Effect of Variation Conditions of The Extraction Process of Morinda Citrifolia L Leaves Using Ultrasound-Assisted Extraction Method (UAE)

Buanasari¹, Sonia Murniana Sri Dharmayanti¹ and Suryaningsih¹

ABSTRACT
Background: Morinda citrifolia L. is widely used as traditional medicine for various diseases. The benefits of noni are studied from the seeds, fruit, leaves and root bark. This leaf active compound is rich in flavonoids, so an effective extraction process is needed to extract it. Conventional extraction generally takes a long time and involves a thermal process that can damage the compound, so it requires extraction with the latest methods, one of which is the use of ultrasonic waves.

Aim: This study aims to examine the effect of variations in extraction process conditions on present yield, DPPH scavenging activity, flavonoid content and phenol content of Morinda citrifolia L leaves by varying the solids-solvent ratio (1:10, 1:20, 1:30, dan 1:40 g/mL), and extraction temperature (25, 35, 45, and 55ºC).

Method: The process uses the ultrasonic assisted extraction method with 50 %V ethanol for 60 minutes.

Result: The highest yield was obtained in the extraction with a solids-solvent ratio of 1:40 g/mL, at an extraction temperature of 55ºC, which was 32.29±0.066%. The highest flavonoid content (173.41±0.615 mg quercetin equivalent/g extract), phenol content (197.00±0.148 mg gallic acid equivalents/g extract) and DPPH scavenging activity (97.65±0.912%) was obtained in the extraction with a solids-solvent ratio of 1:30 g/mL, at an extraction temperature of 25ºC. The best extract measured antioxidant activity and IC₅₀ values obtained with 23.21 µg/mL.

Conclusion: The use of the ultrasonic assisted extraction method by selecting the optimal operating conditions greatly increases the amount of active compound uptake required.

Keywords: Antioxidant, DPPH, flavonoid, Morinda citrifolia, UAE

BACKGROUND
Morinda citrifolia L. has been widely used as a traditional medicine for various diseases. Several studies reported on the benefits of noni both seeds, root, fruit¹,¹¹, leaves² and bark. This plant is capable of being an antidyslipidemic³, antioxidant⁴,⁵, healing wounds caused by diabetes⁶,⁷, hepatoprotector⁸, inhibiting ACE activity⁹, analgesics¹⁰, hypoglycemia⁷, anti-inflammatory and cancer chemopreventive¹¹,¹².

Noni leaves contain five flavonol glycosides, namely: quercetin-3-O-β-D-glucopyranoside;kaemperol-3-O-αL-rhamnopyrosil-(1→6)-β-D-glucopyranoside;quercetin-3-Oα-L-ramnopyrosil(1→6)-β-D-glucopyranoside;quercetin-3-O-β-D-glucopyranosil-(1→2)-[α-L-ramnopyranosil-(1→6)]-βD-glucopyranoside; and kaemperol-3-O-β-D-glucopyranosil (1→2)-[α-L-ramnopyranosil-(1→6)] β-D-galakopyranoside¹³. Polyphenolic compounds such as flavonoid compounds (including flavonols) are able to inhibit autodisassembly through a radical scavenging mechanism by donating one electron from an unpaired electron in free radicals so that the number of free radicals is reduced¹⁴.

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Previous studies have extracted many of the active substances from noni by maceration, soxhletation, and microwave-assisted extraction methods. Previous research has examined the effects of four extraction methods namely; solvent extraction (SE), microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE) on phenolic components and antioxidant activity of noni leaf extract. The MAE extract contains high flavonoids, but UAE shows the highest antioxidant activity and can be recommended for the restoration of the strong natural antioxidant components of M. citrifolia leaves. The ultrasound assisted-extraction (UAE) method has been widely used in research to increase yield and extraction effectiveness. In the UAE extraction process, there are many factors involved such as particle size, type of solvent, pH, time, and temperature. Temperature, ratio solids-solvent and time extraction are the most important factors because they affect the components extracted. The advantage of the ultrasonic extraction method is that it can speed up the extraction process, maintain the efficacy of the active substance, and increase the yield produced compared to conventional extraction.

UAE is very influenced by temperature and the amount of solvent which can affect the antioxidant content in the form of phenols and flavonoids, if the temperature is raised it will reduce the antioxidant content, and vice versa. It’s very important to find the optimum conditions that can maintain the active content of noni leaves and maintain its antioxidant activity. So, in this study, noni leaf extraction was carried out using the UAE method with variations in temperature and solid-solvent ratio.

METHODS

Materials
The main material of this research is noni leaves (Morinda citrifolia L.) obtained from Semarang area, Central Java. Other materials which used: methanol (Merck, 98% purity 1060092500), 1,1 Diphenyl 2-Picryl Hydrazyl/DPPH (Sigma-Aldrich, 90% purity, D9132), gallic acid (Sigma-Aldrich, 98% purity, 398225), quercetin hydrate (Sigma-Aldrich, 95% purity, 337951), folin-ciocalteu’s phenol reagent (Merck, HC57024401), and ethanol (Merck, 96% purity).

The tools which used in this study are analytical balances (Sartorius), oven (Memmert), moisture analyser (Radwag MAC50), digital ultrasonic cleaner (PS-10A), UV-Vis spectrophotometer (Shimadzu 2480), rotary evaporator (Scilogex), and vacuum pump.

Raw material preparation
Noni leaves are washed clean. The leaves are aerated without exposure to sunlight, then continued drying for 10-12 days. After drying, the leaves are uniformed with a size of 100 mesh and a moisture content of less than 10%W.

Ultrasonic-assisted Extraction (UAE)
The pollinated materials were extracted by ultrasound assisted-extraction with various solids-solvent ratios (1:10, 1:20, 1:30 and 1:40 g / mL) and extraction temperatures of 25, 35, 45 and 55°C, using ethanol solvent (50 %V) for 60 minutes. During the extraction process, constant stirring was carried out with a homogenizer (8,000 rpm). The extract was filtered, and stored in a refrigerator at a temperature of 2-8°C before being examined.

Analysis of total flavonoid content (TFC)
Modified aluminium chloride colorimetric method. Standard solution (quercetin solution) and of each extract (0.5 mL) were mixed with 1.5 mL ethanol (95%), 0.1 mL aluminium chloride (10%), 0.1 mL potassium acetate (1M) and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of
the reaction mixture was measured by a spectrophotometer.

**Analysis of total phenolic content (TPC)**

As a standard solution used 100 µg/mL gallic acid and 1,000 µg/mL extract solution of 1.0 ml each added with 1.0 ml of folin-ciocalteu reagent diluted with distilled water, then added with 3.0 ml of 20% sodium carbonate, then vortexed for 1 minute. The solution is allowed to stand for 30 minutes. Furthermore, a spectrophotometric examination is carried out at the maximum wavelength.

**DPPH scavenging activity (DPPH-SA)**

The 1.0 mL extract solution was added with 4.0 mL of 100 µg/mL DPPH solution. The absorbance was measured with a spectrophotometer (Shimadzu 2480) at a wavelength of 514 nm. The extract activity at DPPH is expressed as:

\[
\text{DPPH scavenging activity (\%)} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\% \quad \text{......................................... (1)}
\]

**RESULTS**

This study analyzed the effect of variations in the extraction process conditions on current yield, total flavonoid content (TFC), total phenolic content (TPC) and antioxidant activity of noni leaves by varying the solids-solvent ratio (1:10, 1:20, 1:30, and 1:40 g/mL), and extraction temperatures (25, 35, 45, and 55ºC). The process uses an ultrasonic assisted extraction method with ethanol 50%v for 60 minutes. The results of this study are shown in Table 1.

<table>
<thead>
<tr>
<th>Solid:solvent ratio (g:mL)</th>
<th>Temperature (ºC)</th>
<th>Yield (%)</th>
<th>TFC mg QE/g extract</th>
<th>TPC mg GAE/g extract</th>
<th>DPPH-SA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>25</td>
<td>12.19±0.141</td>
<td>100.89±0.134</td>
<td>97.20±0.233</td>
<td>83.53±0.750</td>
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<td>35</td>
<td>17.72±0.205</td>
<td>105.12±0.120</td>
<td>54.85±1.011</td>
<td>77.92±1.294</td>
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<td>45</td>
<td>18.52±0.092</td>
<td>115.63±0.785</td>
<td>55.72±0.714</td>
<td>95.75±1.054</td>
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<td>55</td>
<td>20.24±0.127</td>
<td>124.98±0.622</td>
<td>61.49±1.068</td>
<td>93.14±1.605</td>
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<tr>
<td>1:20</td>
<td>25</td>
<td>18.58±0.064</td>
<td>102.78±0.636</td>
<td>134.64±0.856</td>
<td>90.76±0.346</td>
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<td>35</td>
<td>20.37±0.057</td>
<td>91.49±1.584</td>
<td>108.89±0.926</td>
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<td>45</td>
<td>19.79±0.057</td>
<td>165.14±1.167</td>
<td>104.41±0.750</td>
<td>71.02±1.435</td>
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<td>55</td>
<td>22.85±0.219</td>
<td>105.51±0.622</td>
<td>116.43±1.945</td>
<td>84.69±0.976</td>
</tr>
<tr>
<td>1:30</td>
<td>25</td>
<td>20.97±0.042</td>
<td>173.41±0.615</td>
<td>197.00±0.148</td>
<td>97.65±0.912</td>
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<td>35</td>
<td>23.80±0.078</td>
<td>121.04±0.891</td>
<td>186.43±1.690</td>
<td>77.57±0.806</td>
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<td>45</td>
<td>27.16±0.156</td>
<td>170.85±1.075</td>
<td>127.10±0.742</td>
<td>84.86±1.216</td>
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<td>28.27±0.085</td>
<td>125.73±1.216</td>
<td>159.92±0.827</td>
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<td>1:40</td>
<td>25</td>
<td>28.06±0.064</td>
<td>104.89±0.170</td>
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<td>75.86±1.216</td>
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<td>24.06±0.049</td>
<td>130.34±0.654</td>
<td>71.03±1.050</td>
<td>89.41±1.132</td>
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<td>55</td>
<td>32.29±0.066</td>
<td>98.90±0.672</td>
<td>67.82±0.964</td>
<td>90.96±1.365</td>
</tr>
</tbody>
</table>

Variations in the ratio of solids-solvent (gr:mL), extraction temperature (ºC), affect yield, levels of the total flavonoids content, total phenol content and antioxidant activity of noni leaf ethanol extract. The higher temperature of the ultrasound extraction will increase the solubility of the active substance. Whereas the greater the amount of solvent, the more compounds that diffuse into the solvent.

**DISCUSSION**

*Effect of the solid-liquid ratio and temperature on extract yield*
The more solvent ratio is added, the greater the pressure applied so that the plasmolysis process will be bigger and cause more cell fluid to come out. The higher the temperature on the ultrasound will increase the solubility of the active substance. If the amount of solvent added is greater, the contact of the material with the solvent which functions as an extraction medium is also greater so that it has the potential to maximize the yield of the extract\textsuperscript{22,23}. The percent yield results for various variations are presented in Figure 1.

![Figure 1. Effect of the solid-liquid ratio and temperature on extract yield.](image)

The best yields were found in ultrasound extraction at a ratio of 1:40 and at a temperature of 55ºC at 32.29±0.066%. So, it can be concluded that the higher the temperature and the ratio of the material to the solvent, the greater the yield.

**Effect of the solid-liquid ratio and temperature on total flavonoid content (TFC)**

The total flavonoid content was determined by the AlCl\textsubscript{3} method with quercetin as a standard. The use of AlCl\textsubscript{3} in determining the total flavonoid content is based on the reaction of Al\textsuperscript{3+} metal with the hydroxy (OH) group which causes a visible wavelength shift to produce complex yellow compounds. The thicker the yellow color produced, the higher the concentration of flavonoids in the sample\textsuperscript{24}. The addition of acetic acid is to detect the presence of 7-hydroxyl groups while the incubation treatment for 30 minutes before the measurement is intended so that the reaction runs perfectly, thus providing maximum color intensity. The TFC results for various variations are presented in Figure 2.

The highest TFC value from this study was found at a temperature of 25ºC and a solids-solvent ratio of 1:30 g: mL of 173.41±0.615 mg QE / g of extract. Inversely proportional to the yield, the highest value was obtained at a temperature of 55ºC. This is because flavonoids at high temperatures are damaged. Flavonoids that are damaged at high temperatures include flavonols, anthocyanins, isoflavin, flavanones and catechins.

This is the same as research on the determination of total anthocyanins from rosella flower petals, which is a flavonoid compound and is used as a source of antioxidants, the highest value was obtained at a temperature of 25ºC\textsuperscript{25}.
Effect of the solid-liquid ratio and temperature on Total Phenolic Content (TPC)

Determination of the total phenolic content was carried out by the folin-ciocalteu method. Gallic acid is used to determine the total phenolic content. The folin-ciocalteu reagent is used because phenolic compounds can react with folin-ciocalteu to form a colorized solution whose absorbance can be measured. The principle of measuring phenolic content with folin-ciocalteu reagent is the formation of complex blue compounds. This reagent oxidizes the phenolic or phenolic-hydroxy groups to reduce the phosphomolybdic-phospho-tungsten acid present in the folin-ciocalteu reagent to become a molybdenum-tungsten complex. Phenolic compounds can react with folin-ciocalteu reagent only under alkaline conditions. To create alkaline conditions 20% Sodium Carbonate is used. The blue colour that is formed will be darker, equivalent to the phenolic ion formed, which means that the greater the concentration of phenolic compounds, the more phenolic ions that reduce phosphomolybdate-phospho-tungsten acid to molybdenum-tungsten complex compounds so that the colour gets blue. The TPC results for various variations are presented in Figure 3.

The highest TPC value was obtained at 25ºC with a solid-solvent ratio of 1:30 g: mL (197.00±0.148 mg EAG / g extract). Effect of temperature and solids-solvent ratio to total phenolic and total flavonoid content gave the same conditions. The optimum temperature found was the same as the results of the bitter melon study using the UAE method. The bitter gourd fruit extraction was carried out by varying the extraction temperature (25-65ºC) and the extraction time variation (5-125 minutes). The results of the highest total phenolic content and antioxidant activity were obtained at 25ºC for 5 minutes because the chemical structure of phenolics was stable at room temperature.
**Effect of the solid-liquid ratio and temperature on DPPH Scavenging Activity**

Antioxidant activity was measured by the DPPH method. This method is carried out by measuring DPPH radical scavenging by compounds contained in the noni leaf ethanol extract. The DPPH scavenging activity results for various variations are presented in Figure 4.
The highest DPPH scavenging activity at 25°C with a 1:30 ratio solid-solvent of 97.65±0.912%, it was linearly correlated to its TFC and TPC. The more flavonoids and phenolic compounds contained in noni leaf extract, the antioxidant activity will increase1. The greater the addition of solvent causes the sample to be at a saturation point, causing a decrease in the level of the desired compound because the solvent absorbs energy from the ultrasonic wave first before entering the material matrix, so when it enters the matrix the ultrasonic wave energy decreases so that the extraction runs less optimally23,28.

After checking the DPPH scan activity, then the best extract is checked for its IC50 value. Noni leaf extract with the best TFC, TPC and DPPH-SA values had an IC50 value of 23.21 µg / mL. So it can be concluded that noni leaves have the potential for high antioxidant activity when extracted by the UAE method at optimal temperature conditions and the right amount of solvent.

CONCLUSION
Ultrasonic assisted extraction has good performance to extract the active substance in Morinda citrifolia L. leaves. The highest yield was obtained in the extraction with a solids-solvent ratio of 1:40 g/mL, at an extraction temperature of 55°C, which was 32.29±0.066%. The highest total flavonoid content (173.41±0.615 mg QE/g extract), total phenolic content (197.00±0.148 mg GAE/g extract) and DPPH scavenging activity (97.65±0.912%) was obtained in the extraction with a solids-solvent ratio of 1:30 g/mL, at an extraction temperature of 25°C. The best extract measured antioxidant activity and IC50 values obtained with 23.21 µg/mL.

ACKNOWLEDGEMENT
The authors would like to thank the STIFERA Research Fund for financial support which enabled the development of our research. We also express our deepest gratitude to the Chemical Laboratory of the Sekolah Tinggi Ilmu Farmasi Nusaputra Semarang, for the provision of equipment and support for this research.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
B. analyzed and interpreted the TPC, DPPH-SA data of the extract leaves. SM and S, performed the TFC examination of the extract, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

FUNDING
None

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REFERENCES


