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Antioxidant Activity Test of Outstreched Bark Extract (*Campnosperma Brevipetiolatum Volkens*) using the DPPH Method

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ABSTRACT

Outstretched bark (*Campnosperma brevipetiolatum Volken*) is rich in tannins, flavonoids, alkaloids, and saponins. Herbal plants contain flavonoids, which are excellent antioxidants. Determine the amounts of phytochemical substances present in the sample and assess the antioxidant activity using the DPPH (1.1-diphenyl, 2-picrylhydrazil) technique at concentrations of 50, 100, 150, 200, 250, and 300 parts per million. The presenance of alkaloids alkaloids, saponins, and tannins was detected in the phytochemical screening findings against ethanol extracts of outstretched bark. 96% ethanol solvent is used in the maceration technique of extraction. Vitamin C is the positive control utilized. According to the findings of tests for antioxidant activity, ethanol extract at 11.25 ppm has a very high potential for antioxidant activity because its IC_{50} value is 3.55 ppm.

KEYWORDS: Antioxidants, DPPH, *Campnosperma brevipetiolatum* Volkens, Phytochemical Screening, Extract

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I. INTRODUCTION

A wide variety of biological natural resources, including species of medicinal plants, are available in Indonesia. The community uses medicinal plants as traditional medicine because they are widely available, reasonably priced, and easily produced by humans. Increasing the community's use of medicinal herbs that function as antioxidants is one of the alternative medicines. Chemical substances known as antioxidants can prevent free radicals from oxidizing processes [1].

For instance, Papua is home to a large and highly valuable orchid trade. Many alkaloid chemicals, flavonoids, glycosides, carbohydrates, and other phytochemical components found in orchids are known to have applications in traditional medicine [2].

Free radicals are defined as atoms or molecules that have one or more unpaired electrons in their outermost orbitals. Because they are incredibly unstable and reactive, free radicals want to react with other molecules to become stable. High-reactivity radicals can start a chain reaction in a single formation, producing abnormal chemicals and starting a chain reaction that may damage important human cells [3]. Free radicals can be fought using antioxidants [4].

Antioxidants are electron-donor chemicals that, by attaching to free radicals and highly reactive molecules, can inhibit oxidation processes. Antioxidants defend the organism against oxidative damage by combating free radicals. They also keep the fat/oil from oxidizing, which allows it to serve as a preservative [5].

Flavonoid substances are polyphenolic chemicals found in plants including tea, fruits, and vegetables that serve as natural antioxidants. Flavonoid compounds have the capacity to directly reduce oxygen free radicals such as superoxide, which is produced by the enzyme xanthine oxidase. Flavanoids contain anti-atherosclerosis, anti-thrombogenic, anti-inflammatory, anticancer, antiviral, and antiosteoporosis characteristics in addition to being antioxidants [6].

Research on the Anarcadiaceae family showed that ethanol extract of cashew fruit pulp has an IC_{50} value of 3.61 ppm [7]. When compared to the synthetic antioxidant BHA which has an IC_{50} of 5.23 ppm ethanol extract has enormous potential to be developed as a natural antioxidant.

The Maybrat tribe employs empirical usage by piercing the bark slightly inward, collecting the sap, and then applying the sap to the hair [8].

Antioxidants are important for hair health because they may regenerate and repair damaged hair cells, build skin tissue favorable to hair development, and improve blood circulation needed by hair to become strong and not dull [9].

II. MATERIAL AND METHODS

A. Appliances

A rotatory evaporator, vial, oven, hot plate, analytical balance, spatula, jar, blender, T-1601 Uv-Vis spectrophotometer, aluminum foil, filter paper, tissue, and extraction container were utilized in this study.

B. Materials

Outstretched bark (*Campnosperma brevipetiolatum Volkens*), Vitamin C, DPPH, distilled water, filter paper, aluminum foil, HCl, Meyer reagent, Dragendroff reagent, magnesium powder, amyl alcohol, $FeCl_3$ (Iron (III) chloride), NaOH, and sulfuric acid.

C. Research Methods

Simplicia Preparation

The bark was thoroughly cleansed under running water before being sun-dried with a black fabric serving as both a foundation and a cover. After that, the bark was wet-sorted. Wet sorting is used to separate the bark from impurities. After 14 days of aeration, non-perishable simplicia is produced. Before the simplicia is pulverized into powder, undesired foreign particles are removed by dry sorting.

Extract Preparation

A total of 400 g of Simplicia powder was weighed and then macerated in up to 1.2 L of 96% ethanol for three days, stirring once every 24 hours. Then, filter and collect the Maserati before employing a rotating vacuum to evaporate the solvent at 50°C to obtain a thick extract. The extract yield is then estimated using the formula:

$$\% \text{ extract yield} = \frac{Weight\ of\ extract}{Weight\ of\ simplisia}\ x\ 100\%$$

D. Phytochemical Screening

Phytochemical screening of extract includes the examination of alkaloids, flavonoids, saponins, tannins, and terpenoids.

Alkaloid Test

Outstretched bark extract was weighed and combined with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water before being heated for 2 minutes in a water bath, cooled, and filtered. Filtrate for an alkaloid test, divided into two sections with Dragendroff and Mayer reagents in each. The positive reaction of alkaloids with Dragendroff reagent resulted in a brick red precipitate, whereas the positive reaction with Meyer reagent resulted in a white precipitate [10].

Flavonoid Test

After weighing 0.5 g of outstretched bark extract, combine it with 5 mL of hot water and strain the mixture. Mix in 1 mL of the hydrochloric acid-ethanol (1:1) solution and 2 mL of amyl alcohol after adding the magnesium powder. A positive reaction is indicated by the generation of orange, yellow, or red color attracted to the amyl alcohol layer [11].

Saponin Test

Weigh 0.5 g of outstretched bark extract into a test tube, add 10 mL of boiling water, and shake for 10 seconds. The presence of saponin compounds is indicated by the formation of a persistent foam, 1 cm high, that does not evaporate when 2 N hydrochloric acid is applied for at least 10 minutes.

Tannin Test

After weighing 0.5 g of outstretched bark extract, it was diluted with 10 mL of distilled water and filtered. The filtrate was diluted with distilled water until it took on no color. After obtaining a total of 2 mL of solution, 1-2 drops of 1% $FeCl_3$ reagent were added. The occurrence of green- or blue-black color shifts is indicative of the presence of tannin molecules.

Triterpenoids Test

A 0.5 g extract of outstretched bark was combined with 20 mL ethanol, 2 mL chloroform, and 3 mL concentrated H_2SO_4. A change in the color of the solution to red indicates the presence of terpenoid compounds.

E. Antioxidant Activity Testing

Preparation of DPPH Solution

DPPH powder was weighed to 4 mg and dissolved in ethanol p.a. before being placed in a 100 mL volumetric flask, ethanol was poured to the limit mark, and the mixture was dissolved until homogeneous.

Preparation of Positive Control Solution

Vitamin C was employed as a positive control. The positive control of stock solution with a concentration of 100 ppm was prepared by weighing 5 mg and dissolving it in 50 mL of ethanol, which was then dissolved until homogenous.

Positive control series solutions of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were created by pipetting 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, and 1 mL of the stock solution and adding ethanol to a total volume of 10 mL.

Preparation of Blank Solution (Comparison Solution)

4 mL of ethanol was poured into a test tube with a 1:1 DPPH solution, stirred until homogeneous, and incubated for 30 minutes as the blank solution.

Preparation of Extract Sample Solution

A total of 50 mg of extract was weighed and dissolved with ethanol p.a before being placed in a 50 mL volumetric flask, filled to the limit mark with ethanol, and shaken until homogeneous.

Preparation of Test Solution

To perform a 1000 ppm stock solution concentration test, weigh 50 mg of the sample and dissolve it in 50 mL of ethanol until homogeneous. Make a 1000 ppm concentration test stock solution. Pipette 0.5 mL, 1 mL, 1.5 mL, 2 mL, 2.5 mL, and 3 mL of stock solution and mix with ethanol to make a total of 10 mL of test series solution 0, 50, 100, 150, 200, 250, and 300.

Antioxidant Activity Testing

To the DPPH solution, 5 mL of each extract's test solution at various concentrations was added, stirred until homogeneous, and incubated for 30 minutes. The absorbance of the activity was then measured at 517 nm with a UV-Vis spectrophotometer. The extract sample test solution and the positive control sample test solution were replicated five times each.

F. Antioxidant Data Analysis

Determination of Percent (%) Inhibition

To calculate the percent inhibition against DPPH radicals, the absorbance of the sample is compared to the absorbance of DPPH. To compute the percentage of antioxidant activity, use the following formula:

Percent (%) Inhibition = $\frac{Abs\ of\ Blanko - Abs\ of\ Sample}{Blanko}$ x 100

Abs

Determination of IC_{50} and AAI Values

After determining the percentage of inhibition from each concentration series, linear regression calculations were performed using the equation y = Bx + A, where x is the concentration (ppm) and y is the percent inhibition (%). An antioxidant's IC_{50} (inhibitory concentration 50%) value is the concentration of the sample that can lower the DPPH radical by 50%. The IC_{50} value is derived from the x value by replacing y with a value of 50.

According to a particular study [12], the IC_{50} value determines the qualities of an antioxidant molecule. When the IC_{50} value is less than 50 ppm, antioxidant activity is extremely high, strong when it is between 50-100 ppm, moderate when it is between 100-150 ppm, and weak when it is between 150-200 ppm.

Furthermore, using the following formula, the AAI (antioxidant activity index) value might be used to compute the antioxidant activity index:

Percent (%) Inhibition = $\frac{Abs\ of\ Blanko - Abs\ of\ Sample}{Blanko}$ x 100%

According to Vasic et al. (2012), an AAI value of < 0.5 indicates a weak antioxidant, an AAI value of 0.51 - 1 indicates a moderate antioxidant, an AAI value of 1 - 2 indicates a strong antioxidant, and an AAI value of > 2 indicates a very powerful antioxidant.

III. RESULTS AND DISCUSSION

A. Result of Extraction

Table 1. Extraction results of outstretched bark

Sample	Simplicia Weight (g)	Volume of Solvent (mL)	Extract Weight Obtained (g)	Extract Yield (% b/v)
Campnosperma brevipetiolatum Volkens	400	1.200	41	10.25

The extraction operation is carried out using cold extraction, namely the maceration method, as given in Table 1. The maceration method of the simplicial of outstretched bark as much as 400 g was soaked three times in 24 hours in 96% ethanol as much as 1.2 L and agitated once every 24 hours before filtering with filter paper. The filtrate was then evaporated in a rotating vacuum evaporator at 50°C until a thick extract was obtained.

The selectivity and low cost of ethanol as a solvent motivate its adoption. Furthermore, the majority of the chemical components contained in Simplicia, such as alkaloids, essential oils, glycosides, curcumin, coumarin, anthraquinones, flavonoids, steroids, rosin, fatty chlorophyll, Malam, tannins, saponins, could be extracted using ethanol. The extraction technique of outstretched bark (*Campnosperma brevipetiolatum* Volkens) generated a thick extract of 41 g with a percent yield of 10.25%. The yield is the weight of the acquired sample divided by the weight of the starting sample.

B. Phytochemical Screening Results

The table below shows the findings of phytochemical screening of an ethanol extract of outstretched bark.

Table 2. Phytochemical Screening Results

Sample Test	Compounds	Extract	Descriptions
Outstretched Bark	Alkaloids	+	A brick red precipitate occurred in the presence of Dragendrof reagent, but a white precipitate formed in the presence of Meyer reagent.
	Flavonoids	+	Yellow color formation on an amyl alcohol layer.
	Saponins	+	Form of stable foam.
	Tannins	+	Blue-blackish hue formation.
	Triterpenoids	-	There was no development of a brownish ring.

Description: (+) present; (-) absent

Table 2 demonstrates that the phytochemical identification of outstretched bark extract indicates that it contains alkaloid, flavonoid, saponin, and tannin components.

The formation of yellow molecules in amyl alcohol solution detects flavonoids. Flavonoids comprise natural phenolic compounds that have antioxidant properties as well

as pharmacological bioactivity. These compounds can be found in stems, leaves, blossoms, and fruits. Flavonoids work as antioxidants in the human body, making them ideal for cancer prevention. Flavonoids have been proven to preserve cell integrity, boost vitamin C efficacy, reduce inflammation, and slow bone loss [13].

Positive Mayer test findings for alkaloids are characterized by the production of a brick-red precipitate, which is thought to be an alkaloid potassium complex. When mercury (II) chloride interacts with potassium iodide, a red precipitate of mercury (II) iodide is formed. Excess potassium iodide results in the formation of potassium tetraiodomercurate (II). Alkaloids contain nitrogen atoms with free electron pairs that can be used to form coordinate covalent bonds with metal ions [14].

During the saponins test, a consistent foam is produced. The presence of foam in the saponin test suggests the existence of glycosides, which when broken down into glucose and other compounds can produce foam in water [15].

Tannin testing uses $FeCl_3$, which reacts with one of the hydroxyl groups found in tannins. $FeCl_3$'s occupation is to hydrolyze the tannin group, which results in a blue-black color change, and condense tannin, which results in a greenish-black color shift [16].

In triterpenoid testing, the ability of compounds to create colors with concentrated H_2SO_4 in anhydrous acetic acid solvent is used [16]. Steroids are indicated by a blue-green ring, while triterpenoids are indicated by a brownish ring. The phytochemical screening results show negative extracts on triterpenoids, implying no brownish ring.

C. Antioxidant Activity Test with DPPH

To assess antioxidant activity, the free radical diphenylpicrylhydrazyl (DPPH) is utilized. DPPH is a stable free radical that functions by delocalizing free electrons in a molecule, rendering it less reactive than other free radicals. The emergence of an evident purple (violet) tint in the absorbance band indicates this delocalization process [12]. The DPPH approach employs the spectrophotometric concept to assess antioxidant activity. As measured at a visible light wavelength of around 517 nm, the DPPH molecule (in ethanol) shows a dark purple hue. A molecule is regarded to have antioxidant activity if it can donate hydrogen atoms to the interaction of DPPH to produce reduced DPPH, which is distinguished by the loss of purple hue (becomes pale yellow).

The concentration that results in a 50% drop in DPPH activity [17], also known as the IC_{50} value, is often used to express antioxidant activity. Because DPPH has a wavelength of 517 nm, measurements were done at that wavelength. Because of the presence of unpaired electrons, DPPH absorbs strongly at 517 nm. When electrons are linked as a result of free radical capture, absorbance reduces [18].

Table 3. Shows the results of antioxidant activity tests on outstretched bark extract

Test	Blank	Concentration (ppm)	Absorbance Value					Average Absorbance of the Sample	% Inhibition
			R1	R2	R3	R4	R5		
Vitamin C	0.502	2	0.67	0.67	0.67	0.67	0.67	0.67	15.93
		4	0.54	0.54	0.53	0.53	0.53	0.53	19.32
		6	0.34	0.33	0.33	0.33	0.33	0.33	33.26
		8	0.19	0.19	0.19	0.19	0.19	0.23	61.75
		10	0.12	0.11	0.11	0.11	0.11	0.11	76.89
Extract	0.502	0	0	0	0	0	0	0	0
		50	0.473	0.408	0.389	0.388	0.383	0.408	18.72
		100	0.463	0.420	0.391	0.368	0.368	0.402	19.92
		150	0.399	0.395	0.385	0.378	0.355	0.382	23.90
		200	0.388	0.382	0.377	0.376	0.375	0.379	24.50
		250	0.378	0.377	0.377	0.362	0.362	0.369	26.49
		300	0.357	0.355	0.355	0.353	0.333	0.346	31.07

Table 3 shows that with increasing concentration, there is a drop in absorbance and a continuous increase in % inhibition. The percent inhibition is calculated by subtracting the absorbance of the negative control from the absorbance of the sample as measured by a UV-Vis spectrophotometer. While the drop in absorbance occurs because the test solution dampens DPPH, dampening happens because of the presence

of chemicals that react as a radical catcher, reducing DPPH to generate reduced DPPH [12].

D. Values of Samples and Positive Controls

Table 4. IC_{50} values of samples and positive control

Sample of Outstretched Bark	IC_{50} Value (ppm)	AAI Value
Extract	11.24	3.55
Vitamin C	7.04	6.98

Table 4 shows that the extract has very substantial antioxidant properties, with an IC_{50} value of 11.24 ppm. The IC_{50} value for vitamin C, the positive control, is 7.04 ppm. Vitamin C is used as a positive control since it is a natural antioxidant capable of absorbing free radicals.

Furthermore, the Activity Antioxidant Index (AAI) value was determined to classify the antioxidant characteristics using the DPPH technique. The AAI value is calculated by comparing the concentration of the DPPH solution to the IC_{50} value of the extract and the vitamin C control. Determined antioxidant activity based on the AAI value [19]. If the AAI value is less than 0.5, it is defined as a weak antioxidant; if the AAI value is more than 0.5-1, it is considered a moderate antioxidant; if the AAI value is greater than 1-2, it is considered as a strong antioxidant; and if the AAI value is greater than 2, it is considered as a very strong antioxidant. The AAI value of vitamin C extract and control is shown in Table 4. According to the AAI value data in the table, the extract has extremely high antioxidant capacity because it has an AAI value greater than 2, with an AAI value of 3.55.

According to the study's findings, the extract shows very significant antioxidant activity, due to the presence of chemicals that attract antioxidants, notably flavonoids. Because hydroxyl groups in flavonoid compounds may release protons in the form of hydrogen ions, they function as antioxidants. Because hydrogen ions only contain one proton and no electrons, they link to radical electrons on nitrogen atoms in DPPH compounds to form reduced DPPH [20]. Flavonoids work as antioxidants in two ways: directly and indirectly. Flavonoids operate directly as antioxidants by contributing hydrogen ions to stabilize reactive free radicals and serve as scavengers to neutralize free radicals [21]. Flavonoids act as antioxidants in the body indirectly by increasing the expression of endogenous antioxidant genes through a variety of mechanisms, including increasing antioxidant gene expression through nuclear factor erythroid 2 related factor 2 (Nrf2) activity, which increases genes involved in the synthesis of endogenous antioxidant enzymes such as SOD (superoxide dismutase) [22].

CONCLUSIONS

Flavonoids, alkaloids, tannins, and saponins were identified in an ethanol extract of outstretched bark (*Campnosperma brevipetiolatum Volkens*). Outstretched bark extract exhibits strong antioxidant activity, with an IC_{50} value of 50 ppm, at approximately 11.24 ppm. The extract of outstretched bark has a very high Antioxidant Activity Index (AAI) value, which is 3.55.

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