Comparison study for extraction of bioactive flavonoids from moringa oleifera, apple, onion, and ascorbic acid (orange) by using microwave-assisted, ultrasound-assisted and maceration methods

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Abstract. Flavonoid compounds are widely applied in the nutrition and pharmacy fields. Flavonoids have anti-cancer, anti-microbial, and antioxidant properties. This study compared the extraction of flavonoid compounds from moringa oleifera, apple, onion and orange using maceration, ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE) methods. The extraction process using ethanol solvent with a concentration of 96% ethanol. Total flavonoid content (TF) was analyzed using the colorimetric method. The results of this research showed that extraction using the combination of microwave-ultrasonic had the highest yield. The results showed that the extraction with the combination of microwave-ultrasound-assisted extraction (MUAE) processes produced the highest yield of flavonoid. The TF analyzed showed that the contains of flavonoids in orange extract were higher than the flavonoids in moringa, onion and apple. In the MUAE process, the total flavonoid content of moringa oleifera, apple, onion, and orange was 2.140, 1.975, 3.923, and 6.080 mg QE / g, respectively. Orange extract tends to the highest contain flavonoids than onion, moringa oleifera, and apples. High flavonoids contain indicate that these samples can be used as antioxidants.

1. Introduction
Moringa oleifera is recognized as a nutritious plant worldwide. The World Health Organization (WHO) launched Moringa as an alternative food to address nutritional (malnutritional) problems. Moringa leaves are recommended in Africa and Asia as a nutrient-rich supplement for breastfeeding mothers and children during infancy [1]. Moringa has been used in herbal medicine to prevent more than 300 diseases, and different parts of the moringa plant that function as a cardiac and circulatory stimulant have anti-tumor, antipyretic, antiepileptic, anti-inflammatory, antilucreative, diuretic, antihypertensive, lower cholesterol, antioxidant, antidiabetic, antibacterial, and antifungal [2]. Components such as flavonoids are responsible for one of the antioxidant effects of moringa [3]. Flavonoids have vasodilatory, antibacterial, antiviral, anti-cancer and anti-inflammatory functions [4]. Flavonoids are also used to extract ethanol and methanol. The common methods of extraction include dipping, percolation, reflux, continuous reflux etc. The high concentration alcohol (90–95 percent) is used to
extract free flavonoids and the alcohol is used to extract flavonoid glycosides at a concentration of about 60 percent [5].

The objective of extraction is to remove one constituent from a solid or liquid by means of a liquid solvent [6]. Conventional solvent extraction (CSE) such as maceration involves longer time and a greater number of solvents often have the downside of oxidation, hydrolysis and ionization damage to phenolic compounds during the extraction process [7,8]. An alternative approach to replace the traditional extraction process is the use of ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE). The UAE method uses sound waves above human hearing thresholds (almost 20 kHz) to assist with the extraction process [8]. The UAE method uses acoustic waves in the kilohertz range that pass through the solvent to create cavitation bubbles. The shockwave damage to the plant cell wall enhances the mass transfer of phenolic compounds across cell membranes to a solution when the cavitation bubbles burst on the surface of the plant sample matrix [9]. The advantages of UAE: faster time, reduce solvent requirements and can be easily combined with other extraction methods. UAE can be conducted at room temperature, so the oxidation and decomposition from samples can be avoided. The UAE is commonly used for the insulation of various natural products [10,11].

MAE is one of the advanced extraction processes in which flavonoids have been increasingly classified, analyzed and assessed. To extract analytes from a sample matrix, MAE harnesses microwave-heated solvents. The effectiveness of this process is related to the use of elevated temperature and solvent or solvent mixture. In relation to their dielectric constant (ε), microwaves penetrate the molecules: The greater the constant, the higher the absorption, [12]. Some of the major benefits of MAE include reduced extraction time and solvent use, and higher yield with high quality targeted compounds. MAE is more effective in some situations than traditional solvent extraction methods. In terms of yield, the extraction of flavonoids from biomaterials with various physicochemical characteristics is also superior or comparable to many advanced extraction methods and is easy to apply [13,14]. The efficiency of MAE is influenced by the polarity, structure, and behavior of solvents and compounds studied under microwave conditions. Either polar / nonpolar or insoluble / water-soluble may be flavonoids. The solubility and interaction of these compounds with water / solvents also affect their efficiency in extraction [14].

The maceration method, UEA, and MAE were influenced by several factors such as extraction time, power, frequency, and extraction temperature. To the best of our knowledge, the research which comparing the different extraction methods, such as maceration, ultrasonic (UEA), and microwave (MAE) methods on flavonoid extraction has not been widely reported. This study aimed to study effectiveness of the maceration method, ultrasonic (UEA), and microwave (MAE) methods for the flavonoids extraction of moringa, apple, onion, and ascorbic acid (oranges). The data from this research can be used in the development of natural material extraction, especially for bio-medicinal substances.

2. Materials and Methods.

2.1. Materials
Moringa oleifera powder was purchased from Herbilogy, Bogor, Indonesia. Other materials such as apple powder were purchased from (MSH Rempah, 3331926527), onion powder (MSH Rempah, 3331923714), and orange powder (MSH Rempah, 3330230579). The flavonoid content in moringa oleifera, apple, onion, and orange were extracted with 96% ethanol (Sigma, CAS Number 64-17-5). The extraction was carried out with maceration, ultrasound, and microwave methods. The flavonoid content was analyzed using aluminum chloride colorimetric methods. Flavonoid test with AlCl₃ 2% solution (Sigma, CAS Number: 7446-70-0), potassium acetate 120 mM (Sigma, CAS Number: 127-08-2). The quercetin standard supplied from (Sigma, CAS Number: 117-39-5).
2.2. Maceration
The sample powder of *moringa oleifera*, apple, onion, and orange was extracted via a maceration technique as defined by Mahdi et al. [15]. The maceration extraction process uses a variable extraction time of 5, 10, 15, 20, 25, 30 hours. The maceration extraction process uses ethanol solvent (96 percent ethanol in water), and the ratio between biomass and solvent was (1:1 w/v). The maceration extraction process was carried out at a temperature of 27 °C and atmospheric pressure. The extracts were collected in amber glass bottles to determine the flavonoid content.

2.3. Ultrasonic-assisted extraction
The extraction of flavonoids from powder samples was carried out using the UEA technique as defined by Jitan et al. [9]. The ethanol sample and solvent were put in a beaker glass with a ratio of 1:1 (weight/volume). Furthermore, the sample is placed in an ultrasonic equipment (Ultrasonic machine, type JP-010S, China), where the ultrasonic process was controlled at 30 °C and 40 kHz. Studies have been carried out to determine the optimal extraction time (0, 5, 10, 15, 20, 25, 30 minutes). The extract was filtered through Whatman 41 filter paper. The extract was collected in an amber glass bottle and stored at cooling temperature for further testing.

2.4. Microwave-assisted extraction
With minor modifications, the extraction of flavonoids from the sample powder was carried out using the MAE technique as defined by Jitan et al. [9]. Sample extraction and solvent (96 percent ethanol in water as a solvent (L) to biomass (g) ratio of 1:1) are stored in a boiling flask put in a microwave (Beko-MGC20100S) at a temperature of 30 °C with a power of 200 W. Studies have been carried out to determine the optimum time of extraction (0, 5, 10, 15, 20, 25, 30 min). The extracts were filtered through Whatman filter paper 41. The extracts were collected in amber glass bottles and stored at refrigeration temperatures. A schematic representation of microwaves and ultrasound is shown in Fig 1.

![Schematic representation of the MAE and UAE.](image)

2.5. Determination of the value of total flavonoid (TF)
The content of TF was calculated using Denny and Mammen [16], a colorimetric aluminum chloride alteration assay. First, it tested 100 mL of reference solutions for ethanolic quercetin (variation from 5 to 100 mg / L) and 100 mL of each sample. Total flavonoid analysis was performed using an ultraviolet
visible (UV-vis) spectrophotometer (Shimadzu UV-1601), at 431.5 nm absorbance. The TF were expressed as Quercetin Equivalent (QE) mg per g of the mass of the sample.

3. Results

3.1. Maceration

To evaluate the impact of maceration time on the total flavonoid material, this study was performed. The results of total flavonoids in moringa oleifera, apple, onion, and the orange extract is presented in Figure 2. In general, the total flavonoid content was significantly affected by maceration time.

![Figure 2. Total flavonoid from Moringa oleifera, apple, onion and orange extract with maceration method.](image)

Total flavonoids will increase with increasing extraction time. Total flavonoid content on moringa extract, apple extract, onion extract, and the orange extract was calculated and expressed as µg of Quercetin Equivalent (QE) per mg of the sample weight. During the 25 hours of maceration process, the total flavonoids extracted from Moringa oleifera, apple, onion, and orange were 0.962, 0.887, 1.763, and 2.733 mg QE/g sample, respectively. The total flavonoid content in the orange extract is the highest compared to other ingredients. However, total flavonoids extracted from Moringa oleifera are higher than apple extract. This is because the level of flavonoids in the orange extract is higher than others’ extract, Table 1 shows the total flavonoid reference for each sample.

The total flavonoid results have similarity with research by Sulastri et al [17], that have the total flavonoid content of 0.81 – 0.96 mg QE/g. Naeem et al [18] reported that the optimal operating conditions for extracting moringa leaves were at an extraction time of 60 minutes and a temperature of 90 °C. From the table, we can conclude the ethanol has slightly more flavonoid content than methanol as solvent. The % of solvent ethanol can get the flavonoid content more because the polar side from the solvent can extract more flavonoid from the sample. The differences in levels of total flavonoids in moringa extract are caused by differences in plant origin, the influence of extraction methods, temperature extraction, and others. Several references regarding maceration extraction of flavonoid content are shown in Table 1.
**Table 1.** Total flavonoid content with maceration method.

| Solvent          | Temperature °C and time | Type       | Total Flavonoid (mg/g) | Reference                        |
|------------------|-------------------------|------------|------------------------|----------------------------------|
| Moringa extract  | 95% ethanol 45 °C, 8 h  | Leaf       | 0.442                  | Mahdi et al, 2016 [15]           |
|                  | 50% ethanol            |            | 0.120                  |                                  |
|                  | Water 100 °C, 30 min    | Powder     | 0.010 – 0.251          | Vongsak et al, 2013 [19]         |
|                  | 96% ethanol 27 °C, 30 h | Powder     | 0.962                  | *our research                    |
| Apple extract    | Methanol 85 °C, 24 h    | Fruit peel | 0.028                  | Hegazy, 2017 [20]                |
|                  | Ethanol 27 °C, 30 h     | Powder     | 0.887                  | *our research                    |
|                  | DCM 27 °C, 30 h         | Powder     | 0.052 – 0.280          | Bystricka et al, 2015 [21]       |
|                  | Acetone 96 % ethanol 27 | Powder     | 1.763                  | *our research                    |
|                  | N-Hexane 27 °C          | Powder     | 0.3 – 3.1              | Ghasemi et al, 2009 [22]         |
|                  | Ethyl acetate 27 °C, 30 | Powder     | 2.733                  | *our research                    |

The flavonoid that we studied has specific spectra. The UV spectra of flavonoid quercetin depicted in Figure 3. This spectrum has two peaks of absorption at 431.5 nm and 299.0 nm in the wavelength region of 240 - 400 nm. The first peak at a wavelength of 431.5 nm caused by the cinnamoyl group, and the second peak at a wavelength of 299.0 nm caused by the benzoyl group can be seen in Figure 4.

![Figure 3](image1.png)

**Figure 3.** The UV-visible absorption spectra flavonoid quercetin.

![Figure 4](image2.png)

**Figure 4.** The structure of flavonoid quercetin.
3.2. **Ultrasound-assisted extraction (UAE)**

Research was carried out to determine the extraction time that affects the total flavonoid content using the ultrasound process. Figure 5 displays the overall flavonoid content of moringa, apple, onion, and orange extracts using ultrasound techniques. These processes were performed at 40 kHz frequency and ambient temperature.

![Figure 5. Total flavonoid from *Moringa oleifera*, apple, onion, and orange extract with Ultrasound-assisted extraction (UAE).](image)

Extraction using ultrasound indicated that there was a higher and faster to extract the flavonoid content in the sample. During 30 minutes of extraction with the Ultrasound process, the total flavonoids extracted from moringa oleifera, apple, onion, and orange were 1.316, 1.214, 2.412, and 3.740 mg QE/g sample, respectively. Santos et al [23] reported that the temperature rise during ultrasound-assisted extraction leads to an increase in the pressure of solvent vapor resulting in a lower cavitation force resulting in lower bioactive compound yields. Furthermore, it was confirmed that lower temperatures are preferred for bioactive compound extraction [24]. This indicates that the use of lower temperatures and shorter extraction times during the UAE process will lead to insufficient extraction of bioactive compounds, while longer extraction times will lead to higher temperature ultrasonic destruction of bioactive compounds. The heat sensitivity of the bioactive compounds and longer ultrasonic exposure can be due to this [25].

Dadi et al [25] extracted flavonoid 8.8 – 20.4 mg QE/g sample with condition 35 kHz, 160 W, A temperature of 40 degrees for 20 minutes. The state was the perfect combination of time and temperature to extract bioactive Moringa compounds. Khan et al [26] obtained the contents of hesperidin and naringin by UAE were considerably higher (0.025 and 0.007 mg /g, respectively) than CSE (0.014 and 0.005 mg /g, respectively). Mohammadpour et al [27] use ultrasound extraction with an optimum at power 20 kHz, 348 W, temperature 30 °C, and extraction time 30 min. Pollini et al [28] use ultrasound for Moringa oleifera with condition 60 °C for 60 min to get a total flavonoid 2.6 – 5.4 mg QE/g sample. The factor that affects the total flavonoid in ultrasound methods is temperature, frequency, power, and processing time.
3.3. Microwave-assisted extraction (MAE)

The microwave method for calculating the extraction time, which affects the total flavonoid content, was used to conduct this study. The total flavonoid content of moringa, apple, onion and orange extract is shown in Figure 6 using microwave methods. These processes were performed at 200 W power and ambient temperature.

![Figure 6. Total flavonoid from Moringa oleifera, apple, onion, and orange extract with Microwave-assisted extraction (MAE).](image)

Total flavonoids extracted from Moringa oleifera, apple, onion and orange during 30 minutes of extraction with microwave process were 1.451, 1.338, 2.659, and 4.124 mg QE/g sample, respectively. Dahmoune et al. [29] contrasted three phenolic compound extraction methods: maceration, UAE, and MAE. The ultrasound reported by the authors causes cavitation disruption in plant cells. In the sample, the particles are resistant to ultrasonic [30]. The increase in pressure in the pores of the cells induces a quicker split compared to the regulation. Nevertheless, MAE causes more serious degradation of tissue via the operation of microwaves. Furthermore, MAE dehydrates and decreases the mechanical strength of cellulose, allowing the solvent to enter the cellular channels easily [31]. Cellular damage and a damaged microstructure that helps to rapidly release the solvent are caused by microwave heating. Compared to traditional extraction techniques, MAE requires less time, less energy and less organic solvent input to achieve higher yields [32]. The MAE process obtained high flavonoid content by 1.33 mg /g in comparison with maceration at 1.11 mg /g and Soxhlet extraction at 1.19 mg /g, respectively [33]. Hayat et al. [34] compared CSE, MAE, and UAE. MAE contains the highest amount at 0.024 mg /g compared to UAE at 0.023 mg /g and CSE at 0.020 mg /g. Miri et al. [35] compared CSE, UAE, MAE, and Supercritical CO2 extraction (SC-CO2) for extraction flavonoid content. The MAE offers the highest degree of TF (0.227 mg /g) than CSE (0.161 mg /g), UAE (0.211 mg /g), and SC-CO2 (0.124 mg /g).

The total flavonoid results tend to be smaller than the research by Potisate and Phoungchandang [36] with the result of total flavonoids 1.156 – 5.746 mg QE/g sample or 64.10 % condition 900 W for 15 min compared with tray drying at 60 °C for 60 min get total flavonoids 3.236 mg QE/g sample. Porto et al. [37], with Moringa olivera microwave pretreatment has the optimal condition for 30 and 60 s time at 100 W. Zhao et al. [33] studied that flavonoid content with microwave at an increase in power from 200 to 500 W as well increase the flavonoid content. The flavonoid content dropped steadily as the power of radiation continued to increase, which was inevitable because the degradation of samples could be caused by excessive microwave power (> 500 W). Izza et al. [38], studied that the microwave condition
at 60 °C with a solvent ratio of 1:8 and an extraction time of 3 minutes could yield the highest Moringa seed extract. Sari et al. [39], that the optimal extraction condition was 414 watts of microwave power, 50.33 percent ethanol concentration and 7.89 minutes of time extraction yielded a predicted value of 0.2963 percent of total flavonoid content. Increase of total flavonoid complying with the correct regulation. This suggests microwaves would damage Moringa's intact cell structure, increasing the release of flavonoid compounds.

3.4. Microwave Ultrasound-assisted extraction (MUAE)
This research was carried out to determine the microwave combination extraction time and the ultrasound method that affects the total flavonoid material. Figure 7 displays the total flavonoid content of moringa, apple, onion and orange extracts using MUAE techniques. These processes were performed at 40 Hz frequency and 200 W power and ambient temperature.

![Figure 7. Total flavonoid from Moringa oleifera, apple, onion, and orange extract with Microwave Ultrasound-assisted extraction (MUAE).](image)

Total flavonoids extracted from Moringa oleifera, apple, onion and orange during 20 minutes of extraction with MUAE process were 2.140, 1.975, 3.923, and 6.080 mg QE/g sample, respectively. Conventional solvent extraction (CSE) yields low relative to UAE, and MAE. Using ultrasound and microwave energy actually speeds up this process. The intensification of ultrasonic extraction efficiency was due to the spread of ultrasonic pressure waves through the solvent and the resulting phenomenon of cavitation [40]. Nevertheless, Microwave irradiation accelerates the breakdown of cells by causing the plant or fruit walls to suddenly rise in temperature and increase internal pressure [41]. During microwave heating processing, weak hydrogen bonds can be broken due to dipole rotation of the molecules. The biomaterial exerts tremendous pressure influencing the biological tissues' physical properties [35]. In addition, the rise in yield obtained by MAE can be explained by the collapse of larger glucoside-bound flavonoid compounds into free compounds with their original molecules and which can react with aluminum chloride assay. Comparison of MAE with other methods available in Table.2.
Table 2. Comparison of Total Flavonoid (mg/g) by CSE Method - UAE - MAE

| Solvent         | CSE       | UAE       | MAE       | Reference                  |
|-----------------|-----------|-----------|-----------|----------------------------|
| 80% ethanol     | 0.161 ± 0.01 | 0.211 ± 0.01 | 0.227 ± 0.01 | M’hiri et al [35]          |
| 42% ethanol     | 6.95 ± 0.20 | 18.99 ± 1.31 | 11.5 ± 0.01 | Madi et al [42]            |
| 100% methanol   | 5.62 ± 0.01 | 26.36 ± 0.01 | 22.40 ± 0.01 | Rocchetti et al [43]       |
| 100% ethanol    | 70.13 ± 4.43 | 86.69 ± 1.67 | 135.18 ± 2.9 | Ling et al [44]            |
| Water           | 21.54 ± 0.52 | 23.86 ± 1.92 | 24.64 ± 2.36 | Ince et al [45]            |
| 50% ethanol     | 5.5 ± 0.10  | 5.5 ± 0.10  | 5.8 ± 0.20  | Gharekhani et al [46]      |
| 99.6% ethanol   | 1.7 ± 0.00  | 1.7 ± 0.00  | 1.9 ± 0.00  | Keshavarz-Rezaei [47]      |
| 100% methanol   | 15.46 ± 1.98 | 21.08 ± 6.88 | 19.13 ± 3.01 | Kothari et al [48]         |
| 96% ethanol     | 0.962 ± 0.25 | 1.316 ± 0.07 | 1.451 ± 0.31 | *our research              |

The total flavonoid results tend to be smaller than the research by Rodrigues et al [49] with optimum MAE - PLE (Pressurized liquid extraction) at 158 °C, 20 min extraction time, 850 W get total flavonoid 2.35 – 13.86 mg QE/g sample. Zhong et al [50] extracted moringa with UAE under condition ultrasound time 5 min, power 200 W, temperature 30 °C, and MAE under condition microwave time 9 min, power 300 W, temperature 40 °C. The UAE and MAE reported a 91% – 94% higher yield in the short extraction cycle compared to traditional extraction (CSE), which yielded 90%. The UAE and MAE could reduce the extraction time without seriously affecting the consistency of the Moringa extract. This shows that the UAE and MAE techniques are the ideal physicochemical properties of Moringa products for achieving high yields, short extraction durations. Combined extraction can reduce extraction time considerably and tend to increase product yield.

4. Conclusion
The sample extracts of *Moringa oleifera*, apple, onion, and orange contain high flavonoids. In the maceration process, the total flavonoid content of moringa oleifera, apple, onion, and orange was 0.962, 0.887, 1.763, and 2.733 mg QE / g, respectively. In the UAE process, the total flavonoid content was 1.316, 1.214, 2.412, and 3.740 mg QE / g, for moringa oleifera, apple, onion, and orange extract, respectively. In the MAE process the total flavonoid content for moringa oleifera, apple, onion, and orange extract was 1.451, 1.338, 2.659, and 4.124 mg QE / g, respectively. In the MUAE process, the total flavonoid content of moringa oleifera, apple, onion, and orange was 2.140, 1.975, 3.923, and 6.080 mg QE / g, respectively. Orange extract tends to the highest contain flavonoids than onion, moringa oleifera, and apples. High flavonoids contain indicate that these samples can be used as antioxidants.

The results showed that the MUAE process obtained higher flavonoid content than UAE and maceration process. In the maceration process, an extraction time of 25 hours is the optimal time to obtain high flavonoid. Extraction time of 30 minutes is the best time for UAE and MAE to obtain high flavonoid. The results showed that the extraction with the combination of microwave-ultrasound processes produced the highest yield of flavonoid. The extraction conditions used a combination of 200 W / 40 Hz power and a temperature of 40 °C, for 20 minutes. These results prove that the combination of extraction methods such as ultrasound and microwave can increase the total flavonoid content.

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**Authors’ background**

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|---------------------------|----------------|---------------------------------|-------------------------------------------|
| Aji Prasetyaningrum        | Senior lecture | Supercritical fluid technology  | https://scholar.google.com/citations?u  
|                           |                |                                 | ser=gLD7TX8AAAAJ&hl=en                   |
| Nur Rokhati                | Senior lecture | Membrane                        | https://scholar.google.co.id/citations?u  
|                           |                |                                 | ser=p1FzhoYAAAAJ&hl=en                   |
| Yudhy Dharmawan            | Senior lecturer| Health Information System       | https://scholar.google.com/citations?u  
|                           |                |                                 | ser=RFWYkxQAAAAJ&hl=en                   |
| Gian Restu Prinanda        | Master student | -                               | -                                         |

**Presentation by**

| Your Name                  | Title*         | Research Field                  |
|---------------------------|----------------|---------------------------------|
| Gian Restu Prinanda        | Master student | -                               |