Pharmacogn J. 2022; 14(2): 432-438
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Optimization of Ultrasound-Assisted Extraction of *Andrographis paniculata* Nees Leaves, Phytochemical Screening, Total Phenolic Content and Anti-Gout Potential Activity

Ismail[1], Candra Irawan[2], Maman Sukiman[3,*], Imalia Dwi Putri[2], Andita Utami[1], M. Ilham Kumala Zalni[3], Ratna Komala Putri[1]

Ismail[1], Candra Irawan[2], Maman Sukiman[3,*], Imalia Dwi Putri[2], Andita Utami[1], M. Ilham Kumala Zalni[3], Ratna Komala Putri[1]

[1]Department of Chemical Analysis, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.
[2]Department of Food Nanotechnology, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.
[3]Department of Industrial Waste Treatment, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.

Correspondence

Maman Sukiman

Department of Industrial Waste Treatment, Politeknik AKA Bogor, Jalan Pangeran Sogiri, Tanah Baru, Bogor 16154, INDONESIA.

E-mail: maman@aka.ac.id

History

- Submission Date: 01-03-2022;
- Review completed: 15-03-2022;
- Accepted Date: 21-03-2022.

DOI : 10.5530/pj.2022.14.55

Article Available online

http://www.phcogj.com/v14/i2

ABSTRACT

Gout is a type of arthritis that causes painful inflammation in one or more joints. In gout, an increase in uric acid in the blood triggers the formation of crystals, causing joint pain. Indonesia is a country rich in the biodiversity of medicinal plant species. Therefore, its flora offers promising therapy for gout, one of which is *Andrographis paniculata* Nees. This study aims to obtain the leaf extract of *A. paniculata* through the application of ultrasound-assisted extraction (UAE) with variations in time and amplitude to produce optimal extraction conditions. Then the extract obtained was subjected to phytochemical screening, a total phenolic content test and uric acid test. The results of phytochemical screening of *A. paniculata* leaf extract using UAE contained saponins, phenols, tannins and alkaloids. The high total phenolic content has an effect on the high potential for reducing uric acid levels. Sample B with a time variation of 35 minutes and an amplitude of 65% showed the highest total phenolic content and potential for reducing uric acid levels compared to the other samples, which were 1104.53 ± 0.5 mg GAE/g extract and 72.81±0.2 %, respectively. From the results of the study, it can be concluded that the UAE extract from the leaves of *A. paniculata* has good potential as an anti-gout agent.

Key words: *Andrographis paniculata* Nees, Anti-gout, Phytochemical screening, Total phenolic content, Ultrasound-Assisted Extraction.

INTRODUCTION

Gout has become more common in the last 50 years, particularly in developing country.[1] There were 41.2 million prevalent instances of gout worldwide. According to the 2007-2016 National Health and Nutrition Examination Survey (NHANES) data estimated the prevalence of gout among African Americans, Caucasians and Hispanics to be 4.8 percent, 4 percent and 2 percent, respectively, when stratified by race.[2] Asians are 2.7 times more likely than Caucasians to be diagnosed with gout.[3] Gout frequency varies widely among Asian countries and new data from China and South Korea show that it is on the rise.[4] While in developing countries such as Indonesia, cases of acid veins are increasing year by year. That matter is supported by Riskesdas data in 2018, which shows the prevalence of gout disease when viewed from the characteristics of age, with a high prevalence at the age of 75 years (54.8%).[5]

Gout is a kind of inflammatory arthritis caused by crystals of monosodium urate (MSU) interacting with tissue.[6] Purine catabolism is catalyzed by the enzyme xanthine oxidase.[7] The oxidative hydroxylation of hypoxanthine to xanthine to uric acid is catalyzed by xanthine oxidase, resulting in severe inflammation.[8] Uricase is an enzyme that catalyzes the further conversion of uric acid to the highly soluble allantoin discharged in the urine. Unfortunately, uricase is not a functioning human enzyme, which can lead to hyperuricemia.[9] Tophi, joint abnormalities and kidney stones have also been linked to gout.[10,11] Gout can be treated with a variety of medications, including colchicine, steroids, nonsteroidal anti-inflammatory medicines (ibuprofen, naproxen, indomethacin and aspirin), cyclooxygenase 2 (COX-2) inhibitors (etoricoxib) and allopurinol.[12,13] For example, allopurinol, the most commonly used xanthine oxidase inhibitor for gout,[14] produces nephrolithiasis, hypersensitivity response, Stevens-Johnson syndrome, renal damage, allergic reactions and deadly liver necrosis.[15,16]

Recently, there has been renewed interest in treating disease using medicinal plants[17] and research on medicinal plants has grown internationally[18,19] owing to their propensity to contain chemical components that can have physiological effects on the human body. Traditional medicine developed from numerous medicinal plants is now widely explored and utilized as a foundation for discovering novel molecules to treat a variety of ailments.[20] One of the native plants of Indonesia is *Andrographis paniculata* Nees which is empirically believed to have antioxidant, antidiabetic, anti-inflammatory and anti-hyperlipidemic activity.[21,22] Furthermore, studies show that secondary metabolites of this plant, such as tannins, saponins, phenolic compounds, flavonoids, terpenoids and alkaloids,[23] play an important role as antioxidants, anti-inflammatory, antibacterial, cytotoxic agents and anti-gout.[24,25]

The selection of an extraction process to produce plant extracts is a vital initial step in the research of medicinal plants. Various extraction techniques have been developed to acquire procedures that are

Cite this article: Ismail, Irawan C, Sukiman M, Putri ID, Utami A, Zalni MIK, et al. Optimization of Ultrasound-Assisted Extraction of *Andrographis paniculata* Nees Leaves, Phytochemical Screening, Total Phenolic Content and Anti-Gout Potential Activity. Pharmacogn J. 2022;14(2): 432-438.

more environmentally friendly, minimize solvent consumption, avoid chemical degradation due to heat use, shorten extraction periods, boost reaction speeds and increase extract yield and quality.[26-28] Ultrasonic sonification is one of them.[29,30] The use of ultrasonic waves delivered through the solvent induces a cavitation effect, which provides a mechanical effect, allowing the solvent to penetrate deeper into the sample matrix and increasing the contact surface area between the solid and liquid phases. As a result, the solute quickly diffuses from the solid phase into the solvent.[31-33]

The anti-gout activity of *A. paniculata* leaf extract using the UAE method has not been reported. In this study, the UAE method will be used to make this extract with variations in time and amplitude conditions to get the best conditions so that the total phenolic content and the highest anti-uric acid activity results are obtained.

METHODS

Simplicia setup

The plant was identified in the Herbarium Bogoriense, Botanical Field of the National Research and Innovation Agency, Cibinong, Bogor Regency, West Java, based on the accuracy of its identity. *Andrographis paniculata* Nees leaf samples were obtained in Sukabumi, West Java (Figure 1). Simplicia was blended, then stored in a closed, dry container that was labeled and kept out of direct sunlight.

Extraction of simplicia

The technique pertains to Irawan's research,[34] which was conducted utilizing the UAE method. An amount of 7 grams of dry leaf powder was weighed and placed in a 250 mL beaker glass, with 4 repetitions. The solvent was then added, along with 70 percent of technical ethanol, until the dry leaf powder was completely immersed and everything was well combined. Simplicia were extracted with UAE using time (minutes) and amplitudo (%) variation at 30 minutes-60% (A), 35 minutes-65% (B), 45 minutes-60% (C) and 45 minutes-65% (D). The liquid extract was separated from the dregs by filtering the extraction findings, which were then placed in a weighted beaker. Each beaker has a capacity of 250 mL. To acquire the A. *paniculata* leaf extract yield, the filtrate in the 250 mL beaker was removed by removing the ethanol solvent by evaporation using an oven at a temperature setting of 40 °C and leaving it until all of the ethanol had evaporated. The extract was weighed and the percent yield value was computed.

Figure 1: The leaves of *Andrographis paniculata* nees.

Phytochemical screening

Phytochemical screening of the UAE extract of *A. paniculata* leaves was performed at each modification of time (minutes) and amplitude (percent). The phytochemical screening tests comprised alkaloids, flavonoids, phenols, saponins and tannins.

Total phenolic

The total phenolic content of the extract was determined by the Folin-Ciocalteu method, referring to a previous study conducted by Irawan.[35] A total of 400 µL of crude extract (1 mg/mL) was pipetted and put into a 10 mL volumetric flask. Then add distilled water up to 5 mL, homogenized, then add 1 mL of Folin–Ciocalteu reagent, homogenized and allowed to stand for 3 minutes. Then 2.5 mL of 10% (w/v) sodium carbonate was added and homogenized again. The mixture was allowed to stand for 60 minutes in the dark and the absorbance was measured by a visible spectrophotometer at a wavelength of 650 nm. The total phenolic content was calculated from the gallic acid calibration curve (concentrations 2, 4, 6 and 8 mg/L). The result is expressed as mg of gallic acid equivalent per gram of dry weight. The gallic acid calibration curve was used to calculate the total phenolic content. The result is reported in milligrams of gallic acid equivalent per gram of dry weight.

Uric acid test

A sample of mother liquor with a concentration of 1,000 mg/L was obtained by weighing 5 mg of extract, then dissolving it with methanol pa in a 5 mL volumetric flask. The solution was pipetted to 40 µL, the solution was transferred to a 5 ml volumetric flask, 40 µL of 6 mg/dL uric acid standard was added, and the flask was allowed to stand for 5 minutes. The solution was treated for 5 minutes with 0.25 mL of 1 TBHBA uric acid reagent before being treated for 30 minutes with 62.5 mL of 1 TBHBA uric acid reagent at 20-25 °C. A visible spectrophotometer was used to measure the absorbance of the solution at 513 nm of absorption. The same procedure was used on uric acid standards, allopurinol as a positive control and blanks. The absorbance was measured and the uric acid content of the sample was estimated.[35]

RESULT AND DISCUSSION

Ultrasonic-Assisted Extraction

Extraction of *A. paniculata* leaves using the UAE method with 70% ethanol as the solvent. During the extraction, variations are made to the amplitude and time parameters used. The amplitude variations used are 60% and 65%, and the time variations are 30, 35 and 45 minutes. Table 1 shows the extraction results of *A. paniculata* leaves.

Table 1 shows that the variation of the time parameter with amplitude causes an increase in the extraction yield. A yield of 8.13% was produced in 35 minutes at 65% amplitude. The increase in extraction time and amplitude allows more evidence to enter the cell, causing the cell wall to break more easily, so that more substance can be extracted.[36]

Phytochemical screening

Table 2 shows the results of a phytochemical screening test for an ethanolic extract of *A. paniculata* leaves with varying duration and amplitude. The phytochemical screening revealed that saponins, phenols, tannins and alkaloids are present in the ethanolic extract of the *A. paniculata* leaves.

Saponins have been found to have antioxidant properties.[37] Several biological effects of phenolic compounds have been investigated, including antioxidant, anti-carcinogenic, alpha-glucosidase activity inhibitor, anti-inflammatory and free radical scavenging properties.[38,39] Tannins have been shown to have strong antioxidant, immunomodulatory and antibacterial properties.[40] Because alkaloids

Table 1: The yield (%) of crude extract from UAE methods.

Sample	The Various parameter of Time (minutes)-amplitude (%)	Sample Weight (g)	Crude Extract Weight (g)	Yield (%)
A	30-60	21.1245	1.5419	7.30
B	35-65	21.4867	1.7477	**8.13**
C	45-60	21.5213	1.9120	8.88
D	45-65	21.2238	1.6797	7.91

Table 2: Phytochemical screening results of ethanolic extract of *Andrographis paniculata*.

No	Phytochemical groups	Various parameter			
		Time (minutes) – Amplitude (%)			
		30 – 60 A	35 – 65 B	45 – 60 C	45 – 65 D
1	Saponins	++	++	+	++
2	Phenols	++	++	++	++
3	Tannin	++	++	++	++
4	Flavonoids	-	-	-	-
5	Alkaloids				
	• Dragendrof's	+	+	+	+
	• Mayer	+	+	+	+

Description: ++++ = Very Strong Reaction, +++ = Strong Reaction
++ = Medium Reaction, + =Weak Reaction, - = No Reaction

Table 3: Total phenolic content of ethanolic extract of *Andrographis paniculata* Nees leaves.

Sample	Total Phenolic Content (mg GAE/g extract)
A (30 minutes - 60%)	842.20 ± 0.6
B (35 minutes - 65%)	**1104.53 ± 0.5**
C (45 minutes - 60%)	988.97 ± 0.2
D (45 minutes - 65%)	889.39 ± 0.5

Table 4: The reduction of uric acid (%) after incubated with allopurinol and various of *Andrographis paniculata* Nees leaf extract.

Sample	Absorbance	Concentration of Uric Acid (mg/L)		Reducing Uric Acid Level (%)
Uric Acid Standard (0.5 mg/L)	0.0602	0.5000		
		react with sample	the rest of reaction	
Allopurinol (0.5 mg/L)	0.0584	0.0146 ± 0.0004	0.4853 ± 0.0004	2.92 ± 0.09
A (30 minutes - 60%)	0.0189	0.3432 ± 0.0004	0.1567 ± 0.0004	68.65 ± 0.09
B (35 minutes - 65%)	0.0164	0.3640 ± 0.0008	0.1360 ± 0.0008	**72.81 ± 0.2**
C (45 minutes - 60%)	0.0183	0.3477 ± 0.0003	0.1522 ± 0.0003	69.55 ± 0.07
D (45 minutes - 65%)	0.0220	0.3171 ± 0.0002	0.1828 ± 0.0002	63.43 ± 0.03

have been shown to have a variety of pharmacological actions, such as anticancer, antihyperglycemic and antibacterial antioxidant activity, they are frequently utilized as natural remedies to treat a variety of ailments.[41]

Andrographis paniculata is a good source of phytochemicals like saponins, phenolics, flavonoids, alkaloids and tannins. These phytochemicals play an important role in promoting pharmaceutical drug preparation and are used for curing various health ailments.[42]

Phenolic content of ethanol extract

Total phenolic content (TPC), as determined by the Folin-Ciocalteu method, was reported as gallic acid equivalents (mg GA/g sample). This analysis was used to investigate contribution in antioxidant activity of the plant extracts. The standard curve of gallic acid can be seen in Figure 2 with the linear regression equation y = 0.0961x + 0.1079 and an R^2 value of 0.9781.

Table 3 shows the total phenol concentration in *A. paniculata* leaves with variations and amplitudes calculated from the preceding equation. It can be noted that sample B had the greatest total phenol concentration when compared to the other samples, with 1104.53 ± 0.5 mg GAE/g extract. This high concentration is linked to the findings of phytochemical screening, namely tannin compounds. Tannins are polyphenol-containing macromolecular molecules.[42]

The presence of active metabolites such as phenol and flavonoid concentration in plant extract is determined by the solvent utilized. Plant phenolic compounds have an aromatic ring with one or more hydroxyl groups.[43] It is composed of two benzene rings separated by a propane unit. Because of their excellent structural chemistry, they may scavenge harmful free radicals such as super oxide and hydroxyl radicals as a result of their scavenging activity, or chelating process, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory actions, antioxidants provide protection against cardiovascular disease, certain types of cancer and age-related degeneration of cell components.[44]

Antigout potential activity

To investigate the capacity of *A. paniculata* leaf extract to decrease uric acid, anti-uric acid activity was performed *in vitro* using TBHBA

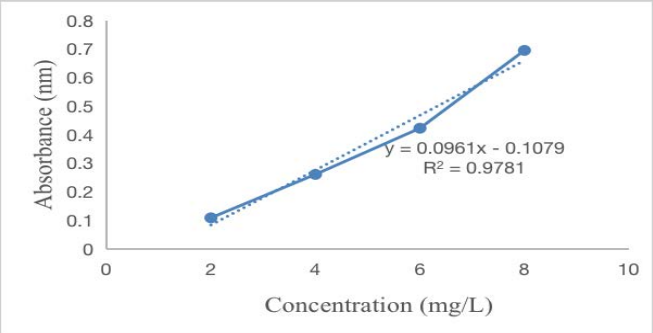


Figure 2: Curve for gallic acid used as a standard.

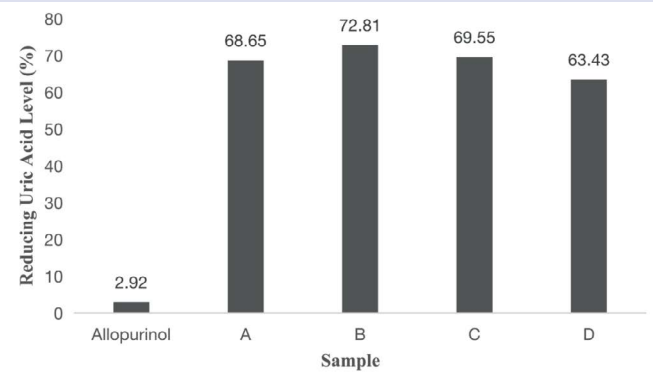


Figure 3: Graph of the reducing uric acid level (%) various of *Andrographis paniculata* Nees leaf extract where (A) with 30 minutes extraction time- 60% of amplitude, (B) with 35 minutes extraction time- 65% of amplitude, (C) with 45 minutes extraction time- 60% of amplitude, (D) with 45 minutes extraction time- 65% of amplitude.

reagent and pure uric acid as a standard. The uricase enzyme oxidizes uric acid to form allantoin and peroxide molecules, which serve as the foundation for the *in vitro* anti-uric acid test. The peroxidase product formed then interacts with 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to create a quinonemin compound that can be detected at 546 nm with a visible spectrophotometer.[45] Table 4 and Figure 3 illustrate the decrease in uric acid (percentage) following incubation with allopurinol and various *A. paniculata* leaf extracts.

Based on research, a variety of leaf extracts of *A. paniculata* may reduce uric acid better than allopurinol. The results of the uric acid test in sample B, with an extraction treatment of 35 minutes and an amplitude of 65%, showed a decrease in uric acid levels of 72.81 ± 0.2 %. In the previous test, sample B also had the highest yield and total phenol content compared to other samples, so sample B showed the highest potential as an anti-gout.

Polyphenols and flavonoids have been proven to reduce uric acid levels by acting as antioxidants, reducing free radicals and inhibiting numerous enzymes such as xanthine oxidase, cyclooxygenase and lipoxygenase.[46] Polyphenols are substrates for xanthine oxidase. Polyphenols inhibit uric acid production because xanthine oxidase preferentially oxidizes them rather than xanthine.[47]

CONCLUSION

Based on the results of the study, sample B of *A. paniculata* leaf extract with a time variation of 35 minutes and an amplitude of 65% resulted in the highest yield of 8.13%. The results of phytochemical screening of A. paniculata leaf extract using UAE contained saponins, phenols, tannins and alkaloids. The high total phenolic content has an effect on the high potential for reducing uric acid levels. Sample B showed the highest total phenol content and potential for reducing uric acid levels compared to the other samples, which were 1104.53 ± 0.5 mg GAE/g extract and 72.81±0.2 %, respectively. From the results of the study, it can be concluded that the UAE extract from the leaves of *A. paniculata* has good potential as an anti-gout agent.

ACKNOWLEDGEMENT

This work was supported by the Industrial Resources Development Agency of the Ministry of Industry, Indonesia.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Safiri S, Kolahi AA, Cross M, Carson-Chahhoud K, Hoy D, Almasi-Hashiani A, *et al*. Prevalence, Incidence and Years Lived with Disability Due to Gout and Its Attributable Risk Factors for 195 Countries and Territories 1990-2017: A Systematic Analysis of the Global Burden of Disease Study 2017. Arthritis Rheumatol. 2020;72(11):1916-1927.
2. Chen-Xu M, Yokose C, Rai SK, Pillinger MH, Choi HK. Contemporary Prevalence of Gout and Hyperuricemia in the United States and Decadal Trends: The National Health and Nutrition Examination Survey, 2007-2016. Arthritis Rheumatol. 2019;71(6):991-999.
3. Krishnan E, Lienesch D, Kwoh CK. Gout in ambulatory care settings in the United States. J Rheumatol. 2008;35(3):498-501.
4. Riskesdas K. Hasil Utama Riset Kesehata Dasar (RISKESDAS). Journal of Physics A: Mathematical and Theoretical. 2018;44(8):1-200.
5. Zeng SY. Changes in the prevalence of rheumatic diseases in Shantou, China, in the past three decades: a COPCORD study. PLoS ONE. 2015;10(9):e0138492.
6. Kuo CF, Grainge MJ, Zhang W, Doherty M. Global epidemiology of gout: prevalence, incidence and risk factors. Nat Rev Rheumatol. 2015;11(11):649-662.
7. Dalbeth N, Lauterio TJ, Wolfe HR. Mechanism of action of colchicine in the treatment of gout. Clin Ther. 2014;36(10):1465-1479.
8. Nile SH, Khobragade CN. *In vitro* anti-inflammatory and xanthine oxidase inhibitory activity of *Tephrosia purpurea* shoot extract. Nat Prod Commun. 2011;6(10):1437-1440.
9. Nile, SH, Kumar B, Park SW. *In vitro* evaluation of selected benzimidazole derivatives as an antioxidant and xanthine oxidase inhibitors. Chem Biol Drug Des. 2013;82(3):290-295.
10. Yamanaka H. The Guideline Revising Committee of the Japanese Society of gout and nucleic acid metabolism. Essence of the revised guideline for the management of hyperuricemia and gout. Japan Med Assoc J. 2012;55(4):324-329.
11. Teh CL, Chew KF, Ling GR. Acute gout in hospitalized patients in Sarawak general hospital. Med J Malaysia. 2014;69(3):126-128.
12. Nguyen MTT, Awale S, Tezuka Y, Le Tran Q, Watanabe H, Kadota S. Xanthine oxidase inhibitory activity of Vietnamese medicinal plants. Biol Pharm Bull. 2004;27(9):1414-1421.
13. Strazzullo P, Puig JG. Uric acid and oxidative stress: relative impact on cardiovascular risk. Nutr Metab Cardiovasc Dis. 2007;17(6):409-414.
14. Pacher P, Nivorozhkin A, Szabó C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev. 2006;58(1):87-114.
15. Wong YP, Ng RC, Chuah SP, Koh RY, Ling APK. Antioxidant and xanthine oxidase inhibitory activities of *Swietenia macrophylla* and *Punica granatum*, in International Conference on Biological, Environment and Food Engineering (Bali). 2014.

16. Kong LD, Yang C, Ge F, Wang HD, Guo YS. A Chinese herbal medicine Ermiao wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. J Ethnopharmacol. 2004;93(2-3):325-330.

17. Unno T, Sugimoto A, Kakuda T. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia speciosa* (L.) Pers. J Ethnopharmacol. 2004;93(2-3):391-395.

18. Tapsell LC, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS. Supplement-health benefits of herbs and spices: the past, the present, the future. Med J Aust. 2006;185(S4):4-24.

19. Triggiani V, Resta F, Guastamacchia E, Sabbà C, Licchelli B, Ghiyasaldin S. Role of antioxidants, essential fatty acids, carnitine, vitamins, phytochemicals and trace elements in the treatment of diabetes mellitus and its chronic complications. Endocr Metab Immune Disord Drug Targets. 2006;6(1):77-93.

20. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical Constituents of Some Nigerian Medicinal Plants. African Journal of Biotechnology. 2005;4(7):685-688.

21. Therasa S, Sobiya G, Parimala S. Leaves of *Andrographis paniculata* is an antioxidant and anticancer agent. Asian Journal of Pharmaceutical and Clinical Research. 2020;213-217.

22. Fardiyah, Qonitah, Taslim, Suyanta, Agus, Suprapto, *et al*. New potential and characterization of *Andrographis paniculata* L. Ness plant extracts as photoprotective agent. Arabian Journal of Chemistry. 2020;10:015.

23. Akbar S. *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Altern Med Rev. 2011;16(1):66-77

24. Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas AI, Hufeisen SJ, *et al*. Predicting new molecular targets for known drugs. Nature. 2009;462(7270):175-181.

25. Xie L, Xie L, Kinnings SL, Bourne PE. Novel computational approaches to polypharmacology as a means to define responses to individual drugs. Annu Rev Pharmacol Toxicol. 2012;52:361-379.

26. Bonfigle M, Godoy E, Reinheimer MA, Scenn NJ. Comparison between Conventional and Ultrasound-Assisted Techniques for Extraction of Anthocyanins from Grape Pomace. Journal of Food Enginering. 2017;207:56-72.

27. Zhang QW, Lin GL, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018;13:20.

28. Aziza NAA, Hashama R, Sarmidi MR, Suhaimi SH, Idris MKH. A review on extraction techniques and therapeutic value of polar bioactives from Asian medicinal herbs: Case study on *Orthosiphon aristatus*, *Eurycoma longifolia* and *Andrographis paniculata*. Saudi Pharmaceutical Journal. 2021;29(2):143-165.

29. Wang L, Weller CL. Recent Advances in Extraction of Nutraceuticals from Plants, Trends in Food Science & Technology. 2006;17:300-312.

30. Luna SRLD, Ramírez-Garza RE, Saldívar SOS. Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. Scientific World Journal. 2020;2020:1-38.

31. Płotka-Wasylka J, Rutkowska M, Owczarek K, Tobiszewski M, Namieśnik J. Extraction with environmentally friendly solvents. Trends in Analytical Chemistry. 2017;91.

32. Azmir J, Zainudin ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. Journal of Food Engineering. 2013;117:426-436.

33. Rostagno MA, Palma M, Barroso CG. Ultrasound Assisted Extraction of Soy Isoflflavones. Journal of Chromatography A. 2003;1012:119-128.

34. Irawan C, Elya B, Hanafi M, Saputri FC. Application of Ultrasound-Assisted Extraction on the Stem Bark of *Rhinachantus nasutus* (L.) Kurz, Total Phenolic, and Its Potential as Antioxidant and Inhibitor of Alpha-Glucosidase Enzyme Activity. Pharmacogn J. 2021;13(5):1-7.

35. Irawan C, Utami A, Styani E, Putri ID, Putri RK, Dewanta A, *et al*. Potential of Ethanolic Extract from Ripe *Musa balbisiana* Colla Fruit Using Ultrasound-Assisted Extraction as an Antioxidant and Anti-Gout. Pharmacognosy Journal. 2021;13(6):1332-1340.

36. Nishad J, Saha S, Kaur C. Enzyme- and Ultrasound-Assisted Extraction of Polyphenol from *Citur sinensis* (cv.Malta) peel : A Comparative Study. Journal of Food Processing and Preservation. 2019.

37. Yoshiki Y, Kudou S, Okubo K. Relationship Between Chemical Structures and Biological Activities of Triterpenoid Saponins from Soybean. Bioscience, Biotechnology and Biochemistry. 1998;62(12):2291-2299.

38. Chiou WF, Lin JJ, Chen CF. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with lipopolysaccharide. British Journal of Pharmacology. 1998;125(2):327-334.

39. Chiou WF, Chen CF, Lin CC. Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide. British Journal of Pharmacology. 2000;129(8):1553-1560.

40. Kataky A, Handique P. Micropropagation and screening of antioxidant potential of *Andrographis paniculata* (Burm. f) Nees. Journal of Hill Agriculture. 2010;1(1):13-18.

41. Hassan SB, Gullbo J, Hu K, Berenjian S, Morein B, Nygren P. The Nano-Particulate quillaja Saponin BBE is Selectively Active Towards Renal Cell Carcinoma. Anticancer Res. 2013;33(1):143-51.

42. Usman H, Osuji JC. Phytochemical and *in vitro* antimicrobial assay of the leaf extract of *Newbouldia leavis*. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(4):476-480.

43. Sulaiman SF, Sajak AAB, Supriatno KLO, Seow EM. Effect of solvents in extracting polyphenols and antioxidants of selected raw vegetables. Journal of Food Composition and Analysis. 2011;24(4-5):506-515.

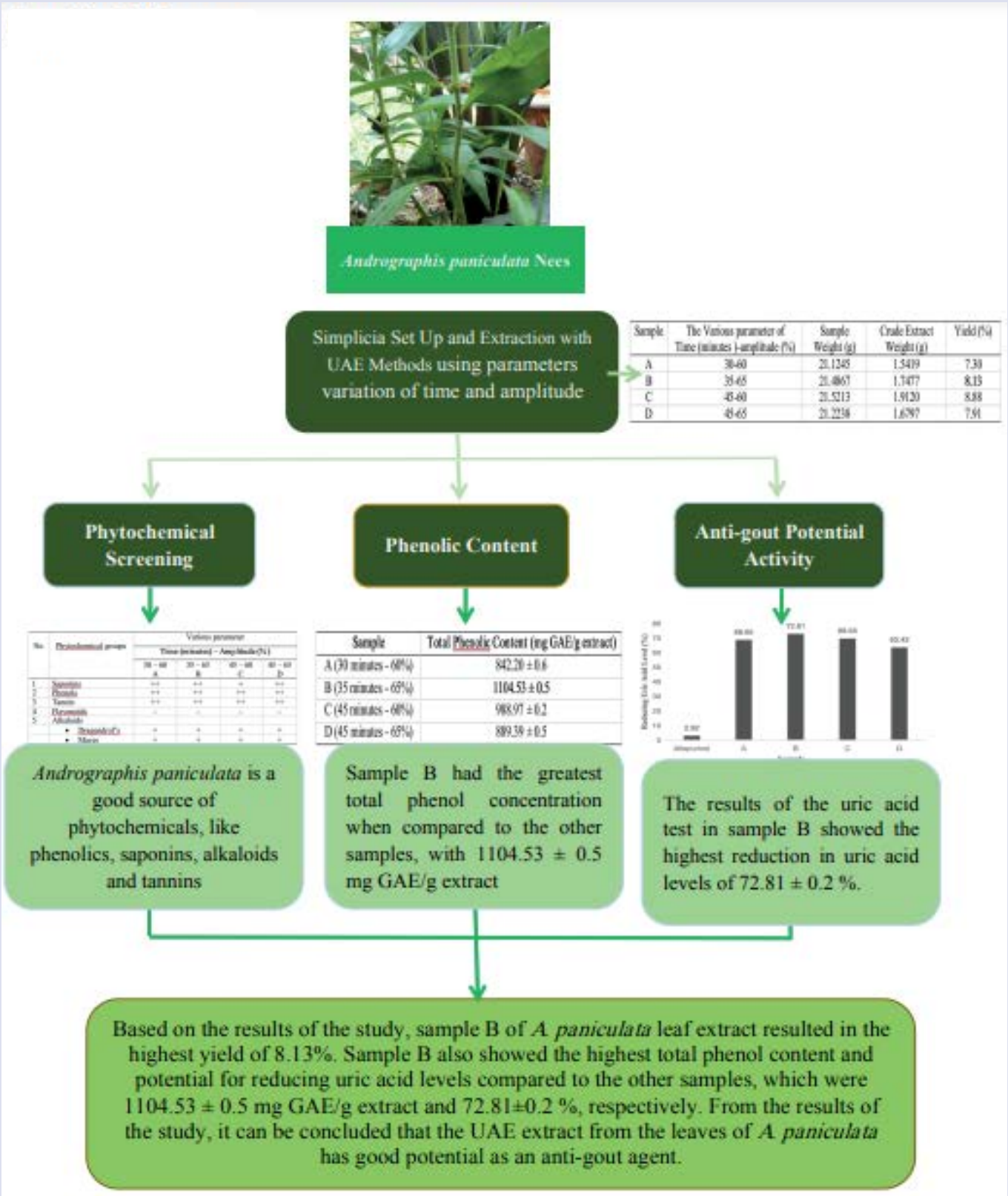
44. Clavin M, Gorzalczany S, Macho A, Munoz E, Ferraro G, Acevedo C, *et al*. Anti-inflammatory activity of flavonoids from *Eupatorium arnottianum*. Ethnopharmacological communication. 2007;112(3):585-589.

45. Styani E, Hanafi, Sulistiawaty L, Irawan C, Putri ID. Liquid Chromatograph-Mass Spectrophotometer and Anti Uric Acid Potential Studies of Ethyl Acetate Extract of *Archidendron bubalinum* (Jack) I.C. Nielsen Fruit Seed Shell. International Conference on Science and Technology. 2018;293-297.

46. Musaa KH, Abdullaha A, Subramaniamb V. Flavonoid Profile and Antioxidant Activity of Pink Guava. Skin. 2015;172(36b):308-357a.

47. Al-Azzawie HF, Abd SA. Effects of Crude Flavonoids from Ginger (*Zingiber officinale*), on Serum Uric Acid Levels, Biomarkers of Oxidative Stress and Xanthine Oxidase Activity in Oxonate-Induced Hyperuricemic Rats. International Journal. 2015;3(10):1033-1039.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Ismail is a Doctoral Natural Resources and Environmental Management Student at IPB University, Bogor, West Java 16143, Indonesia and Lecturer at Chemical Analysis Department, Politeknik AKA Bogor. He has research experience in field of Natural Resources and Modelling.

Candra Irawan is a Lecturer at the Department of Food Nanotechnology, Politeknik AKA Bogor, Indonesia. He has research experience in the field of Phytochemistry and Natural Product.

Maman Sukiman is a Lecturer at the Department of Industrial Waste Treatment, Politeknik AKA Bogor, Indonesia. He has research experience in the field of Natural Product and Chemical Environment.

Imalia Dwi Putri a lecturer at D-IV Department of Food Nanotechnology, Politeknik AKA Bogor, Indonesia. Research focus in functional foods, antioxidants from various plant extracts and halal food.

Andita Utami is a Lecturer at the Department of Chemical Analysis, Politeknik AKA Bogor, Indonesia. She has research experience in the field of Natural Product and Antioxidants.

M. Ilham Kumala Zalni is a College student at Politeknik AKA Bogor. He participates in research on natural products.

Ratna Komala Putri is a laboratory staff at Politeknik AKA Bogor. Ratna continuing her Postgraduate study at IPB University, Indonesia, majoring Food Science. She is focusing on research in the field of Functional Foods, Microbiology and Food Technology.

Cite this article: Ismail, Irawan C, Sukiman M, Putri ID, Utami A, Zalni MIK, et al. Optimization of Ultrasound-Assisted Extraction of *Andrographis paniculata* Nees Leaves, Phytochemical Screening, Total Phenolic Content and Anti-Gout Potential Activity. Pharmacogn J. 2022;14(2): 432-438.