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DETERMINATION OF TOTAL PHYTOCHEMICAL COMPOUNDS FROM ETHANOL EXTRACT NANGKA (*Artocarpus heterophyllus* Lam.) LEAVES AND ANTIOXIDANT ACTIVITY FROM NORTH SUMATERA, INDONESIA

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ABSTRACT

Nangka (*Artocarpus heterophyllus* Lam.) has various nutritional contents and abundant bioactive compounds. Potential bioactive compounds including phenolic and flavonoid categorized as antioxidants. This study aimed to determine the levels of phenolics and flavonoids and to test their potential activity as antioxidants from the ethanol extract of nangka leaves. The extraction process used ethanol by maceration method, colorimetric determination of phenolic, flavonoid, and tannins content, and determination of antioxidant activity by 2,2-dipicryl -1-hydrazil (DPPH) method using UV-Vis spectrophotometry. Determination of phenolic content was measured at a maximum wavelength of 765 nm with gallic acid as the standard, flavonoid content was measured at a maximum wavelength of 431 nm with quercetin as standard, tannins content was measured at a maximum wavelength of 745 nm with tannic acid as standard and the determination of antioxidant activity at a maximum wavelength of 517 nm with positive control of vitamin C. The results showed that the ethanolic extract of nangka leaves has phenolic content with a total phenolic content of 27.654±0.054 mg GAE/g d.w ethanolic, flavonoid content of 2.978 ±0.192 mg QE/g d.w ethanolic, tannins content 0.46 ± 0.017 mg TAE/g d.w ethanolic extract and antioxidant activity (IC_{50}) 25.561±0.055 ppm which categorized as a very strong antioxidant

Keywords: Antioxidant, DPPH, Flavonoid, Nangka Leaves, Phenolic, and Tannins

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INTRODUCTION

Determination of the levels of various groups of bioactive compounds and activities contained in a plant is the center of attention of researchers in developing their potential as herbal medicines based on natural ingredients.[1] Since ancient times the use of plants for various treatments have been used. Groups of bioactive compounds that are often studied are phenolic groups, flavonoids, and tannins which are considered to provide activity with various benefits.[2,3] The content of these bioactive compounds is considered to have an important role in health and is able to reduce and minimize the adverse effects caused by free radicals. Free radicals are highly reactive species that can induce oxidative reactions in molecules such as carbohydrates, lipids, and proteins, and trigger the emergence of various diseases.[4] Various potential activities reported from the content of these secondary metabolites include anticancer, obesity prevention, antidiabetic anticarcinogenic, antimutagenic, anti-inflammatory, antiviral, antimicrobial, antimutagenic, antitumor, and many more.[5] Free radicals are atomic species or reactive compounds that cause oxidative reactions and cause damage to cells and organs, proteins, lipids, and DNA in the body.[6] The damage caused by free radicals causes various diseases such as atherosclerosis, Chronic Obstructive Pulmonary Disease (COPD), Alzheimer's disease and cancer,[7,8] premature aging, cancer, liver disorders, neurodegenerative

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diseases, and kidney disease.[9] To stop the effects and prevent the occurrence of various diseases caused by free radicals, antioxidants are needed. Antioxidants protect the body from the effects and dangers of free radicals.[10] Antioxidants can be obtained from within (endogenous) and from outside (exogenous) the body. Endogenous antioxidants are produced from the work of enzymatic metabolism in the body, while exogenous antioxidants are obtained from outside the body such as vitamins C, A, and E, carotenoids, xanthophylls, flavonoids, phenolics, and polyphenols. One of the plants whose content is determined by the total content of bioactive compounds (phenolic, flavonoid, tannin) and antioxidant activity is nangka (*Artocarpus heterophyllus* Lam.) leaves.

EXPERIMENTAL

Sample Preparation

Nangka (*Artocarpus heterophyllus* Lam.) leaves were obtained from Rumamis village, Barus Jahe sub-district, Karo district, Sumatra province, Indonesia. The samples used were fresh leaves in good condition from plants that had already borne fruit. Taxonomic tests on samples were carried out by botanists at the Herbanennse Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara (No. 5109/MEDA/2020). The sample was cleaned in running water to remove impurities, then dried in a sample drying cabinet at a temperature of 50^{o}C. The dry sample was powdered to obtain Simplicia powder and stored in the pharmacognosy laboratory before use. The purpose of pollination was to increase the surface area of the sample that touched the solvent so that the extraction process would be more optimal.[11]

Sample Extraction Process

500 g Simplicia powder of nangka (*Artocarpus heterophyllus* Lam.) leaves was extracted using ethanol (98%) with maceration method for 3 days. Samples that have been immersed in the macerator container are occasionally stirred to maximize the process of extracting the bioactive content. After reaching it, it was filtered using Whatman paper No. 1, so that the ethanol extract of nangka leaves was obtained. The extract obtained was concentrated using a rotary vacuum evaporator at medium speed at a temperature of 50^{o}C.[12]

Phytochemical Screening Compounds

The thick extract was screened for phytochemicals to identify groups of phenolic, flavonoid, and tannins compounds using standard reagents.[6,11,12]

Determination of Total Phenolic Compounds

Determination of total phenolic compounds was carried out by colorimetry using the Folin-Ciocalteu reagent and Na_2CO_3 solution (7%) that was measured using UV-Vis spectrophotometry at a modified maximum wavelength of 765 nm. Gallic acid solution is used as the standard solution with various concentrations of 25 ppm, 50 ppm, 75 ppm, 125 ppm, and 200 ppm with methanol solvent (p.a.). Briefly, the procedure carried out was to take 0.2 mL of gallic acid from each concentration and then add 1 mL of Folin-Ciocalteu and leave it for 5 minutes. Following the addition of 4 mL of Na_2CO_3 solution (7%), each of them was filled to the mark of 10 mL with distilled water. The solution was incubated for 30 minutes, and then the absorbance was measured at a maximum wavelength of 765 nm.[13,14,15] The linear equation obtained is $y = 0.0037x + 0.0084$; $R^2 = 0.9501$. The determination of phenolic content in the sample followed the same steps as a concentration of 1000 ppm ethanol extract of nangka leaves. Phenolic content is expressed in mg of gallic acid equivalent per gram of ethanol extract (mg GAE/g d.w. ethanolic extract).

Determination of Total Flavonoid Compounds

The determination of total flavonoid compounds was carried out by colorimetry using the reagent $AlCl_3$ at 10% and measured using UV-Vis spectrophotometry at a modified maximum wavelength of 431 nm. Quercetin solution as a standard solution with concentrations of 25 µg/mL, 50 µg/mL, 75 µg/mL, 125 µg/mL, and 200 µg/mL in methanol (p.a.). Briefly, the procedure carried out was to take 1 mL of quercetin from each concentration, add 0.2 mL of $AlCl_3$ 10%, and then add distilled water to each of the 10 mL marks. The solution was incubated for 30 minutes, and then the absorbance was measured at a maximum wavelength of 431 nm.[14,15,16] The linear equation obtained is $y = 0.0006x + 0.0044$; $R^2 = 0.9411$. The determination of flavonoid levels in the sample followed the same steps as a concentration of 1000 ppm ethanol extract of nangka leaves. Flavonoid content is expressed in mg of quercetin equivalent per gram of ethanol extract (mg QE/g d.w. ethanolic extract).

Determination of Total Tannin Compounds

Determination of total tannin compounds was carried out colorimetrically using Folin Ciocalteu reagent, saturated Na_2CO_3, and measured using UV-Vis spectrophotometry at a modified maximum wavelength of 745 nm. The standard solution used in the determination of total tannins is tannic acid, with a concentration variation of 0.4, 1.0, 1.6, 2.0, 4.0, 8.0, 12.0, and 14.0 ppm. In the modified procedure[6], each concentration of the standard solution was taken in 200 L, and then 200 L of Folin-Ciocalteu reagent was added, followed by 5 minutes of standing. Then add 100 µL of saturated Na_2CO_3 and add distilled water up to 5 mL. The mixture was incubated for 35 minutes, and the absorbance was measured at 745 nm. The linear equation obtained is $y = 0.0694x + 0.0334$; $R^2 = 0.9989$. The determination of tannin levels in the sample followed the same steps as a concentration of 1000 ppm ethanol extract of nangka leaves. The tannin content was expressed as mg of tannic acid equivalent per gram by weight of the dry sample used (mg TAE/g d.w. ethanolic extract).

Determination of Antioxidant Activity

The antioxidant activity of an ethanol extract of leaves was determined using a 1000 ppm concentration as the mother liquor. Then, the concentration was varied to 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. Each concentration was taken at 1 mL and then added to 1 mL of DPPH (0.4 mM) and ethanol (p.a.) until it reached 5 mL. Samples were incubated for 30 minutes, after which the absorbance was measured using UV-Vis spectrophotometry at a maximum wavelength of 517 nm. Measurements were replicated three times.[16,17,18]

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The results of qualitative phytochemical screening of an ethanol extract of nangka leaves were positive for phenolic, flavonoid, and tannin compounds (Table-1). This information is also the basis for the quantitative determination of phenolic, flavonoid, and tannin content using spectrophotometric methods.

Table-1: Phytochemical Screening of an Ethanol Extract of Leaves

Phytochemical compounds	Reagent	Results
Phenolic	$FeCl_3$ 5%	+
Flavonoid	Shinoda test	+
Tannins	$FeCl_3$ 1% (aqua dest)	+

Total Phenolic, Flavonoid, and Tannin Content

Determination of the total levels of phytochemical components of the ethanolic extract of nangka leaves for phenolics, flavonoids, and tannins by colorimetric method using UV-Vis spectrophotometry at the maximum wavelength (λ_{max}) of each standard. Determination of total phenolic using gallic acid as a standard was measured at λ_{max} 765 nm and obtained a total content of 27.654 ± 0.054 mg GAE/g d.w ethanol extract. Determination of flavonoids using quercetin as a standard was measured at λ_{max} 431 nm and obtained a total content of 2.978 ± 0.192 mg QE/g d.w ethanolic extract. Determination of tannins using tannic acid as a standard was measured at λ_{max} 745 nm and obtained a total content of 0.46 ± 0.017 mg TAE/g d.w ethanolic extract. Phenolic compounds are components of secondary metabolites that are abundant in plants and are divided into two parts, namely the simple phenolic group and the polyphenol group. The simple phenolic groups include phenolic acids and coumarins, while the polyphenol groups are flavonoids, stilbenes, lignans, and tannins. These two groups are produced from the shikimate pathway and acetic acid in the biosynthesis process.[19]

This content is contained in the ethanolic extract of nangka leaves which is responsible for providing activity as a natural antioxidant in deactivating free radicals. The ability of phenolic compounds and flavonoids in deactivating free radicals is due to the presence of a hydroxyl group attached to phenol which has the ability to release proton radicals (hydrogen) and form stable phenoxyl radicals.[20] In addition, the class of tannin compounds is reported to have strong activity in deactivating free radicals as the molecular weight increases. This is because the tannins also have hydroxyl groups in their compounds, but when the hydroxyl groups are dimethoxylated or glycosylated, they reduce their activity in deactivating free

radicals.[21] The phytochemical content obtained from nangka leaves was supported by the ability of the ethanol solvent to extract.

Antioxidant Activity Test

Testing of antioxidant activity using the DPPH method since this method is relatively easier, cheaper, faster, and has a better sensitivity level compared to other methods.[22] The antioxidant activity value of the ethanol extract of nangka leaves is shown in Fig.-1.

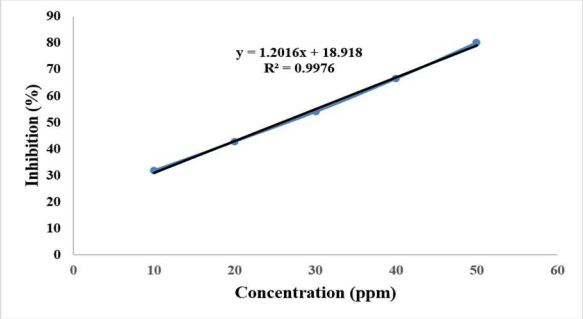

Fig.-1: The Results of Determining the Antioxidant Activity of the Ethanol Extract of Nangka Leaves

The antioxidant activity value (IC_{50}) of nangka leaves ethanol extract was 25.867±0.055 ppm. The IC_{50} value obtained is in line with the presence of phenolic, flavonoid, and tannin content in the extract, which shows a positive singularity as an antioxidant.

CONCLUSION

The ethanolic extract of nangka leaves contain phenolic bioactive compounds with a total content of 27.654 ± 0.054 mg GAE/g dry weight ethanolic; flavonoids with a total content of 2.978 ± 0.192 mg QE/g dry weight ethanolic; tannins with a total content of 0.46 ± 0.017 mg TAE/g dry weight ethanolic extract; and antioxidant activity (IC_{50}) of 25.867 ± 0.055 ppm.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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