Analysis quantitative of flavonoid content in moringa leaves comes from Sigi Biromaru, Palu, Central Sulawesi

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Abstract. Moringa is a multifunctional vegetable plant so that all parts of this Moringa plant can be used as a food source because it contains active compounds and complete nutrition. Moringa is widely used because it is beneficial to health. Many compounds in Moringa leaves are very beneficial for health, one of which is flavonoid compounds. An analysis of the flavonoid content in Moringa leaves has been analyzed. Analysis of flavonoid content was carried out by extracting Moringa leaves with methanol-HCl 1\% solvent. The extract was analyzed qualitatively and quantitatively using a UV-Vis spectrophotometer. The results of the analysis showed flavonoid in young and old leaves in 100 g of the samples respectively 0.0839 mg and 0.0327 mg.

1. Introduction
Moringa plants in Indonesia are known by different names in each region, among them are named kelor (Javanese, Sundanese, Lampung, Central Sulawesi), moranggih (Madura), maltong (Flores), Keloro (Boges), ongge (Bima), morang or barunggai (Sumatra), kou fo (Timor), drumstick (India, South America, Arab and Southeast Asia) [1-3]. Moringa plants besides nutritious also function as herbal medicines, so that until now it has been known in 82 countries in the world with different names. Among other things in England known by several names moringa, horseradish tree, drumstick tree. In Bangladesh we are called sajina, in Colombia the name is mrm, name kelor, moringa (Indonesia), iih'um (Laos), merenggai, gengonggae, kelor (Malaysia), dandalonbin (Myanmar), malunggay (Philippines), marum, phakihum, makharkom (Thailand) and chum ngay (Vietnam).

World Health Organization (WHO), has introduced Moringa plants as a source of nutrition, so it can be used for malnutrition in Africa and in Asia. Moringa leaves are recommended as nutritional supplements for children and nursing mothers [4]. Moringa plants are almost all useful and nutritious parts of the plant, so the moringa gets the nickname of the mother's best friendi and miracea tree [4].

Moringa is not only rich in nutrients, but also has functional properties, this is because all Moringa plants have benefits and benefits for human health [5]. Moringa can also be used as a cosmetic ingredient, for example in Palu, Central Sulawesi the seeds and leaves are used as raw material for powder for sunscreen, this is caused by Moringa leaves and fruit containing secondary metabolites including flavonoids, alkaloids, steroids and anthocyanins [6].

The latest findings of the function of Moringa leaves as pharmacological, namely antimicrobial, antifungal, anti-pertensive, anti-hyperglycemic, antitumor, anticancer, anti-inflammatory [4, 7]. Moringa leaf n-hexane extract can function as an antimicrobial especially against esherichia coli.
Moringa leaf extract can be used as an inhibitor of antibacterial activity (*Pseudomonas aeruginosa*) in fresh fish [8, 9].

Moringa fruit extract (*Moringa oleifera*) from Sigi Regency, Palu, Central Sulawesi has a inhibitory effect on the growth of albicans candida fungi. Moringa fruit extract using aquades solvent has a greater inhibitory power than 70% ethanol and hexane solvents, this is due to the presence of alkaloids, flavonoids in Moringa fruit which inhibit the growth of candida albicans fungi [6].

Based on the previous results, thick extracts of Moringa fruit originating in SigiBiromaru Regency, Palu, contain primary and secondary metabolites, to complete the information on the use of Moringa plants in the field of phytopharmaca, a study of secondary metabolite compounds, such as flavonoids in leaves is needed. young and old moringa. Flavonoid levels in Moringa leaves originating from the Sigi region have not been determined, so it is very urgent to do a study. The purpose of this study was to determine the levels of flavonoids in young and old Moringa leaves from Sigi Regency, Palu, Central Sulawesi. This research is very important because people in the area believe that the leaves and fruit of Moringa are healing all diseases.

2. Materials and Methods

2.1. Materials and Tools used

The non-chemical research materials used were young and old moringa leaves taken from the SigiBiromaru area, Palu, Central Sulawesi. The chemicals used are filter paper, ammonium hydroxide, acetic acid, distilled water, TLC plate, hexane, methanol, hydrochloric acid, ethyl acetate, 10% FeCl3 solution, pH 1 buffer solution and pH 4.5 (all chemicals used proanalysis).

The tools used are drip pipette, measuring cup 250 and 10 mL, 1000 mL beaker, 500 mL Erlenmeyer, test tube, stirrer, funnel, digital balance, cuvette, UV-Vis spectrophotometer (Miltonroy 3000 array) and rotary evaporator (R-214) and micro pipette (SOLOREK Switzerland).

2.2 Research Procedure

2.2.1 Sample preparation. Moringa leaf samples used in the study were young and old leaves. Sampling is done in the morning between six and seven in the morning. The next step is the two samples in different places are picked leaves (leaves separated from the stem), then aerated each for three days at room temperature. After drying the two samples are mashed, and so that the results obtained are large, the same particle is sieved. The results of this sample preparation will be analyzed qualitatively and quantitatively the levels of flavonoids.

2.2.2 Flavonoid Extraction. Young moringa leaves (preparation results) were weighed as much as 20 g, put into Erlenmeyer 500 mL, added with hexane solvents as much as 200 mL, closed, then stirred with a shaker for 2 hours, then let stand for 24 hours, then filtered. The residue was re-extracted with an ethyl setat solvent of 200 mL with the same extraction time of 2 hours and left to stand for 24 hours. The extraction was filtered, then the residue was re-extracted with 1% methanol-HCl solvent mixture. Extraction is done in the same way and time, then filtering is done to separate the extracts and residues [10]. Old moringa leaf samples are carried out with the same procedure as young moringa leaves. The next step is the extraction (extract) of young and old moringa leaves analyzed by the qualitative and quantitative levels of flavonoids.

2.2.3 Identification of Flavonoids (Qualitative Analysis). Results of extraction of young and old moringa leaves, taken as much as 2 mL each, put into a different test tube, then each tube is heated for approximately 5 minutes. After being heated, each added with 1 g of metal Mg and 5 drops of
concentrated HCl. If each sample solution is formed in orange to red, it positively contains flavonoids [6, 11].

The extraction of young and old moringa leaves is taken as much as 1 mL each, then put in a 10 mL volumetric flask. The next step is diluted using an alcohol solvent to the boundary mark, after which the wavelength of the measured using a UV-Vis spectrophotometer [12]. The results of extracting young and old moringa leaves are taken several drops each, then each drop on a different TLC plate. After that it was waited for a while until it dried, then the TLC plate was each steamed with ammonia [13].

2.2.4 Quantitative Analysis of Flavonoids

Young and old moringa leaf extracts were taken as much as 50 μmL each, put into 2 different volumetric flasks, then each diluted to 5 mL with a buffer solution pH 1.0 and pH 4.5. Then the absorbance was measured using a UV-Vis spectrophotometer at wavelength (maximum) 520 nm and 700 nm. The levels of flavonoids were determined using the formula as described by Lee et al. [14].

3. Result and Discussion

3.1. Results of sample preparation

The drying process is carried out at room temperature to keep the flavonoid content from being damaged [15]. The maceration extraction process is carried out using solvents from non-polar (n-hexane), then semi-polar (ethyl acetate) and polar solvents is a mixture of methanol-HCl 1%. Flavonoids are polar compounds, so this compounds will be interested in polar solvents, namely 1% methanol-HCl mixture solvent [10].

3.2 Qualitative Flavonoid Analysis

Flavonoids are the largest secondary metabolites in plants. These compounds include polyphenol compounds which have a basic structure C6-C3-C6. The structure of simple flavonoids is shown in Figure 1.

![Figure 1. Structure of chaemferol flavonoid compounds](image)

The results of the analysis of flavonoids in each sample (young and old Moringa leaves), were stated positively, because the results of testing the second leaf extract used concentrated HCl and magnesium metal pieces (Mg), resulting in an orange color [16]. The reactions that occur are illustrated as in Figure 2.

The young Moringa leaf extract was analyzed by UV-Vis spectra, which had strong absorption in the maximal area of 213 nm, 279 nm, 332 nm and 522 nm. Old moringa leaves appear absorption in the area of aks max 207 nm, 282 nm, 336 and 519 nm.

Flavonoids have absorption bands around 220 max 220-270 nm and other strong bands at max higher. Maximum uptake at the max of various flavonoids as follows: anthocyanin 500-530 nm, flavone and flavonol 330-375 nm, chalcone and auron 370-470 nm, flavonone 250-300 nm, leukuantocyanidin and ketekin 280 nm, and isoflavones 310-330 nm. Based on the results of analysis with UV-Vis spectrophotometers, young and old Moringa leaf extracts are thought to contain flavonoids [10, 17].
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**Figure 2.** Reaction of flavonoids with concentrated HCl and magnesium metal

**Figure 3.** Reaction between anthocyanin cyanidin and NH$_3$ vapor [10].

Anthocyanin has a typical absorption in the wavelength region ($\lambda_{max}$) between 465-560 nm. Based on the results of UV-Vis analysis and reaction with NH$_3$ vapor in young and old Moringa leaves containing flavonoids of anthocyanin types [18, 19]. The results of quantitative analysis of flavonoids in young and old Moringa leaves are presented in Table 1.

**Table 1.** The results of the analysis using UV-Vis levels of flavonoid extract of young and old Moringa leaf extract.

| Moringa leaf sample/100 g | Absorbance | Flavonoid levels (mg/ g) |
|--------------------------|------------|--------------------------|
|                          | pH = 1,0   | pH = 4,5                 |
| max                      | 520 nm     | 700 nm                   |
| Young                    | 1,745      | 0,850                    |
| Old                      | 1,191      | 0,737                    |

Young and old Moringa leaves in a qualitative analysis detected anthocyanin, so that in determining the total flavonoids using UV-Vis in the area of $\lambda_{max}$ (wavelength max) 520 and 700 nm. The wavelength of 520 nm is the max of a cyanidin-3-glucoside anthocyanin type. The results of qualitative studies with UV-Vis uptake in the max 522 and 519 nm area, these results indicate the presence of cyanidin-3-glucoside anthocyanin. Measurements at max 700 nm aim to correct or turbidity the solution being
analyzed [14]. Measurements in acidic conditions are carried out, because anthocyanins are stable in acidic conditions.

The content of young Moringa leaf flavonoids was 0.0839 mg/g, after the leaves grew old the flavonoid levels decreased to 0.0327 mg/g. This event is thought to be due to the biosynthetic process in Moringa leaves. The results of the study are in accordance with the results of a study conducted by Janna et al. [20]. The anthocyanin content in the flower of Melastoma malabothricum which has blossomed and which has withered has decreased anthocyanin levels.

4. Conclusion

Moringa leaves from Sigi Regency, Palu, Central Sulawesi, after analysis of flavonoid content, the young leaves were obtained as much as 0.0839 mg/g, more than the old Moringa leaves (0.0327 mg/g). The results of the analysis indicate that the Moringa leaves contain anthocyanin-type flavonoids. It is recommended that further research is needed on the analysis of anthocyanin content contained in Moringa leaves, so that it will be the reason that Moringa leaves can function as herbal medicines, because anthocyanin has been shown to function as an antioxidant.

References

[1] Duke J A 2018 CRC handbook of nuts (Boca Raton, FL: CRC Press)
[2] Haslinah A 2002 ILTEK 7 995-9.
[3] Tekle A, Belay A, Kelem K, Wodajo B and Tesfaye Y 2015 Eur. J. Nutrit. Food Safety 1100-1.
[4] Aminah S, Ramdhan T, Yanis M 2015 Buletin Pertanian Perkotaan 5 35-44.
[5] Fahey J W 2005 Trees for Life Journal 1 2125-85.
[6] Nuryanti S, Puspisasari D J 2017 AIP Conference Proceedings (Yogyakarta, Indonesia: AIP Publishing) p 020006
[7] Nepolean P, Anitha J, Emilin R 2009 Curr. Biotica 3 33-7.
[8] Cowan M M 1999 Clin. Microbiol. Rev. 12 564-82.
[9] Widowati I, Efifyti S, Wahyuningsyas S 2014 Petita-Jurnal Penelitian Mahasiswa UNY 9.
[10] Nuryanti S, Matsjeh S, Anwar C, Raharjo T J 2010 Agritech 30.
[11] Sayeed M A, Hosain M S, Chowdhury M E H, Haque M 2012 Journal of Pharmacogn. Phytochem. 1 94.
[12] Nuryanti S, Matsjeh S, Anwar C, Raharjo T J 2012 Indones. J. Chem. 12 167-71.
[13] Nuryanti S 2013 Prosiding Seminar Sains dan Matematika Nasional (Palu) (Palu, Indonesia: Universitas Tadulako).
[14] Lee J, Durst R W, Wrolstad R E 2005 J. AOAC Int. 88 1269-78.
[15] Pourmorad F, Hosseinimehr S, Shahabimajd N 2006 African journal of biotechnology 5.
[16] Nugrahanri R, Andayani Y, Hakim A 2016 Jurnal Penelitian Pendidikan IPA 2.
[17] Wijoyo H 2003 Makara Sains 7 2-6.
[18] Degenhardt A, Knapp H, Winterhalter P 2000 J. Agric. Food Chem. 48 338-43.
[19] Aaby K, Hvattum E, Skrede G 2004 J. Agric. Food Chem. 52 4595-603.
[20] Janna O, Khairul A, Maziah M, Mohd Y 2006 Afr. J. Biotechnol. 5 170-4.