Buanasari *et al. J. Sci. Technol. Res. Pharm. (2021) I: (1)p25-33*
https://doi.org/10.15294/jstrp.v1i1.44348
ISSN 2776-0685

Received: 03 Januari 2021 Accepted: 15 February 2021
Published online: 26 February 2021

Journal of Science and Technology Research for Pharmacy

RESEARCH Open Access

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

Buanasari[1,*], Sonia Murniana Sri Dharmayanti[1] and Suryaningsih[1]

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 IC_{50} 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

BACKGROUD

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, fruit[1,1], leaves[2] and bark. This plant is capable of being an antidyslipidemic[3], antioxidant[4,5], healing wounds caused by diabetes[6,7], hepatoprotector[8], inhibiting ACE activity[9], analgesics[10], hypoglycemia[7], anti-inflammatory and cancer chemopreventive[11,12] .

Noni leaves contain five flavonol glycosides, namely: quercetin-3-O-β-D-glucopyranoside;kaemperol-3-O-αL-ramnopirosil-(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-ramnopiranosil-(1→6) β-D-galakopyranoside[13]. Polyphenolic compounds such as flavonoid compounds (including flavonols) are able to inhibit autoxidation through a radical scavenging mechanism by donating one electron from an unpaired electron in free radicals so that the number of free radicals is reduced[14].

*Correspondence: buanasari.only@gmail.com
[1] Chemistry Department, D3 Pharmacy, Sekolah Tinggi Ilmu Farmasi Nusaputera, Semarang, Indonesia
Full list of author information is available at the end of the article

Previous studies have extracted many of the active substances from noni by maceration[15], soxhletation, and microwave-assisted extraction methods[16]. 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[17]. 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[18,19].

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 55ºC, 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 2-8ºC before being examined.

Analysis of total flavonoid content (TFC)

Modified aluminium chloride colorimetric method[20]. 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[4].

DPPH scavenging activity (DPPH-SA)

The 1.0 mL extract solution was added with 4.0 mL of 100 µg/mL DPPH solution[21]. The absorbance was measured with a spectrophotometer (Shimadzu 2480) at a wavelength of 514 nm. The extract activity at DPPH is expressed as:

$$\text{DPPH } \textit{scavenging activity } (\%) = \frac{\text{A blank} - \text{A sample}}{\text{A Blank}} \text{ x } 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

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 1. The result of present yield, TFC, TPC and antioxidant activity of ethanolic extract leaves

Solid:solvent ratio (g:mL)	Temperature (ºC)	Yield (%)	TFC mg QE/g extract	TPC mg GAE/g extract	DPPH-SA (%)
1:10	25	12.19±0.141	100.89±0.134	97.20±0.233	83.53±0.750
	35	17.72±0.205	105.12±0.120	54.85±1.011	77.92±1.294
	45	18.52±0.092	115.63±0.785	55.72±0.714	95.75±1.054
	55	20.24±0.127	124.98±0.622	61.49±1.068	93.14±1.605
1:20	25	18.58±0.064	102.78±0.636	134.64±0.856	90.76±0.346
	35	20.37±0.057	91.49±1.584	108.89±0.926	82.93±1.315
	45	19.79±0.057	165.14±1.167	104.41±0.750	71.02±1.435
	55	22.85±0.219	105.51±0.622	116.43±1.945	84.69±0.976
1:30	25	20.97±0.042	173.41±0.615	197.00±0.148	97.65±0.912
	35	23.80±0.078	121.04±0.891	186.43±1.690	77.57±0.806
	45	27.16±0.156	170.85±1.075	127.10±0.742	84.86±1.216
	55	28.27±0.085	125.73±1.216	159.92±0.827	93.52±0.735
1:40	25	28.06±0.064	104.89±0.170	76.29±1.068	86.90±1.273
	35	28.70±0.212	119.88±0.891	75.86±1.216	77.72±1.011
	45	24.06±0.049	130.34±0.654	71.03±1.050	89.41±1.132
	55	32.29±0.066	98.90±0.672	67.82±0.964	90.96±1.365

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

https://doi.org/10.15294/jstrp.v1i1.44348
ISSN 2776-0685

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[22,23]. The percent yield results for various variations are presented in Figure 1.

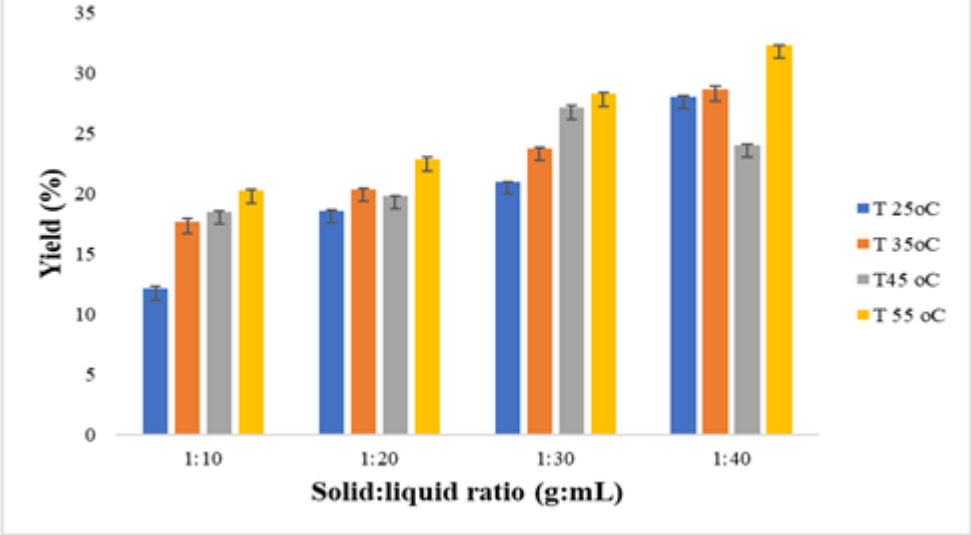

Figure 1. Effect of the solid-liquid ratio and temperature on extract yield.

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 $AlCl_3$ method with quercetin as a standard. The use of $AlCl_3$ in determining the total flavonoid content is based on the reaction of Al^{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[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[25].

https://doi.org/10.15294/jstrp.v1i1.44348
ISSN 2776-0685

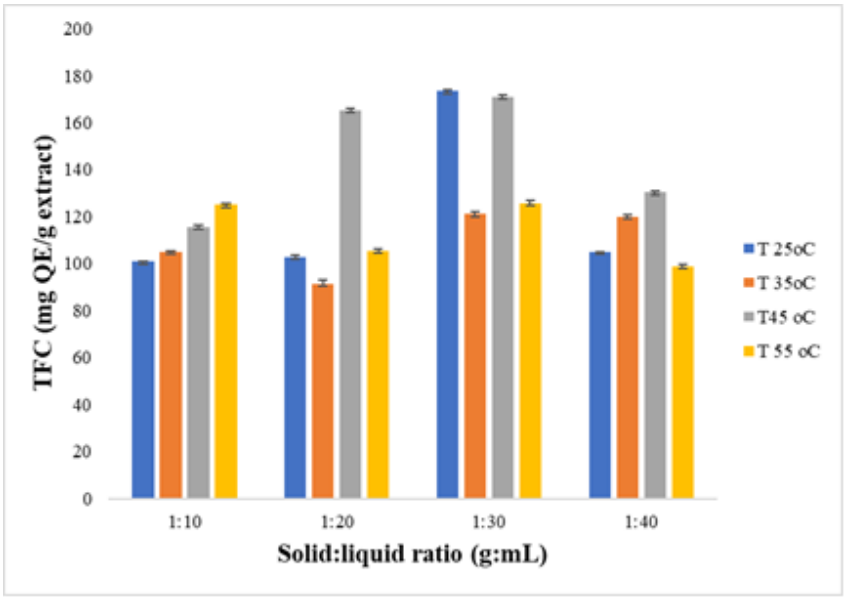

Figure 2. Effect of the solid-liquid ratio and temperature on total flavonoid content (TFC).

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[26]. The TPC results for various variations are presented in Figure 3.

The highest TPC value was obtained at 25^{o}C 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 (25-65^{o}C) and the extraction time variation (5-125 minutes). The results of the highest total phenolic content and antioxidant activity were obtained at 25^{o}C for 5 minutes because the chemical structure of phenolics was stable at room temperature[27].

https://doi.org/10.15294/jstrp.v1i1.44348
ISSN 2776-0685

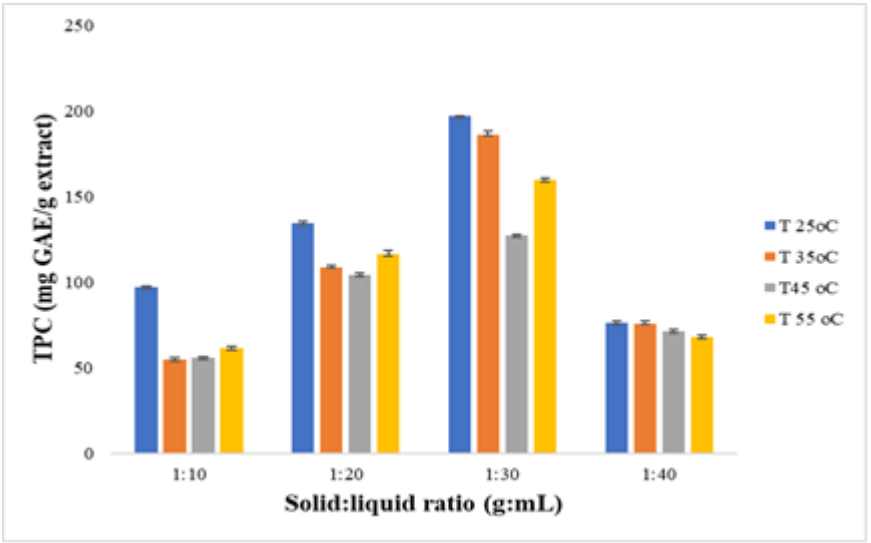

Figure 3. Effect of the solid-liquid ratio and temperature on total phenolic content (TPC).

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.

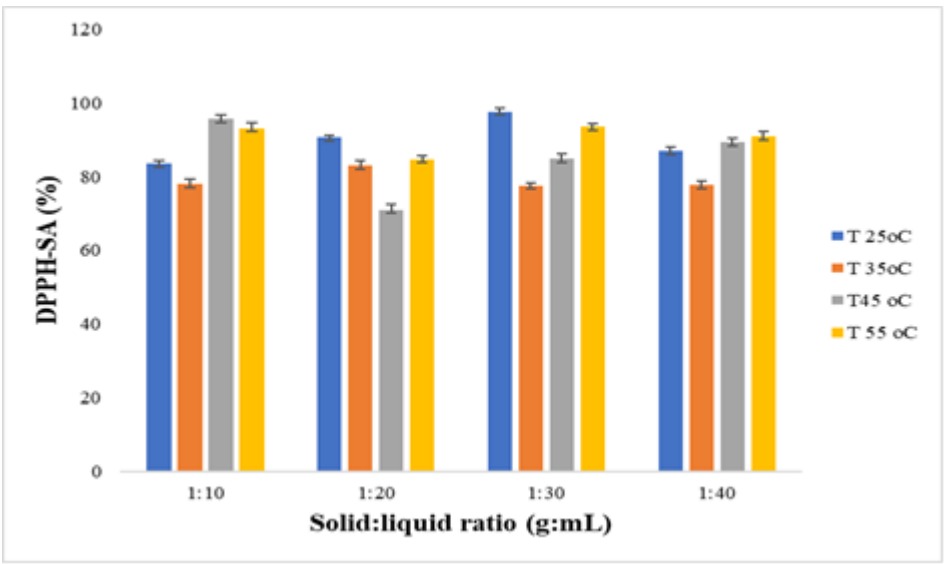

Figure 4. Effect of the solid-liquid ratio and temperature on DPPH scavenging activity.

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 increase[1]. 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 optimally[23,28].

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 IC_{50} 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 55ºC, 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 25ºC. The best extract measured antioxidant activity and IC_{50} 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 Nusaputera 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

AUTHOR DETAILS

[1]Chemistry Department, D3 Pharmacy, Sekolah Tinggi Ilmu Farmasi Nusaputera, Semarang, Indonesia

REFERENCES

1. Zin ZM, Abdul-Hamid A, Osman A. Antioxidative activity of extracts from Mengkudu (Morinda citrifolia L.) root, fruit and leaf. *Food Chemistry*. 2002;78(2):227-231. doi:10.1016/S0308-8146(01)00402-2
2. Mohamad Shalan NAA, Mustapha NM, Mohamed S. Chronic toxicity evaluation of Morinda citrifolia fruit and leaf in mice. *Regulatory Toxicology and Pharmacology*. 2017;83:46-53. doi:10.1016/j.yrtph.2016.11.022

3. Mandukhail S, Aziz N, Gilani A-H. Studies on antidyslipidemic effects of Morinda citrifolia (Noni) fruit, leaves and root extracts. *Lipids Health Dis*. 2010;9(1):88. doi:10.1186/1476-511X-9-88

4. Krishnaiah D, Bono A, Sarbatly R, Anisuzzaman SM. Antioxidant activity and total phenolic content of an isolated Morinda citrifolia L. methanolic extract from Poly-ethersulphone (PES) membrane separator. *Journal of King Saud University - Engineering Sciences*. 2015;27(1):63-67. doi:10.1016/j.jksues.2013.01.002

5. Piaru SP, Mahmud R, Abdul Majid AMS, Mahmoud Nassar ZD. Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia. *Asian Pacific Journal of Tropical Medicine*. 2012;5(4):294-298. doi:10.1016/S1995-7645(12)60042-X

6. Nayak BS, Isitor GN, Maxwell A, Bhogadi V, Ramdath DD. Wound-healing activity of Morinda citrifolia fruit juice on diabetes-induced rats. *Journal of Wound Care*. 2007;16(2):83-86. doi:10.12968/jowc.2007.16.2.27006

7. Kamiya K, Hamabe W, Harada S, Murakami R, Tokuyama S, Satake T. Chemical Constituents of Morinda citrifolia Roots Exhibit Hypoglycemic Effects in Streptozotocin-Induced Diabetic Mice. *Biol Pharm Bull*. 2008;31(5):935-938. doi:10.1248/bpb.31.935

8. Wang M-Y, Nowicki D, Anderson G, Jensen J, West B. Liver Protective Effects of Morinda citrifolia (Noni). *Plant Foods Hum Nutr*. 2008;63(2):59-63. doi:10.1007/s11130-008-0070-3

9. Yamaguchi S, Ohnishi J, Sogawa M, Maru I, Ohta Y, Tsukada Y. Inhibition of Angiotensin I Converting Enzyme by Noni(Morinda citrifolia) Juice. *Journal Of The Japanese Society For Food Science And Technology*. 2002;49(9):624-627. doi:10.3136/nskkk.49.624

10. Basar S, Uhlenhut K, Högger P, Schöne F, Westendorf J. Analgesic and antiinflammatory activity of *Morinda citrifolia* L. (Noni) fruit: Antiinflammatory Activity Of *Morinda Citrifolia*. *Phytother Res*. 2010;24(1):38-42. doi:10.1002/ptr.2863

11. Akihisa T, Matsumoto K, Tokuda H, et al. Anti-inflammatory and Potential Cancer Chemopreventive Constituents of the Fruits of *Morinda citrifolia* (Noni). *J Nat Prod*. 2007;70(5):754-757. doi:10.1021/np068065o

12. Rajivgandhi G, Saravanan K, Ramachandran G, et al. Enhanced anti-cancer activity of chitosan loaded Morinda citrifolia essential oil against A549 human lung cancer cells. *International Journal of Biological Macromolecules*. 2020;164:4010-4021. doi:10.1016/j.ijbiomac.2020.08.169

13. Sang S, Cheng X, Zhu N, et al. Flavonol Glycosides and Novel Iridoid Glycoside from the Leaves of *Morinda citrifolia*. *J Agric Food Chem*. 2001;49(9):4478-4481. doi:10.1021/jf010492e

14. Pokorny J, ed. *Antioxidants in Food: Practical Applications*. Reprint. Woodhead; 2003.

15. Li J, Niu D, Zhang Y, Zeng X-A. Physicochemical properties, antioxidant and antiproliferative activities of polysaccharides from Morinda citrifolia L. (Noni) based on different extraction methods. *International Journal of Biological Macromolecules*. 2020;150:114-121. doi:10.1016/j.ijbiomac.2019.12.157

16. Suktham K, Daisuk P, Shotipruk A. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia L. (Rubiaceae): Errata and review of technological development and prospects. *Separation and Purification Technology*. 2021;256:117844. doi:10.1016/j.seppur.2020.117844

17. Pak-Dek MS, Osman A, Sahib NG, et al. Effects of extraction techniques on phenolic components and antioxidant activity of Mengkudu (Morinda citrifolia L.) leaf extracts. *Journal of Medicinal Plants Research*. 2011;5:5050-5057.

18. Ghafoor K, Choi YH, Jeon JY, Jo IH. Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds, Antioxidants, and Anthocyanins from Grape (*Vitis vinifera*) Seeds. *J Agric Food Chem*.

2009;57(11):4988-4994. doi:10.1021/jf9001439

19. Buanasari B, Sugiyo W, Fitriani N, Suryaningsih S. Potential of Chitosan From Local Crab (Portunus Pelagicus) to Enhance Storability of Musa Paradisiaca L. *JBAT*. 2019;8(1):41-46. doi:10.15294/jbat.v8i1.16423
20. Das N, Islam ME, Jahan N, et al. Antioxidant activities of ethanol extracts and fractions of Crescentia cujete leaves and stem bark and the involvement of phenolic compounds. *BMC Complement Altern Med*. 2014;14(1):45. doi:10.1186/1472-6882-14-45
21. Shui G, Leong LP. Residue from star fruit as valuable source for functional food ingredients and antioxidant nutraceuticals. *Food Chemistry*. 2006;97(2):277-284. doi:10.1016/j.foodchem.2005.03.048
22. Bakti AA, Triyasmono L, Rizki MI. Penentuan Kadar Flavonoid Total dan Uji Antioksidan Ekstrak Etanol Daun Kasturi (Mangifera casturi Kosterm.) dengan Metode DPPH. *JPS*. 2017;4(1). doi:10.20527/jps.v4i1.5762
23. Buanasari, Palupi PD, Serang Y, Pramudono B, Sumardiono S. Development of ultrasonic-assisted extraction of antioxidant compounds from Petai (*Parkia speciosa Hassk.*) leaves. *IOP Conf Ser: Mater Sci Eng*. 2018;349:012009. doi:10.1088/1757-899X/349/1/012009
24. Wong YS, Sia CM, Khoo HE, Ang YK, Chang SK, Yim HS. Influence of extraction conditions on antioxidant properties of passion fruit (Passiflora edulis) peel. *Acta Sci Pol Technol Aliment*. 2014;13(3):257-265. doi:10.17306/J.AFS.2014.3.4
25. Suzery M, Lestari S, Cahyono B. Penentuan Total Antosianin Dari Kelopak Bunga Rosela (Hibiscus sabdariffa L) Dengan Metode Maserasi Dan Sokshletasi. *Jurnal Sains Dan Matematika*. 2010;18(1):1-6.
26. Alfian R, Susanti H. Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah (Hibiscus sabdariffa Linn) Dengan Variasi Tempat Tumbuh Secara Spektrofotometri. *Pharmaciana*. 2012;2(1). doi:10.12928/pharmaciana.v2i1.655
27. Sutanto H, Himawan E, Kusumocahyo SP. Ultrasound Assisted Extraction of Bitter Gourd Fruit (Momordica charantia) and Vacuum Evaporation to Concentrate the Extract. *Procedia Chemistry*. 2015;16:251-257. doi:10.1016/j.proche.2015.12.048
28. Handayani H, Sriherfyna FH, Yunianta. Ekstraksi Antioksidan Daun Sirsak Metode Ultrasonic Bath (Kajian Rasio Bahan : Pelarut Dan Lama Ekstraksi. *Jurnal Pangan dan Agroindustri*. 2016;4(1):262-272.