark extract expressed as the ability to scavenge 50% of free radical ABTS-+ (IC50) and TEAC (Trolox equivalent antioxidant capacity).

Formula	IC50 (µg/mL)	TEAC (mM)
FI	71.41	0.143
FI	45.07	0.166
FIII	16.50	0.194

Table 3 shows the antioxidant activity of formulas (I, II, and III) of akway bark extract lotion preparations expressed as the ability to counteract 50% of ABTS free radicals (IC50) and TEAC (antioxidant capacity equivalent to Trolox). Formula III, with the highest concentration of akway bark extract, 2%, showed a higher antioxidant capacity, expressed by an IC50 value of 16.50 and a TEAC value of 0.194. At the same time, the lowest was Formula I, with an IC50 value of 71.41 and a TEAC value of 0.143.

The ability of the akway bark extract lotion preparation formula to counteract 50% ABTS free radicals and show antioxidant capacity, one of which is due to the content of secondary metabolites in akway bark extract. Based on research by Tethool and Purwaningsih (2019), the ethanol extract of akway bark contains alkaloid, saponin, triterpenoid, flavonoid, and tannin compounds.

Based on the flavonoid structure, it is known that there is more than one phenol group (OH- and aromatic groups). It has a conjugated double bond so that it can ward off free radicals (Rice-Evans & Packer, 2003), based on research conducted by Heim. Et al. (2002), a double bond and carbonyl function in the heterocycle or polymerization of the nuclear structure increases activity by affording a more stable flavonoid radical through conjugation and electron delocalization.

Other secondary metabolite compounds that potentially have antioxidant activity are alkaloids and saponins, where the mechanism of alkaloids as antioxidants is by donating H atoms to free radicals (Chung & Shin, 2007); in the research of Yin et al (2016) Alkaliod from Aconitum handelianum (The aconitine-type C19-diterpenoid alkaloid) could serve as potential secondary antioxidants for their strong binding effects to metal ions.

The mechanism of saponins as antioxidants is through the formation of hydroperoxides that function as secondary antioxidants, preventing the formation of more reactive oxidation products and damaging lipid peroxides. Based on the research of Yinget al. (2014), Radix Trichosanthis saponins in n-butanol fraction might be a potential antioxidant candidate, as CCl4-induced oxidative stress has been found to be alleviated.

IV. CONCLUSION

The conclusions of this study include the following:

1. Formulations I, II, and III have antioxidant capacity with IC50 values of 71.41, 45.07, and 16.50 ppm,

respectively, while TEAC values are 0.143, 0.166, and 0.194 mM, respectively.

2. Formula III is the formula with the highest antioxidant capacity.

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