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ANTIOXIDANT ACTIVITY OF TEGINING GANANG (*Senna hirsuta* L) LEAF EXTRACT USING DPPH METHOD

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ABSTRACT

Tegining ganang (*Senna hirsuta* L) is a commodity plants on Lombok Island and is traditionally used to treat minor wounds, severe wounds, and skin infections. This study aimed to evaluate the antibacterial and antioxidant activities of ethanol, ethyl acetate, and n-hexane extracts of Tegining ganang leaves against *Staphylococcus aureus* and *Escherichia coli* using the DPPH method. The results showed that the ethyl acetate extract exhibited the highest antibacterial activity. *Escherichia coli* with an inhibition zone of 8.16 mm. For *Staphylococcus aureus*, the inhibition zone was 1.16 mm. However, all three extracts demonstrated weak antifungal activity. Phytochemical screening revealed the presence of alkaloids and flavonoids in all the extracts. Additionally, the ethanol and ethyl acetate extracts contained phenolic compounds and tannins, which were absent in the n-hexane extract. In the antioxidant activity assay, the 96% ethanol extract demonstrated excellent antioxidant activity with average absorbance values of 0,496 ± 0,018 at 10 ppm, 0,438 ± 0,008 at 20 ppm, 0,357 ± 0,003 at 30 ppm, and 0,326 ± 0,002 at 40 ppm, resulting in an IC_{50} value of 16,67 ppm. The ethyl acetate extract had an absorbance of 0,537 ± 0,010 at 10 ppm, 0,487 ± 0,002 at 20 ppm, 0,443 ± 0,011 at 30 ppm, 0,392 ± 0,003 at 40 ppm, and 0,383 ± 0,005 at 50 ppm, with an IC_{50} of 28,50 ppm. The n-hexane extract demonstrated modest antioxidant activity with absorbance values of 0,602 ± 0,036, 0, 541 ± 0, 001 at 20 ppm, 0, 547 ± 0, 018 at 30 ppm, and 0, 512 ± 0, 001 at 10, 20, 30, and 40 ppm, respectively, with an IC_{50} of 70,42 ppm. With The IC_{50} value was 7.8 ppm.

Keywords: *Tegining-ganang*, antioxidant, DPPH

INTRODUCTION

Lombok is a small island located in eastern Indonesia that falls within a tropical climate zone. Tropical climates can lead to several tropical diseases, particularly those related to skin conditions. The skin is the outermost part of the human body and covers the entire external body surface (Kumarahadi *et al.*, 2020). It is the largest organ in the human body, with an area of approximately 2 m² (Adha, Sumijan, and Nurcahyo, 2021). The skin acts as the primary barrier against viruses, bacteria, and other external threats (Adha, Sumija, and Nurcahyo, 2021). Its functions include protecting the body, serving as a sensory organ, and regulating body temperature (Kumarahadi *et al.*, 2020).

One of the factors that causes skin damage is the presence of free radicals (Al-bari, Pratiwi, and Ni, 2024). Free radicals are unstable and highly reactive chemical compounds that contain one or more unpaired electrons. They can be generated through bodily metabolism, cigarette smoke, radical-inducing substances in food, and other environmental pollutants. Additionally, free radicals can be caused by exposure to radiation from electronic

devices such as televisions, mobile phones, and computers (Faizah *et al.*, 2023). Antioxidants are a solution to protect the skin from free radical damage (Martemucci *et al.*, 2022).

One of the plants that is a commodity on Lombok Island is tegining ganang (*Senna hirsuta* L.). The leaves of Tegining Ganang are known for their medicinal properties and are commonly used in Lombok. Based on empirical community experience, these leaves are used to treat internal conditions such as stomach aches, diarrhea, gastritis, heartburn, kidney stones, poisoning, diabetes, and hypertension. They are also used externally to treat animal bites, plant and fish poisoning, skin itching, wounds, hemorrhoids, and other conditions. The chemical compounds found in tegining ganang leaves include citronellal, tumerone, hexadecanoic acid, oxacycloheptadecane, neophytadiene, palmitic acid, stearic acid, anthraquinone glycosides, flavonoids, alkaloids, phytosterols, saponins, tannins, galactomannan, polysaccharides, and terpenoids (Sianturi, Febriani, and Manalu, 2020).

Previous studies have shown that the ethanol extract of tegining ganang leaves possesses strong analgesic activity, as evidenced by an analgesic activity percentage exceeding 50% compared to the positive control (Febriani, Syariifatul and Satrana, 2018). The results of the phytochemical screening slightly differed from previous findings. In earlier research, the detected phytochemical compounds included alkaloids, phenols, flavonoids, tannins, steroids, saponins, and terpenoids (Rahma Yulis *et al.*, 2020). Another study reported the presence of alkaloids, flavonoids, saponins, tannins, and steroids in the leaves. These differences can be attributed to the fact that secondary metabolites are produced by specific organisms under certain environmental conditions influenced by altitude, rainfall, soil fertility, and regional differences, leading to variations in the chemical compound content (Anjani *et al.*, 2024). Based on these studies, tegining ganang leaves have been shown to contain secondary metabolites, particularly flavonoids.

However, research on the use of active compounds from tegining ganang leaf extracts as ingredients in cosmetic products, particularly antioxidant cream formulations, remains limited. Therefore, this study aimed to determine an effective formulation of antioxidant cream preparations containing tegining ganang leaf extract at three different concentrations using the DPPH method.

METHODS

Equipment and Materials

The equipment used in this study includes a maceration extraction apparatus, spectrophotometer UV-Vis (Shimadu UV-1601, japan), oven (*Memmer*t, UN110 Universal Oven 108 L). The materials used in this research consisted of Tegining Ganang leaves *(Senna hirsuta* L.), collected from Presak Village, West Lombok, and identified at the Laboratory of the State Islamic University of Mataram. Other materials included 96% ethanol, ethyl acetate, n-hexane, methanol pro analysis (p.a.), 1,1-diphenyl-2-picrylhydrazyl/DPPH p.a. (Sigma-Aldrich), quercetin (Sigma-Aldrich), filter paper, cotton, aluminum foil.

Research Procedure

Sample Collection

The simplisia used in this study consisted of tegining ganang (*Senna hirsuta* L.) leaves collected from Peresak Village, Narmada District, West Lombok Regency, West Nusa Tenggara, Indonesia.

Extraction of Tegining Ganang Leaves

The extraction of tegining ganang leaves was carried out using the maceration method. A total of 541 grams of tegining ganang leaf samples were macerated using 96% ethanol as the solvent. The extraction was repeated thrice for 24 hours each. The sample was then filtered to obtain the filtrate, which was concentrated using a rotary evaporator to

obtain a thick extract. The yield obtained was then weighed and recorded (Almira, Ahidin, and Indawati, 2023).

Phytochemical Screening

One milliliter of 2% HCl and two to three drops of Dragendorff's reagent were added to 0.5 grams of the extracted ganang leaf in a test tube. The presence of alkaloids was indicated by the formation of an orange to reddish-brown precipitate (Almira, Ahidin, and Indawati, 2023). For the flavonoid test, 0.5 grams of the ethanol extract of tegining ganang was added to a test tube, followed by ten drops of concentrated HCl and 0.2 grams of magnesium powder. The presence of flavonoids was indicated by a color change to red, yellow, or orange. To test for tannins, 0.5 grams of the extract and two to three drops of 1% $FeCl_3$ solution were added to a test tube. Tannins were present because of the formation of a dark green to black color (Ibroham, 2020). To perform the phenolic test, three drops of a 0.1% $FeCl_3$ solution were added to a test tube containing 0.5 grams of the extracted ganang leaf. The development of a green, blue-green, or dark blue solution is indicative of the presence of phenolic chemicals (Permata, Iswandi and Saifullah, 2023).

Antioxidant Activity Test (DPPH Method)

Antioxidant activity was measured using DPPH**.** DPPH (1,1-diphenyl-2-picrylhydrazyl) weighing 10 mg was dissolved in ethanol up to the mark in a 100 mL volumetric flask (1000 ppm), and then homogenized. A total of 0.5 mL of DPPH solution was pipetted into a 50 mL volumetric flask, diluted with ethanol p.a., and homogenized. Vitamin C exhibits high antioxidant activity owing to the presence of two hydroxyl groups that facilitate hydrogen donation. A total of 10 mg of Vitamin C was weighed and dissolved in a 10 mL volumetric flask, then diluted with ethanol to prepare the stock solution. Serial dilutions were subsequently performed to obtain Vitamin C solutions at concentrations of 1, 3, 5, 7, and 9 ppm. Each cream formulation was weighed (10 mg) and dissolved in a 10 mL volumetric flask with ethanol p.a. to the mark. Serial dilutions were prepared at concentrations of 1, 3, 5, 7, and 9 ppm, respectively. From each dilution, 2 mL was pipetted into a vial, and DPPH solution was added in a 2:1 ratio. The mixture was incubated at 37 °C and then transferred into a cuvette for absorbance measurement using a UV-Vis spectrophotometer at 517 nm. The percentage inhibition was calculated to determine the IC_{50} value (Zamzam, 2023). After obtaining the absorbance values, the antioxidant activity results of the DPPH assay were interpreted using the IC_{50} parameter. The IC_{50} value represents the concentration required to inhibit 50% of the DPPH activity. The IC_{50} was calculated using the linear regression equation y = bx + a. Data on the percentage of inhibition from the assay are required for this calculation. The percentage of inhibition was determined using the following formula:

$$\%\text{Inhibition} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

(Husain, 2023).

Data Analysis

The absorbance measurements of 96% ethanol, ethyl acetate, and n-hexane extracts of tegining ganang (*Senna hirsuta* L.) leaves, along with quercetin, were obtained using a UV-Vis spectrophotometer (Syifni and Utami, 2024). The data were statistically analyzed using linear regression in Microsoft Excel.

RESULTS AND DISCUSSION

A sample of 3 kg of fresh simplicia was used. The leaves of the tegining ganang were collected in the morning, sorted, and cleaned. The leaves were then dried for three days at 50 ^{0}C in an oven. This drying process aims to reduce the moisture content, preventing the material from being easily infested by mold or fungi (Kusuma, 2024). After drying, the simplicia was ground using a blender. The grinding process was intended to increase the surface area and facilitate the extraction process. The mass of the ground ganang leaves obtained was 791.28 grams.

Figure 1. Tegining – ganang Plant, dilution of quercetin standard solution with a series of concentrations of 2, 4, 6, 8, 10 ppm and dilution of extract solution with a series of concentrations of 10, 20, 30, 40, 50 ppm.

A compound dissolves in a solvent that shares properties similar to of those the compound. This tiered maceration technique is employed to ensure that the solvent can effectively extract all metabolite compounds present in the sample, thereby improving the quality of secondary metabolite identification in research.

Table I. Extract yield from maceration

Extract	Weight (gram)	Yield (%)	Extract characteristics		
			Shape	Color	Smell
Ethanol 96%	27,33	13,66	Viscous	Black	Characteristic odor
Ethyl Acetate	6,1	3,05	Viscous	Black	Characteristic odor

Based on **Table I**, the difference in the weight of the extract obtained from maceration using each solvent indicates that the content of polar secondary metabolites is more abundant in the tegining ganang leaf sample than non-polar and semi-polar secondary metabolites. This can be observed from the highest yield obtained from extraction using ethanol, a polar solvent, while the lowest yield was obtained from extraction using the nonpolar solvent, n-hexane. Flavonoids are an example of polar secondary metabolites.

Results of Antioxidant Activity Test of 96% Ethanol Extract, Ethyl Acetate Extract, and N-Hexane Extract of *Tegining Ganang* Plant Leaves

Owing to its simplicity, speed, ease of use, and short analysis time, the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) radical scavenging method was employed to assess antioxidant activity. Furthermore, the accuracy and usefulness of this approach have been demonstrated (Zamzam, 2023). UV-Vis spectrophotometry was used to measure the absorbance at 516 nm, the maximal wavelength for DPPH, to evaluate antioxidant activity. The DPPH molecule is appropriate for assessing antioxidant activity because it comprises an unstable nitrogen-free radical that can bind to hydrogen ions. The DPPH solution in methanol changes color from deep violet to pale yellow in the presence of antioxidant chemicals in the sample. When

DPPH is reduced, the electron pairs together, resulting in a hue shift (Husna, Kairupan and Lintong, 2022).

The blank solution was treated in the same manner as the sample and standard solutions, but it did not contain the analyte. The purpose of measuring the blank absorbance was to determine the amount of absorption by substances other than the analyte. From the blank measurement, the average absorbance was 0.910. Before measuring the antioxidant activity of the sample, it is necessary to determine the maximum wavelength to understand when absorption reaches its maximum limit, which aims to optimize the absorption of the solution by light (Puspa, 2023).

The maximum wavelength of the blank solution in this study was 516 nm. In this study, quercetin was used for comparison. This is because quercetin is a flavonoid that demonstrates various biological activities and has a strong ability to scavenge free radicals (Hidayah *et al*., 2024). The purpose of using quercetin in this study was to compare the antioxidant activity potential of 96% ethanol, ethyl acetate, and n-hexane extracts to determine whether it is stronger than that of quercetin. A linear regression equation was created between the concentration (ppm) as the abscissa (x-axis) and the antioxidant activity (%) as the ordinate (y-axis). The IC_{50} value was determined from the regression equation. The linear regression obtained from the quercetin standard solution was y = 5.4322x + 7.1941, with an R^2 value of 0.9777.

Table II. Absorbance Measurement, Inhibition Percentage, and IC_{50} of the Standard

Sample	ppm	Absorbance (mean ± SD)	% Inhibition (mean ± SD)	IC_{50} (ppm)
Standard	2	0,774 ± ,0030	14,87 ± 0,15	7,8
	4	0,619 ± 0,002	31,83 ± 0,28	
	6	0,550 ± 0,011	41,28 ± 0,25	
	8	0,440 ± 0,003	51,68 ± 0,45	
	10	0,365 ± 0,005	59,26 ± 0,53	

Based on **Table II**, the results indicate that the lower the concentration used in the antioxidant activity measurement of the quercetin standard solution, the higher the absorbance. Conversely, higher concentrations showed greater antioxidant activity, and as the concentration increased, the free radical inhibition percentage tended to increase. This is because at the highest concentration, the amount of extract used was the greatest, making it more effective at capturing free radical molecules. This statement is closely related to Lambert-Beer's law, which states that the concentration of a sample is directly proportional to its absorbance. Additionally, the presence of the DPPH reagent in the sample influenced the results. According to the linear regression equation y = bx + a, the IC_{50} value obtained was 7.8 ppm, which places quercetin in the category of very strong antioxidant activity, since the IC_{50} value is less than 10 ppm. These concentration variations aimed to determine the concentration of DPPH free radical inhibition and to assess the antioxidant activity of tegining ganang leaf (*Classia planisiliqua* Burm.f) (Budi and Nastiti, 2022).

The absorbance measurements of the 96% ethanol, ethyl acetate, and n-hexane extracts at various concentrations showed that the 96% ethanol extract at a concentration of 10 ppm had the highest absorbance of 0.496, with the lowest inhibition percentage of 45.49%. In contrast, at a concentration of 50 ppm, it had the lowest absorbance of 0.230 and the highest inhibition percentage of 74.72%. Based on the linear regression equation y = bx + a, the IC_{50} obtained was 16.67 ppm, indicating that the 96% ethanol extract falls into the category of strong antioxidants (IC_{50} between 10-50 ppm) (Suriawati, 2023). The ethyl acetate extract at a concentration of 10 ppm exhibited the highest absorbance of 0.537 ± 0,011, with the lowest inhibition percentage of 40.91% ± 0,42. At 50 ppm, the lowest absorbance was 0.381 ± 0,007, with the highest inhibition of 58.09% ± 0,39. The IC_{50} value of the ethyl acetate extract was 28.50 ppm, indicating a strong antioxidant activity.

Meanwhile, the n-hexane extract at 10 ppm showed the highest absorbance of 0.618 ± 0,009 and the lowest inhibition percentage of 32.05% ± 0,38. At 50 ppm, the lowest absorbance was 0.511 ± 0,005, with the highest inhibition percentage of 43.77% ± 0,36. The IC_{50} obtained for the n-hexane extract was 70.42 ppm, classifying its antioxidant activity as moderate (IC_{50} between 50-100 ppm). Compared to quercetin, the IC_{50} values of all three extracts were higher, but they still demonstrated antioxidant activity due to the presence of secondary metabolites such as alkaloids and flavonoids. According to (Anjani *et al.*, 2024), compounds with antioxidant potential can be predicted from polar compounds in the phenolic, flavonoid, and alkaloid groups. Phytochemical screening revealed the presence of alkaloids and flavonoids in all three extracts. The ethanol and ethyl acetate extracts also tested positive for tannins and phenolics, whereas the n-hexane extract did not contain these compounds.

Table III. Meas urement of Absorbance, Percentage of Inhibition, and IC_{50} values of 96% Ethanol extract, Ethyl Acetate extract, and N-Hexane extract of *Tengining Ganang* Leaves (*Senna hirsuta* L)

Sample	ppm	Replications			Average	%Inhibition	IC_{50} (ppm)
		1	2	3			
Ethanol 96%	10	0,485	0,487	0,516	0,496	45,49	16,67
	20	0,438	0,446	0,430	0,438	51,86	
	30	0,359	0,354	0,354	0,357	60,91	
	40	0,328	0,325	0,326	0,326	64,13	
	50	0,230	0,230	0,230	0,230	74,72	
Ethyl Acetate	10	0,550	0,533	0,530	0,537	40,91	28,50
	20	0,488	0,496	0,485	0,489	46,19	
	30	0,430	0,447	0,452	0,443	51,31	
	40	0,393	0,390	0,395	0,392	56,84	
	50	0,375	0,389	0,380	0,381	58,09	
N-Hexane	10	0,629	0,609	0,617	0,618	32,05	70,42
	20	0,579	0,561	0,564	0,568	37,58	
	30	0,540	0,541	0,542	0,541	40,54	
	40	0,537	0,531	0,536	0,534	41,24	
	50	0,511	0,512	0,512	0,512	43,77	

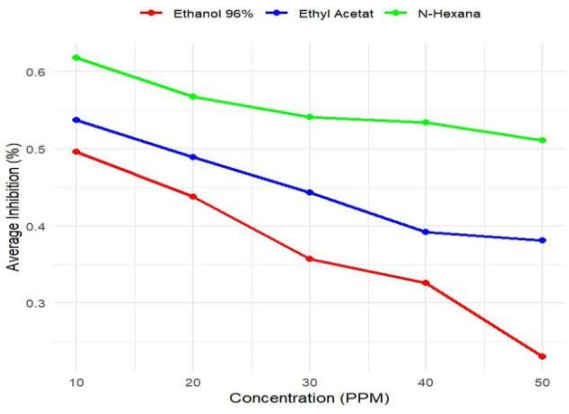

Figure 2. Relationship between sample ethanol, ethyl acetate, n-hexane extract concentration (ppm) and average inhibition

Based on the data and graph, the 96% ethanol extract exhibited the highest antioxidant activity, with an inhibition percentage of 74.72% at 50 ppm and an IC_{50} value of

16.67 ppm, classifying it as a strong antioxidant. The ethyl acetate extract was also categorized as a strong antioxidant, with an IC_{50} value of 28.50 ppm, although its activity was lower than that of the 96% ethanol extract. Meanwhile, the n-hexane extract demonstrated lower antioxidant activity, with an IC_{50} value of 70.42 ppm, placing it in the moderate antioxidant category. This indicates that as the concentration increased, the absorbance values decreased, whereas the inhibition percentage increased. These findings suggest that the 96% ethanol extract is more effective in scavenging free radicals than the ethyl acetate and n-hexane extracts.

Antioxidant Activity

The antioxidant activity of the tegining ganang leaf extracts was evaluated based on their ability to scavenge DPPH radicals. The results showed that the ethanol extract had the highest activity, followed by the ethyl acetate and n-hexane extracts. The IC_{50} values are summarized in **Table IV**.

Table IV. Values of Tegining Ganang Extracts

Extract	IC_{50} (ppm)	Antioxidant Category
Ethanol 96%	16,67	Strong
Ethyl Acetate	28,50	Strong
N-Hexana	70,42	Moderate
Quercetin (Reference)	7,8	Very Strong

The ethanol extract exhibited the strongest antioxidant activity, likely due to its higher phenolic and flavonoid content. The moderate activity of the n-hexane extract suggests that non-polar compounds in tegining ganang contribute less to antioxidant potential. These results align with previous research on plant-based antioxidants, where ethanol extracts generally yield higher antioxidant activity due to their ability to extract a broader range of phenolic compounds. Similar studies on Senna species have reported significant antioxidant properties, supporting the findings of the present study. These findings are consistent with those of previous studies. Plants containing phenolic compounds are an effective alternative for preventing free radical formation and protecting tissues from oxidative damage (Ibroham, 2020). Comer et al. Phenolic compounds in plant extracts significantly contribute to antioxidant activity (Husna, 2022). Senna species contain high levels of phenolic compounds and exhibit significant antioxidant and antibacterial activities, suggesting their potential as natural sources for drug development (Chefo Kengne et al., 2024).

CONCLUSION

Based on the research conducted, the leaf extract of tegining ganang (*Senna hirsuta* L) extracted using 96% ethanol and ethyl acetate solvents contains secondary metabolites such as alkaloids, flavonoids, tannins, and phenolics, which were not detected in the n-hexane extract. The antioxidant activity test using the DPPH method showed that the 96% ethanol extract had the highest antioxidant activity, with an IC_{50} value of 16.67 ppm, categorizing it as a strong antioxidant. The ethyl acetate extract also demonstrated good antioxidant activity, with an IC_{50} value of 28.50 ppm, and was classified as a strong antioxidant. The n-hexane extract exhibited moderate antioxidant activity with an IC_{50} value of 70.42 ppm. In comparison, quercetin showed very strong antioxidant activity with an IC_{50} value of 7.8 ppm. Therefore, it can be concluded that the 96% ethanol extract of tegining ganang leaves has the highest potential as a natural antioxidant source that can be further developed.

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