RESEARCH ARTICLE

Study of Total Phenols, Flavonoids, Antioxidant and Antibacterial Activities from *Dioscorea hispida* Tubers

Masfria[1], Syahputra H[1*], Samosir VC[2], Manalu E[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.*
[2]*Undergraduate Student, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia.*

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ABSTRACT

The tubers of *Dioscorea hispida* Dennst., a Dioscoreaceae plant, contain flavonoid molecules and plant secondary metabolites that are antioxidants and antibacterials. In this study, the total phenolic content and flavonoids from the ethanol extract and fraction (n-hexane and ethyl acetate) of *Dioscorea* tuber were investigated for their antioxidant and antibacterial properties. Fractionation with n-hexane and ethyl acetate followed by diffusion with 96% ethanol. The Folin-Ciocalteau reagent measured total phenol at 765 nm, and the $AlCl_3$ method measured total flavonoid at 440 nm using a visible spectrophotometer. The DPPH assessed the antioxidant activity, whereas the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was determined using agar diffusion on paper discs. The extract, n-hexane fraction, and ethyl acetate exhibited total phenolic content of 33.02, 5.95, and 156.33 mg GAE/g extract, respectively. Similarly, the total flavonoid content was measured at 20.26, 14.31, and 74.02 mg QE/g extract. The extract, n-hexane fraction, and ethyl acetate of the *Dioscorea* tuber exhibited minimum inhibitory concentrations (MICs) of 8.25, 7.76, and 10.30 mg/mL against *S. aureus* and 8.51, 8.31, and 12.05, against *E. coli,* respectively. The findings of this study indicate that the ethanol extract and n-hexane fraction derived from *Dioscorea* tubers exhibited comparatively diminished antioxidant and antibacterial properties in comparison to the ethyl acetate fraction. Furthermore, the ethyl acetate fraction exhibited elevated concentrations of total phenols and total flavonoids in comparison to the remaining fractions.

Keywords: Total phenolic, Flavonoid, Antioxidant, Antibacterial, *Dioscorea hispida* Dennst.

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INTRODUCTION

Cancer, heart disease, stroke, hypercholesterolemia, and diabetes are just a few of the degenerative illnesses that may be brought on by oxidative stress. A lack of antioxidants and excess free radical production are two general conditions that can cause oxidative stress. The phenomenon of oxidative stress occurs due to an imbalance between the body's antioxidant defense mechanism and the production of free radicals, which are commonly referred to as pro-oxidants.

Reactive oxygen species (ROS) and other free radicals are molecules, atoms, or groups whose outer shells contain one or more unpaired electrons, rendering them extremely reactive. The molecules most prevalent free radicals in biological systems within the body. However, there are many more forms. The majority of reactive oxygen species in the body come from the body's regular cellular metabolism (endogenous ROS), while a tiny percentage comes from exposure to radicals or other substances outside the body (exogenous ROS). Reactive oxygen that comes from pollution, radiation, bacterial, fungal, and viral diseases is what the body takes in. Reactive oxygen species can be mitigated and eliminated through the use of antioxidant agents and proactive external measures, specifically antibacterial treatments.[1]

Compounds that are antioxidants include flavonoids, polyphenols, coumarins, vitamin E, and beta-carotene. The antioxidant characteristics of phenolic and flavonoid compounds enable them to effectively mitigate the existence of free radicals by reducing the quantity of hydroxyl groups in their chemical composition.

Furthermore, flavonoid compounds possess antibacterial properties by releasing transduction energy to the bacterial cytoplasmic membrane and impeding bacterial motility. This is achieved through the hydroxyl groups present in the flavonoid structure, which induce alterations in organic components and nutrient transport, ultimately leading to toxic effects on bacteria. Therefore, there is a relationship between total phenol

**Author for Correspondence: hafid@usu.ac.id*

and flavonoids on antioxidant and antibacterial activity, in which flavonoids are found in many plants, one of which is the tuber of *Dioscorea*.[2]

Dioscorea tuber, a variety of tuberous plants, thrives in both natural forest and cultivated plantation settings. The tubers of *hispida* are utilized as supplementary food in Indonesia through a straightforward processing method. The community utilizes the plant for various medicinal purposes, such as managing leprosy, wound healing, fever reduction, anti-rheumatic effects, expectorant properties, and alleviating menstrual pain. The sap has been employed for the treatment of snake bites. *Dioscorea* tubers are known to contain bioactive compounds, including diosgenin, dioscorine alkaloids, furanoid diterpenes, and tannins, which have significant medicinal properties. In addition, *Dioscorea* tubers contain several minerals.[3]

The objective of the investigations was to examine the ethanol extract of *Dioscorea* tuber and its fractions in terms of their overall phenolic and flavonoid content, as well as their antioxidant and antibacterial characteristics, as described before. The UV-vis spectrophotometry method was employed to determine the total phenols and flavonoids. The Folin-Ciocalteau reagent and gallic acid were utilized as references for the total phenol test. In contrast, the $AlCl_3$ and quercetin reagents were employed as references for the total flavonoids test. The DPPH free radical entrapment method was employed for the antioxidant test. In contrast, the agar diffusion method with a paper scavenger was utilized for the antibacterial test against *S. aureus* and *E. coli* bacteria.

MATERIALS AND METHODS

Materials

The research utilized high-quality laboratory glassware manufactured by Iwaki and Pyrex such as petri dishes, flow funnels, incubators, callipers, loop needles, cameras, parchment, spare paper, micropipette (Eppendorf), UV-vis spectrophotometer (Shimadzu UV-1800), autoclave (Fisons), laminar air flow cabinet (Astec HLF 1200L), rotary evaporator (Stuart), water bath incubator (Memmert), electric oven (Fisher), bath water (Yenaco). The high analytical grade is used for all solvents, standards, and reagents. The process of manufacturing is under consideration. The chemical compounds used in this research include DPPH, ascorbic acid, gallic acid, and Folin-Ciocalteu reagent from Smart Lab and E-Merck Production: methanol, 96% ethanol, n-hexane, ethyl acetate, toluene, chloroform, isopropanol, benzene, acetic acid anhydrite, amyl alcohol, α-naphthol, iodine, mercury (II) chloride, nutrient broth, and nutrient agar.

Preparations and Characterization of Simplicia

Tubers of *D. hispida* Dennst from the Pagar Merbu District of the Deli Serdang Regency in the province of Sumatera were utilized as the raw material. Drying using a drying cabinet at ± 40ºC. Subsequently, the tubers are subjected to a blending process, and the resulting product is stored in a securely sealed plastic container. The Simplicia characterization analysis involved doing various determinations, such as quantifying total ash, acid-insoluble ash, water content, ethanol-soluble extract content, and total ash content. The simplicia and ethanol extract of *Dioscorea* tuber were subjected to phytochemical screening, which involved the analysis of several compounds such as alkaloids, glycosides, saponins, tannins, flavonoids, triterpenoids, and steroids.

Preparation of the Extract and Fractionation

The percolation method was employed to prepare the ethanol extract of *Dioscorea* tubers, followed by fractionation using the liquid-liquid extraction method involving n-hexane, ethyl acetate, and water. The concentrated extract was obtained and the fraction was evaporated.[3]

Determination of Total Phenolic and Flavonoid Content

The amount of 25 mg of sample was precisely measured and dissolved in methanol. After that, pipette 1-mL into 2.5 mL aquades and 2.5 mL Folin-Ciocalteau reagent. After allowing it to rest for 5 minutes, 2 mL of Na_2CO_3 was introduced into the mixture and agitated using a vortex. The solution was thereafter incubated for 15 minutes at a temperature of 45ºC and then measured at a wavelength of 765 nm. A calibration curve was established by employing gallic acid at values ranging from 50 to 250 g/mL.[4]

Determining the aggregate flavonoid content present in *Dioscorea* tubers was conducted through a colorimetric method involving the utilization of aluminum chloride. About 2 mL sample solution was put into each pipette, then $AlCl_3$, CH_3COONa, and distilled water. Next, it was incubated for 40 minutes and measured at 440 nm. The calibration curve was determined using quercetin. The quantification was conducted by expressing the overall flavonoid content as milligrams of quercetin equivalent per gram of sample [4].

Determination of Antioxidant Activity

The maximum absorption of a DPPH solution was measured as a control, utilizing a concentration of 40 g/mL in methanol through UV-visible spectrophotometry. The measurements indicate that the highest absorption of DPPH in methanol occurs at a wavelength of 516 nm. Sample concentrations ranging from 20 to 100 g/mL were prepared. In each flask, 1.0 mL of 1 mM DPPH was introduced and diluted with methanol until the designated line was reached. The solution underwent incubation for 30 minutes and was measured at 516 nm [4].

The radical scavenging activity was calculated using the following equation:

$$\text{Scavenging ability (\%)} = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \times 100$$

The positive control was prepared to utilize ascorbic acid. The experiment was conducted in triplicate. Using geometric methods, the IC_{50} values were determined by plotting the inhibition percentage against extract concentrations 20 to 100 g/mL.

Antibacterial Activity

The extract and fraction under investigation were evaluated for their antibacterial properties against *S. aureus* and *E. coli*. The experiment involved the transfer of inoculum into a petri dish containing nutrient agar media. Subsequently, a paper plate was subjected to various concentrations of test solutions (350, 200, 100, 75, 50, and 25 mg/mL) and DMSO as a blank. The specimen was thereafter subjected to incubation at 37°C for 21 hours. Subsequently, the vernier caliper was employed to quantify the extent of inhibition surrounding the paper disk.

RESULTS AND DISCUSSION

Characterization and Phytochemical Screening

The plant was identified at the Herbarium Medanense (2018), University of North Sumatra, which said that the plant used was *D. hispida* Dennst from the family Dioscoreaceae. The characterization of simplicia and phytochemical screening of alkaloids, flavonoids, saponins, and tannins in both simplicia and the extract of *Dioscorea* tuber, see Tables 1 and 2.

The water content of the *Dioscorea* tuber simplicia was determined to be 7.93%. When considering the standards of the simplicia moisture content, it falls within the specified limit of not exceeding 10%. Excessive water content can cause easy microbial growth and hydrolysis of active compounds in simplicia. The simplicia and ethanol extract of *Dioscorea* tuber contains phytochemicals, including alkaloids, glycosides, flavonoids, saponins, steroids and tannins, which are potential sources of biopharmaceuticals to be developed as modern medicinal plants in human life because has potential as an antioxidant and antibacterial.[5]

Extraction and Fractionation

The percolation method was used to extract 600 g of *Dioscorea* tuber simplicia using 96% ethanol solvent, resulting in 24.06 g of *Dioscorea* tuber ethanol extract with a yield percentage of 4.01%. then fractionated to produce 2.51 g of n-hexane, 1.01 g of ethyl acetate and 1.28 g of water fraction.

Analysis of Total Phenol and Flavonoid Content

The linear equation y = 0.0026x + 0.5752 was derived when comparing the total phenol content sample to gallic acid, with a regression coefficient of 0.9977 while the total phenol value had a different value, where the highest concentration was in the ethyl acetate fraction of 156.33 mg GAE/g sample, then the ethanol extract 33.02 mg GAE/g sample, and finally the n-hexane fraction 5.95 mg GAE/g sample. Table 3 displays the available data.

Table 1: Characterization results of *Dioscorea* tuber simplicial

No.	*Characterization*	*Simplicia (%)*
1	Water content	7.93
2	Water soluble essence	13.02
3	Ethanol soluble essence	9.26
4	Ash total	3.45
5	acid-insoluble ash	0.31

Table 2: Results of the *Dioscorea* tuber phytochemical screening examination

No.	*Compound class*	*Simplicia*	*Ethanol extract*
1	Triterpenoids/Steroids	+	+
2	Alkaloids	+	+
3	Flavonoids	+	+
4	Glycosides	+	+
5	Saponins	+	+
6	tannins	+	+

(+) positive = contains a group of compounds

Table 3: Total phenolic and flavonoids

No.	*Sample*	*Total phenol (mg GAE/g sample)*	*Total Flavonoids (mg QE/g sample)*
1	Ethanol Extract	33.02	20.26
2	n-hexane fraction	5.95	14.31
3	Ethyl acetate fraction	156.33	74.02

The flavonoid levels were determined using the comparator quercetin, which yielded a linear equation y = 0.0241x-0.0078 with a regression coefficient of 0.9985. Testing the levels of flavonoids in the sample solution reacted with $AlCl_3$ reagent will produce a yellowish solution due to the formation of a flavonoid-$AlCl_3$ complex, but the sample gives a different color to quercetin due to the presence of other compounds that affect the intense yellow color. The results of the measurement revealed that the ethyl acetate fraction displayed the highest concentration of 74.02 mg QE/g sample, the ethanol extract followed by 20.26 mg QE/g sample, and the n-hexane fraction had a concentration of 14.31 mg QE/g sample. The relevant data is shown in Table 3.

The maximum yield of total phenol and flavonoid content was observed in the ethyl acetate fraction of *Dioscorea* tubers. This can be attributed to the existence of polyphenolic compounds in the ethyl acetate fraction. In contrast, the ethanol extract demonstrated the presence of primary metabolites that possess the capacity to impact the results of the reaction upon the introduction of reagents. Variations in solvent polarity can influence the composition and nature of phenolic compounds extracted, leading to the selective extraction of specific compounds in each solvent employed. The ethyl acetate fraction is believed to contain a polyphenol group with similar polarity to ethyl acetate solvents, such as xanthones and flavonoids, resulting in high levels of total phenol and total flavonoids. Phenolic compounds correlate with the antioxidant activity of a sample. Generally, with an increase in phenolic compounds (simple phenols or polyphenols), the antioxidant activity will also be high. The effective acquisition of hydrogen atoms from phenolic hydroxyl groups by free radicals, which act as donors of hydrogen protons, is responsible for the antioxidant actions of phenolic compounds.

Moreover, the antioxidant activity is influenced by the presence of double bonds and carbonyl groups situated within the heterocyclic ring of the core structure. The stabilization of phenolic radicals through electron conjugation and delocalization can boost antioxidant activity.[6]

Results of Analysis of Antioxidant Activity

The findings from the examination of the ability of extracts and fractions to scavenge free radicals (Table 4) while the results of the analysis of standard ascorbic acid (positive control) data from the analysis of free radical scavenging can be seen in Table 5. The findings from Tables 4 and 5 indicate that *Dioscorea* tuber extract, fraction, and ascorbic acid exhibit free radical inhibition properties. Specifically, the results demonstrate that higher concentrations of the extract, fraction, and ascorbic acid correspond to increased DPPH inhibition activity. This suggests that a greater number of hydrogen atoms are paired with electrons in the radicals. The DPPH-free state results in a reduction in absorption.[7]

Increase in percentage of DPPH free radical inhibition with each subsequent increase in concentration. The rise in the proportion of DPPH inhibition indicates the existence of antioxidant properties in both the ethanol extract and the fraction of *Dioscorea* tuber. The neutralization of DPPH free radicals can be achieved through the interaction of antioxidants with DPPH, which involves either electron transfer or hydrogen donation. The transition of the DPPH free radical from a state of unpaired electrons to a state of paired electrons results in a discernible alteration in the color of the solution, shifting from a dark purple hue to a vivid yellow tint. This alteration can be quantified through stoichiometric means, whereby the quantity of electrons or hydrogen atoms assimilated by the DPPH molecule indicates the presence of antioxidants.[7]

The IC_{50} value of the ethyl acetate fraction is 35.51 µg/mL, indicating a robust antioxidant activity. The difference in IC_{50} values in each sample is due to the distribution of the type and number of secondary metabolite groups that act as antioxidants based on the polarity of the solvent used.[8] The results of the IC_{50} value can be seen in Table 6.

Table 4: Results of free radical scavenging analysis by *Dioscorea* tuber extracts and fractions

Concentration (µg/mL)	*% Inhibition*		
	Ethanol extract	*n-Hexane fraction*	*Ethyl acetate fraction*
20	32.51	10.45	47.02
40	37.71	17.28	64.48
60	43.27	23.94	82.92
80	51.95	30.08	88.37
100	58.02	40.06	91.12

Table 5: Results of analysis of free radical inhibition by ascorbic acid

Concentration (µg/mL)	*%Inhibition*
2	25.71
4	45.61
6	67.67
8	89.76

Table 6: Results of IC_{50} values

No.	*Sample solution*	*IC_{50} (µg/mL)*
1	Ethanol extract	74.98
2	n-hexane fraction	128.15
3	Ethylacetate fraction	35.51
4	Ascorbic acid	4.45

The antioxidant activity comes from the phytochemical compounds contained in extracts and fractions from *Dioscorea* tubers, based on the literature that ethyl acetate attracts semi-polar compounds so that it attracts many components of bioactive compounds such as flavonoids, which flavonoids have antioxidant properties. Flavonoid and phenolic compounds that contain free hydroxyl groups in extracts demonstrate the ability to scavenge radicals and hinder the formation of new free radicals by disrupting chain reactions and transforming them into more stable substances. In contrast, the n-hexane fraction exclusively exhibits an affinity for non-polar molecules, including steroids.[7]

According to the strength category's IC_{50} value, the ethyl acetate fraction of *Dioscorea* tubers and ascorbic acid exhibit very strong antioxidant activity, with the former having an IC_{50} value of less than 50 µg/mL (Table 6). This discrepancy can be attributed to ascorbic acid being a pure compound, whereas the ethyl acetate fraction contains multiple flavonoid and phenolic compounds. Tannins are a class of compounds known for their antioxidant properties. The antioxidant activity of flavonoids, phenolics and tannins is because these three compounds are phenolic compounds, namely compounds with –OH groups attached to aromatic carbons, which are strong in capturing free radicals. The antioxidant activity generated by phenol and flavonoid compounds positively correlates with the number of hydroxyl groups present. Based on the analysis of their structural composition and the number of hydroxyl groups, the hierarchy of antioxidant potency can be arranged in descending order: tannins, flavonoids, and phenolics. Phenol compounds can donate hydrogen atoms, reducing the DPPH radical to a more stable form. The tubers of *Dioscorea* also contain diosgenin compounds. Diosgenin, a steroid saponin, regulates blood glucose levels and exhibits antioxidant properties.

Antibacterial Activity Analysis

The results of the antibacterial activity evaluation performed on *S. aureus* and *E. coli* bacteria are presented in Table 7.

The sequence of extracts and fractions that exhibited the highest level of antibacterial activity were identified as the ethanol extract and fraction. The results of measuring the diameter of the inhibition zone formed in *S. aureus* bacteria are larger than those in *E. coli* bacteria. The differences in the cell wall components of the two bacteria can affect the work of the *Dioscorea* tuber extract as an antibacterial. Antibacterial agents have been observed to exhibit greater efficacy against gram-positive bacteria. The relatively uncomplicated structure

Table 7: Measurement of the inhibition area's diameter (mm)* average bacterial growth

No.	*Concentration (mg/mL)*	*Staphylococcus aureus*			*Escherichia coli*		
		Ethanol extract	*n-Hexane fraction*	*Ethyl acetate fraction*	*Ethanol extract*	*n-hexane fraction*	*Ethyl acetate fraction*
1	350	8.25	7.76	10.3	8.51	8.31	12.05
2	200	7.75	7.38	10.05	7.75	7.50	11.40
3	100	7.65	7.13	9.45	7.28	6.78	9.15
4	75	7.08	6.78	9.05	6.16	6.21	9.00
5	50	6.78	6.40	9.45	6.13	6.11	8.35
6	25	-	6.30	9.00	-	-	6.40
7	Blank DMSO	-	-	-	-	-	-

of the gram-positive cell wall facilitates the penetration of antibacterial agents into the cell and their subsequent interaction with specific targets. The ethanol extract exhibited a comparatively reduced level of antibacterial activity in comparison to the ethyl acetate fraction.[9]

It was discovered that the tubers belonging to the *Dioscorea* species exhibited a substantial concentration of polyphenolic compounds. *Dioscorea* tubers and other components contain diverse phenolic compounds that could account for their antimicrobial properties.[10]

The ethyl acetate fraction exhibited a more significant total phenol content than the total flavonoids. This disparity can be attributed to the fact that the elevated phenol content in the ethyl acetate fraction did not solely comprise flavonoid compounds. The ethyl acetate fraction of *Dioscorea* tuber demonstrated the most significant antioxidant and antibacterial activity when compared to the ethanol extract and n-hexane fraction. Furthermore, a strong correlation was identified between the overall phenol and total flavonoid levels and the aforementioned fraction. Specifically, the total phenol content was measured at 156.33 mg GAE/g extract, while the total flavonoid content was measured at 74.02 mg QE/g extract.

CONCLUSION

The present research showed that the ethanol extract and n-hexane fraction obtained from the *Dioscorea* tuber exhibited somewhat lower levels of antioxidant and antibacterial activity compared to the ethyl acetate fraction. Furthermore, it was observed that the ethyl acetate fraction displayed elevated concentrations of total phenol and total flavonoid content, with measurements of 156.33 mg GAE/G extract and 74.02 mg QE/G extract, respectively.

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