Antioxidant Potential Of Ethanol Extract Of Kendondong Leaves (*Spondias Dulcis*), Characterization And Examination Of Quercetin By TLC Method

Cucu Arum Dwi Cahya[1*], Urip Harahap[2], M. Pandapotan Nasution[3]

[1]Postgraduate Programs, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
[2]Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia
[*]Corresponding Author:
Email: urip@usu.ac.id

Abstract.

Indonesia has a variety of plants that can be used to cure various diseases. Natural therapies offer little negative effect, especially in degenerative diseases, due to decreased function of body cells damaged by oxidation. Antioxidants have a vital role in counteracting free radicals to prevent degenerative diseases. One of the most abundant sources of antioxidant compounds is flavonoids. Kedondong leaf (Spondias dulcis) is one of the plants which is also suspected to contain flavonoids. Spondias species contain triterpenoids, namely oleanolic acid and flavonoids such as quercetin, kaempferol, kaempferida and rhamnetin. Examination of simplicia characteristics, phytochemical screening, examination of quercetin with Thin Layer Chromatography, and determination of antioxidant activity and IC50 value of Kedondong leaves using the DPPH method were carried out in this study. The results of the characteristic examination showed that simplicia met the requirements with a water content of 8.63%, a water-soluble extract content of 19.42%, an ethanol-soluble extract content of 5.17%, a total ash content of 12.43%, an acid-insoluble ash content of 7 .81%. Phytochemical screening showed that the bright kedondong leaves contained flavonoids, alkaloids, glycosides, saponins, tannins, and steroids/triterpenes. Examination of quercetin thin layer chromatography with chloroform: methanol (70: 30) as eluent produced six stains with Rf values (0.5; 1; 1.2; 3; 4.3; 6.6), and compared with quercetin raw material on the sixth stain with an Rf value of 6.6 has the same stain as the ethanol extract of kendondong leaves. The results of antioxidant testing at a 400 – 800 nm wavelength using UV-Vis spectrophotometry obtained a maximum wavelength of 515 nm. IC50 value obtained an IC50 value of 68.6260 ppm, so it can be concluded that the ethanol extract of kendondong leaves has antioxidant activity in the strong category.

Keywords: *Antioxidant, Characterization, Kendondong Leaves, Spondias dulcis, Quercetin, and TLC Method*

I. INTRODUCTION

Indonesia is a tropical country that has a variety of plants that can be used to cure various diseases [1, 2]. The use of natural ingredients as medicine (biopharmaceuticals) has expanded due to the back-to-nature approach adopted by many people and the economic crisis, reducing people's purchasing power for expensive contemporary medicines. Natural therapies offer few adverse effects, especially in degenerative diseases [3]. Degenerative disease is caused by a decrease in the function of human body cells, which can be damaged due to oxidation [4]. Antioxidants have a vital role in body health. Therefore, free radicals are needed, namely antioxidants that can counteract free radicals to prevent degenerative diseases [5]. One of the most abundant sources of antioxidant compounds is flavonoids [6]. Plants that contain flavonoid compounds usually have the property to treat various degenerative diseases such as stroke, rheumatism, heart disease, and cancer [4].

One plant also suspected of containing flavonoids is kedondong leaves (*Spondias dulcis*). According to research, Spondias species contain triterpenoids, namely oleanolic acid and flavonoids such as quercetin, kaempferol, kaempferida and rhamnetin [7]. Pharmacognostic characterization of the ethanol extract of kedondong leaf bark showed the presence of flavonoids and phenolic acids in its constitution. Due to their chemical characteristics, phenolic acids usually can cause primary prevention that inhibits mutation and cancer initiation in extracellular and intracellular media, prevents the uptake of mutagens and carcinogens, and maintains DNA structure [8]. Other studies say that kedondong leaf extract has the potential to be applied as a therapy in the management of TB and MDR-TB [9]. Kedondong leaf extract also shows that the ethanol extract of the stem from kedondong can be helpful as a compound to prevent DNA damage due to mutagenic agents cyclophosphamide and benzopyrene [10]. Kedondong leaves also have the potential to heal burns and have the potential as an anti-bacterial [11, 12].

II. METHODS

Tools and Materials

The tools used were laboratory glassware, drying cabinets, centrifugal rotary evaporator tools, cover glass, parchment paper, crumpets, mortar and tamper, a furnace, a blender, and aluminium foil. The material used is kedondong leaves identified at the Medanense Herbarium (MEDA) Botany Field, University of North Sumatra. The chemicals used were Bouchard at reagent, dragendroff, Mayer, iron (III) chloride, molisch, lead (II) acetate, sulfuric acid, hydrochloric acid, methanol, chloroform-isopropanol, 96% ethanol n-hexane, toluene, chloroform, powder, Magnesium, zinc powder.

Examination of Simplicia Characteristics

Kedondong leaves collected are washed thoroughly with running water, drained than in wet sorting. This material is then dried in a cabinet until dry, sorted and weighed. The material is then mashed using a blender. Simplisia is put in a plastic container, tied, labelled, and then stored in a place protected from sunlight [13]. Examination of simplicia properties includes determination of water content, determination of water-soluble extract content, determination of ethanol-soluble extract content, determination of total ash content, and determination of acid-insoluble ash content [13].

Phytochemical Screening

The phytochemical screening of kedondong leaf simplicia powder includes an examination of compounds belonging to the class of flavonoids, alkaloids, saponins, tannins, glycosides, and steroids/triterpenoids.

Extraction

Making the extract was carried out by maceration using 96% ethanol solvent. Put one part of the simplicia dry powder into the macerator, and add ten parts of the solvent. Soak for the first 6 hours while stirring occasionally, then let stand for 18 hours. Separate macerate by filtration. Repeat the screening process at least once with the same type of solvent, and the total volume of the solvent is half the amount of solvent in the first screening. Collect all the macerate, then vaporize it with a vacuum vaporizer or low-pressure vaporizer using a "rotavapor" until a thick extract is obtained [14].

Thin Layer Chromatography Test

Dab the test solution, the reference solution, and the mixture of the test solution and the reference solution, according to the method indicated in the individual monographs, 1.5 to 2 cm from the bottom edge of the plate, and allow to dry. Use a stencil to determine where to shoot and the compaction distance, and mark the creepage distance. Place the plate on the support rack so the spotting is on the bottom, and insert the rack into the chromatography vessel. Place the vessel lid and allow the mobile phase to propagate to the creepage distance limit. Remove the plate, dry it in the air, and observe the spot in visible light. Measure and record the distance of each spot from the spotting point and record the wavelength for each observed spot.

Determine the Rf value [14].

$$Rf = \frac{Distance\ Traveled\ by\ Substance}{Distance\ Traveled\ by\ Mobile\ Phase}$$

Determination of Antioxidant Activity

A 0.5 M DPPH solution (200 ppm) was prepared first by weighing 10 mg of DPPH powder dissolved in methanol up to 50 mL and obtaining a DPPH solution with a concentration of 200 ppm. Then measure the Maximum Absorption Wavelength of DPPH using a pipette of 1 mL of DPPH standard solution. put into a 5 mL volumetric flask. Then added, methanol up to the mark limit to obtain a solution with a concentration of 40 ppm. The maximum wavelength was measured using a UV-Vis spectrophotometer (400 – 800 nm) and obtained a Maximum Wavelength of 515 nm.

Preparation of Extract Test Solution

Weigh 10 mg of condensed extract and dissolve it with up to 10 mL of methanol. A solution with a concentration of 1000 ppm was obtained. Taken 0.25 mL; 0.5mL; 0.75mL; 1mL; 1.25 mL of the 1000 ppm extract solution. Then added, 1 ml of DPPH solution (200 ppm concentration) at each concentration and added with methanol up to the mark limit (5 mL volumetric flask) and obtained concentrations of 25, 50, 75,

100, and 125 ppm. Incubated for 30 minutes, the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 515 nm.

Determination of DPPH Free Radical Trapping Process

*D*etermination of the free radical entrapment process by the test sample using the DPPH free radical entrapment method, which is calculated using the following formula:

$$Immersion\ Activity\ (\%) = \frac{Abs.control - Abs.sample}{Abs.control} x100\%$$

Description: abs. control = Absorbance does not contain sample

abs sample = Absorbance of sample

IC_{50} Value Calculation

Calculation of the results of the DPPH trapping method is to calculate IC_{50}. This value indicates that plant extracts can cause attenuation of as much as 50% of DPPH activity. This can also be seen from the colour change of the test sample, which is deep purple. DPPH will turn yellowish when added if the extract has a damping effect. The calculation results are entered into the regression equation with sample concentration (ppm) as the abscissa (X-axis) and the per cent damping activity value as the ordinate (Y-axis). The level of antioxidant power can be seen in Table 1.

Table 1. Levels of Antioxidant Strength [15]

Antioxidant Intensity	IC_{50} Level
Very strong	<50 ppm
Strong	50 – 100 ppm
Currently	100 – 250 ppm
Weak	251 – 500 ppm

III. RESULT AND DISCUSSION

Examination Results of the Characteristics of Kedondong Leaves

The results of the simplicia characterization examination, namely examination of water content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content, can be seen in Table 2.

Table 2. Results of Characterization of Kedondong Leaf Simplicia

Parameter	Result
Ash content	12.43%
Acid Insoluble Ash Content	7.81%
Water Soluble Extract Content	19.42%
Ethanol Soluble Extract Content	5.17%
Moisture Content 8.63%	8.63%

Determination of the water content in the simplicia is carried out to determine the amount of water contained in the simplicia used. The results of determining the water content obtained <10%, namely 8.63%. Moisture content that exceeds the requirements of 10% can be a medium for microbial and fungal growth and encourages simplex damage [16, 17].Determination of water-soluble essence content was carried out using two solvents, namely water and ethanol. The determination of water-soluble and ethanol-soluble extracts aims to determine the concentration of the active compounds extracted in the invention from several simplicia powders. The water-soluble extract content of kedondong leaves is 19.42%, while the ethanol-soluble extract content is 5.17%.

Determination of total and acid-insoluble ash content aims to assure that the simplicia does not contain certain heavy metals exceeding the values set for plant simplicia, which can be harmful (toxic) to health. Determine the total ash content, which contains simple inorganic compounds such as Mg, Ca, Na, Zn and K. The ash content is insoluble in acids to determine the levels of inorganic compounds that are insoluble in silicic acid. Total ash is divided into two, namely physiological ash and non-physiological ash. Physiological ash is ash that comes from the plant tissue itself. In contrast, non-physiological ash is the residue after combustion from external materials found on the surface of the simplicia. The ash content of kedondong leaves is 12.43%, and the acid-insoluble ash content is 7.81%.

Results of Phytochemical Screening Examination of Kedondong Leaves

The results of the phytochemical screening examination of kedondong leaves (*Spondias dulcis*) include examination of alkaloid compounds, saponins, tannins, glycosides, and steroids/triterpenoids can be seen in Table 3.

Table 3. Results of Phytochemical Screening

Secondary Metabolites	Reactants	Result
Alkaloids	Dragendroff	+
	Bouchardat	+
	Meyer	+
Flavonoids	Mg Powder + Amyl Alcohol + HCl p	+
Glycosides	Molish+H_2SO_4	+
Saponins	Hot/shaken water	+
Tannins	$FeCl_3$	+
Triterpenes/Steroids	Lieberman-Bourchat	+

The results of the phytochemical examination of cheerful kedondong leaves contain flavonoids, alkaloids, glycosides, saponins, tannins, and steroids/triterpenes. Flavonoids are a class of naturally occurring aromatic compounds. Plant flavonoids can prevent free radicals from forming [18]. Spondias species contain triterpenoids, namely oleanolic acid and flavonoids such as quercetin, kaempferol, kaempferida and rhamnetin [7].

Thin Layer Chromatography Results

The results of the thin layer chromatography test with the eluent chloroform: methanol (70:30) compared to the raw material quercetin obtained six spots with Rf values (0.5; 1; 1.2; 3; 4.3; 6.6). Moreover, compared to the raw material quercetin at spot 6 with an Rf value of 6.6, the ethanol extract of kendondong leaves and quercetin have the same stain, which indicates that the ethanol extract of kendondong leaves contains quercetin. The results of the thin-layer chromatography test can be seen in Figure 1.

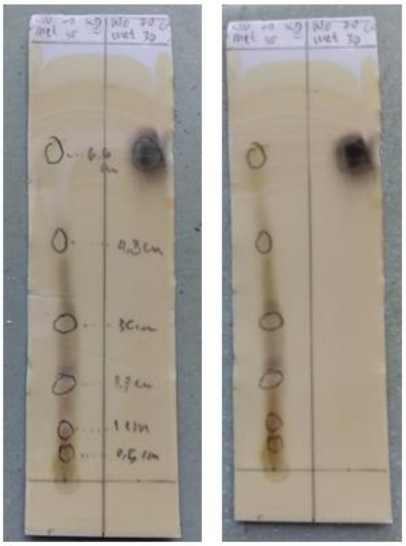

Fig 1. Thin Layer Chromatography Results

Antioxidant Test Results

Measurement of the absorption of the maximum wavelength of DPPH at a wavelength of 400 – 800 nm with UV-Vis spectrophotometry obtained a maximum wavelength of 515 nm (Figure 2). The results of measuring the IC_{50} value obtained the IC_{50} value of the kendondong leaf ethanol extract of 68.6260 ppm, and it has a strong category of antioxidant activity. Antioxidants have a vital role in the health of the human body because their function is to inhibit and neutralize the occurrence of oxidation reactions involving free radicals. Flavonoids can have an antioxidant effect by preventing the generation of ROS, directly capturing ROS or indirectly increasing enzymes [19].

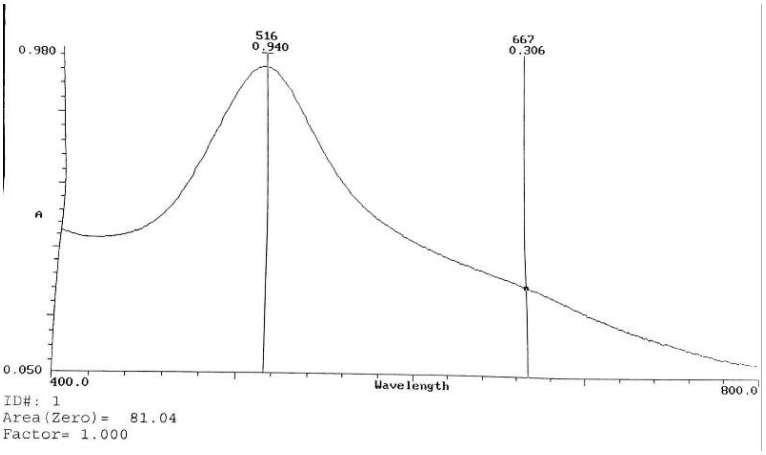

Fig 2. The results of the maximum absorption wavelength of DPPH at a wavelength of 400 – 800 nm with UV-Vis spectrophotometry

IV. CONCLUSION

Based on the discussion and research results, it was concluded that kedondong leaves (*Spondias dulcis*) contain quercetin, and the results of measuring the IC_{50} value obtained a value of 68.6260 ppm in the strong category.

REFERENCES

[1] Hidayat, D.; Hardiansyah, G. *Studi Keanekaragaman Jenis Tumbuhan Obat Di Kawasan IUPHHK PT. Sari Bumi Kusuma Camp Tontang Kabupaten Sintang*. ***J. Vokasi 2012, 8, 61–68***. [Google Scholar]

[2] Sitorus, P., Keliat, J. M., Asfianti, V., Muhammad, M., & Satria, D. (2022). *A Literature Review of Artocarpus lacucha Focusing on the Phytochemical Constituents and Pharmacological Properties of the Plant*. ***Molecules***, 27(20), 6940.

[3] Katili, A.S. *Inventarisasi Tumbuhan Obat Dan Kearifan Lokal Masyarakat Etnis Bune Dalam Memanfaatkan Tumbuhan Obat Di Pinogu, Kabupaten Bonebolango, Provinsi Gorontalo.* ***Pros. Sem. Nas. Masy. Biodiv. Indon. 2015, 1, 78–84. [Google Scholar] [CrossRef]***

[4] Hasanah, N., Dahlia, A. A., & Handayani, V. (2023). *Uji Aktivitas Antioksidan Ekstrak Daun Kedondong Laut (Nothopanax fructicosum (L.) Miq) Dengan Metode Peredaman Radikal Bebas DPPH.* ***Makassar Natural Product Journal*** (MNPJ), 1(1), 10-17.

[5] Jatmika, C, Maggadani, B, P & Hayun 2015, '*Evaluasi aktivitas Antioksidan Senyawa 4-[(E)-2-(4-okso-3-fenilkuinazolin-2il) etenil]-benzensulfonamida dan analognya',* ***Pharm Sci Res***, vol. 2 no. 3.

[6] Banjarnahor, D, S, S, & Artanti, N 2014. *'Antioxidant Properties of Flavonoid.'* ***Med.J.Indones'***, vol. 23 no. 4 pp. 239-244.

[7] Silva D, Araújo AR, Al. E. (2012). *Chemical composition, antioxidant and antibacterial activities of two Spondias species from Northeastern Brazil.* ***PharmBiol,*** 50(6):740–6.

[8] De Flora S and Ferguson LR (2005). *Overview of mechanisms of cancer chemopreventive agents.* ***Mut Res*** 591:8-15.

[9] Gitari,M.N.,Hartawan,I.G.,Dwija,I.B.2017. *Potensi Nanopartikel Alginat- Kitosan-Ekstrak Daun Kedondong Hutan (Spondias Pinnata (L.F.) Kurz.) Dalam Penatalaksanaan Tuberkulosis Dan Multi Drug Resistance Tuberculosis (Mdr-Tb). Bali:* ***Universitas Udayana***

[10] Araujo. (2019). *Protective effects of bark ethanolic extract from Spondias dulcis Forst F. against DNA damage induced by Benzo[a]pyrene and Cyclophosphamide*. ***Genetics and Molecular Biology***

[11] Pakpahan,K.Y.,Yamlean,P.,Jayanto,I.(2020). *Formulasi dan Uji Antibakteri Gel Ekstrak Etanol Daun Kedondong (Spondias dulcis) Terhadap Bakteri Pseudomonas aeruginosa.* ***Pharmacon : Jurnal Ilmiah Farmasi UNSRAT***

[12] Prasongko,E.T.,Lailiyah,M.,Muzayyiddin,W. (2020). *Formulasi dan Uji Efektivitas Gel Ekstrak Daun Kedondong (Spondias dulcis) Terhadap Luka Bakar Pada Tikus Wistar (Ratus novergicus).* ***Jurnal Wiyata***, Vol. 7 No. 1 Tahun 2020

[13] Depkes RI. (1995). ***Materia Medika Indonesia. Jilid VI. Jakarta: Direktorat Jenderal Pengawasan Obat Dan Makanan***. Halaman 297-307, 321-325, 333-337.

[14] Kementerian Kesehatan RI. 2017. ***Farmakope Herbal Indonesia Edisi II***. Jakarta: Kementerian Kesehatan RI.

[15] Fidrianny, I., Darmawati, A., & Sukrasno. (2014). *Antioxidant Capacities from Different Polarities Extracts of Cucurbitaceae Leaves Using FRAP, DPPH Assays and Correlation with Phenolic, Flavonoid, Carotenoid Content.* ***International Journal of Pharmacy and Pharmaceutical Sciences****, 858-862.*

[16] WHO. (1998). ***Quality Control Methods for Herbal Materials***. *Switzerland: Printed in Malta*. Hal. 33-35.

[17] Wiraputra, H., Nainggolan, M., & Sitorus, P. (2018, October). *Senyawa Saponin Hasil Isolasi dari Daun Buni (Antidesma bunius (L.) Spreng.).* ***In Talenta Conference Series: Tropical Medicine (TM)*** (Vol. 1, No. 1, pp. 264-270).

[18] Al-Qiriim TM.,Shahwan M.Zaidi., Uddin, Q. (2002). *Effect of Khat, its constituent and restraint stress on free radical metabolism of rats.****J.Ethnopharm*** 83:245-250

[19] Akhlaghi, F., & Zare-Bidaki, A. (2009). *Influence of graphite content on the dry sliding and oil impregnated sliding wear behavior of Al 2024–graphite composites produced by in situ powder metallurgy method.* ***Wear***, 266(1-2), 37-45.