Pharmacogn J. 2022; 14(2): 308-314
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Optimization of Ultrasound-Assisted Extraction of *Tinospora crispa* Stem, Phytochemical Screening, Total Phenolic Content and Anti Gout Potential Activity

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History

- Submission Date: 21-02-2022;
- Review completed: 06-03-2022;
- Accepted Date: 14-03-2022.

DOI : 10.5530/pj.2022.14.39

Article Available online

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

ABSTRACT

Aims: This study aimed to obtain the stem extract of *Tinospora crispa (L.)* Hook. f & Thomson through the application of ultrasound-assisted extraction (UAE) with variations in time and amplitude to produce optimal extraction conditions. The extract's potential as an anti-gout. **Results:** The yield of crude ethanol extract of *Tinospora crispa (L.)* Hook. f & Thomson obtained from the UAE process ranged from 4.49% to 10.60%. The phytochemical test results of *Tinospora crispa* stem extract contain saponins, tannins and alkaloids. Extract C was treated for 45 minutes and had an amplitude of 60% with a total phenolic content of 981.37 ± 0.7 mg/L and a reduction of uric acid level of 53.22 ± 0.1%. **Conclusion:** The ethanol extract of the *Tinospora crispa* stem from the UAE has the potential as a source of anti-gout.

Key words: *Tinospora crispa (L.)* Hook. f & Thomson Stem, Ultrasound-assisted extraction, Total phenolic content, Anti-gout potential activity.

INTRODUCTION

Gout prevalence varies greatly among Asian nations, and new statistics from China[1] and South Korea indicate that it is continuously growing. According to a 2017 survey, up to 1.1 percent of adults in China had Gout, with prevalence increasing significantly from 1.0 percent in 2000–2005 to 1.3 percent in 2010–2016.[2] In South Korea, the prevalence of gout in the general population increased from 0.35 percent in 2007 to 0.76 percent in 2015, with a further increase to 1.66 percent anticipated in 2025.[3] When uric acid levels in the blood surpass 6.8 mg/dL, monosodium urate (MSU) crystals develop and accumulate in joints, tendons, and other tissues. Gout is caused by crystal buildup.[4,5] Excessive uric acid levels can promote inflammation in blood vessel cells[6,7,9] and have been associated to hyperlipidemia, cardiovascular disease, hypertension, renal disease, diabetes, coronary microvascular dysfunction, and adverse outcomes in postmenopausal women.[4,8,9] Gout can be treated with a variety of therapies. Urate-lowering therapy, one of which involves the use of xanthine oxidase (XO) inhibitors, is the most effective.[10,11] Allopurinol and Febuxostat are the main drugs used to treat gout. However, adverse effects such as renal and gastrointestinal toxicity, cardiovascular safety, hepatitis, and allergic reactions have been reported frequently.[11,12] Consequently, it is imperative to obtain effective and safe naturally-based XO inhibitors for pharmaceutical applications.

Medicinal plants are beneficial to human health. This is due to the presence of chemical components in medicinal plants that can have physiological effects on the human body. Traditional medicine derived from a wide range of medicinal plants is now widely researched and used as a foundation for the discovery of new molecules to cure a wide range of illnesses.[13] *Tinospora crispa* is a native plant of Indonesia. Empirically, T. *crispa* is used for traditional medicine for various diseases such as atherosclerosis, diabetes mellitus, rheumatoid arthritis, analgesics, antipyretics, anti-inflammatory, anticoagulants, tonic, antiperiodic, stomatic and diuretic.[14,15] Furthermore, research indicates that this plant's secondary metabolites, such as alkaloids, flavonoids glycoside, phenolic compounds, saponins, tannins and terpenoids play an essential role as antioxidants, anti-inflammatory, antimalaria and cytotoxic agents.[15,16]

A critical first step in the study of medicinal plants is the selection of an extraction method to obtain plant extracts. One of the most popular extraction methods is maceration. The disadvantage of this method is that it requires a long extraction time as well as a large amount of solvent.[17-19] Various extraction techniques have been developed to obtain methods that are more environmentally friendly, reduce solvent use, prevent compound degradation due to heat use, shorten extraction times, increase reaction rates, and increase extract yield and quality.[20-22] Ultrasonic sonification is one of them.[23-25]

The use of ultrasonic waves passed through the solvent causes a cavitation effect, which produces 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 rapidly diffuses from the solid phase to the solvent.[26-29] The possibility of the targeted compound degrading is relatively low in this method.[20] In this study, the ultrasound-assisted extraction method will be used to prepare this extract. Variations in time and amplitude conditions were used in the extraction to obtain the best conditions for obtaining the highest total phenolic content and anti-uric acid activity yield.

Cite this article: Sukiman M, Irawan C, Ismail, Putri ID, Utami A, Dewanta A, et al. Optimization of Ultrasound-Assisted Extraction of *Tinospora crispa* Stem, Phytochemical Screening, Total Phenolic Content and Anti Gout Potential Activity. Pharmacogn J. 2022;14(2): 308-314.

METHODS

Simplicia setup

The correctness of the identity of the plants utilized was previously determined in the Herbarium Bogoriense, Botany Field of the National Research and Innovation Agency, Cibinong, Bogor Regency, West Java. Farmers in Bogor Regency, West Java, provided *Tinospora crispa* stem samples (Figure 1). Simplicia was crushed in a blender, then stored in a dry container, labeled, and kept out of direct sunlight.

Extraction of simplicia

The method refers to Irawan's research employing the UAE approach.[30] Seven grams of dried stem powder were weighed and placed in a 250 mL beaker glass four times. The solvent was then added, along with 70 percent of technical ethanol, and everything was vigorously mixed until the dry fruit peel powder was completely submerged. Fruit peel simplicia was extracted with UAE at 30 minutes-60 percent, 35 minutes-65 percent, and 45 minutes-65 percent, with time variation parameters (minutes) and amplitude (percent) set at 30 minutes-60 percent, 35 minutes-65 percent, and 45 minutes-65 percent, respectively. The liquid extract was separated from the dregs and deposited in a weighted beaker after filtering. Each beaker has a capacity of 250 milliliters. To achieve a higher yield, the ethanol solvent was removed from the filtrate in the 250 mL beaker by evaporation using an oven set at 40 °C and allowed to evaporate until all of the ethanol had evaporated. The percent yield value was derived by weighing the extract without the solvent.

Phytochemical screening

Phytochemical screening was performed using the UAE method on each variation of time (minutes) and amplitude (percent) of stem extraction. Alkaloids, flavonoids, phenols, saponins and tannins were among the phytochemical screening assays performed.[31]

Total phenolic

Based on prior work by Irawan *et al.*,[32] the Folin–Ciocalteu method was used to determine the total phenolic content of the extract. A total of 400 L of crude extract (1 mg/mL) was pipetted into a volumetric flask with a 10 mL capacity. Then add up to 5 mL of distilled water, homogenize, and then add 1 mL of Folin–Ciocalteu reagent, homogenize, and set aside for 3 minutes. Then 2.5 mL of sodium carbonate (10% w/v) was added and homogenized once more. The absorbance was measured at a wavelength of 650 nm after the mixture had been left in the dark for 60 minutes. The gallic acid calibration curve (concentrations of 0, 4, 6, 8 and 10 mg/L) was used to calculate the total phenolic content. The result is given in milligrams of gallic acid equivalent per gram of dry mass.

Uric acid test

The method used in this study refers to the method previously used by Irawan *et al.*[32] The mother liquor sample with a concentration of 1,000 mg/L was prepared by weighing 5 mg of extract and dissolving it in a 5 mL volumetric flask with methanol pa. The solution was pipetted to 40 µL, 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 with 0.25 mL of 1 TBHBA uric acid reagent for 5 minutes before being treated with 62.5 mL of 1 TBHBA uric acid reagent for 30 minutes at 20-25 °C. A visible spectrophotometer was used to test the solution's absorbance at 513 nm. The same procedure was applied to uric acid standards, allopurinol as a positive control and blanks. The absorbance was measured, and the uric acid level in the sample was determined.

RESULT AND DISCUSSION

Ultrasonic-Assisted Extraction

The UAE method was used to extract *Tinospora crispa* stem, with 70% ethanol as the solvent. Variations in the amplitude and time parameters used in the extraction are made during this stage. The amplitude variations used are 60% and 65%, and the time variations are 30, 35 and 45 minutes. Table 1 shows the results of stem extraction.

Table 1 shows that the variation of the time parameter with increasing amplitude causes an increase in yield. The research that has been done shows that the increase in time along with an increase in the amplitude can increase the extraction yield. The increase in extraction time and amplitude allows more solvent to enter the cell, causing the cell wall to break more easily. As a result, yields increase.[33]

Phytochemical screening

Table 2 shows the results of the phytochemical screening test for ethanolic stem extracts with various variations in time and amplitude. According to phytochemical screening results, saponins, tannins and alkaloids are among the compounds found in the ethanolic extract of *Tinospora crispa* stem.

Saponins have been shown to have antioxidant properties.[34] Tannins have been shown to have powerful antioxidant, immunomodulatory and antibacterial properties.[35-37] Alkaloids have been shown to

Figure 1: The stem samples of *Tinospora crispa*.

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

Sample	The Various parameter of Time (Minutes)-Amplitude (%)	Sample Weight (g)	Crude Extract Weight (g)	Yield (%)
A	30-60	104.4742	4.6868	4.49
B	35-65	104.5621	5.8558	5.60
C	45-60	104.5742	8.9568	8.57
D	45-65	104.6879	11.0933	10.60

Table 2: Phytochemical screening results of ethanolic extract of *Tinospora crispa* stem.

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

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

have a variety of pharmacological activities, including anticancer, antihyperglycemic and antibacterial antioxidant activities and they are widely used as natural medicines to treat a variety of diseases.[37-39]

Total phenolic

The Folin-Ciocalteu method was used in this study to determine total phenol levels in the stem of *Tinospora crispa*. The phosphomolybdic acid and phosphotungstic acid in the Folin-Ciocalteu reagent are reduced by phenolic compounds to form a bluish-purple complex molybdenum-tungsten combination.[40,41] 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. The total phenol content in the stem of T. *crispa* with various time and amplitude obtained from this equation could be seen in Table 3.

Table 3 shows that the total phenolic content in the stem of T. *crispa* for treatments A, B and D was relatively the same, while for treatment C it was lower. However, the total phenolic content identified from the 4 treatments was relatively high. This high content correlates with the results of phytochemical screening, namely the identification of tannin compounds. Tannins are macromolecular compounds from the polyphenol group.[42]

The total phenol content of plants is usually related to their antioxidant activity. Antioxidant activity increases with increasing phenol content.[43] Phenolic compounds can donate protons, so free radicals can be stabilized. These stable radicals are formed as a result of the resonance of the aromatic ring, which results in electron delocalization.[44,45]

Anti-uric acid potential activity test

An anti-uric acid activity was tested in vitro with TBHBA reagent and pure uric acid as a standard to determine the ability of T *crispa* stem extract to reduce uric acid. The uricase enzyme oxidizes uric acid into allantoin and peroxide compounds, which is the basis of the in vitro anti-uric acid test. The peroxidase compound produced then reacts with 2,4,6-tribromo-3-hydroxybenzoic acid (TBHBA) to produce a quinonemin compound that can be measured using a visible spectrophotometer at 546 nm.[46] The reduction of uric acid (%) after being incubated with allopurinol and various T. *crispa* stem extracts is shown in Table 4 and Figure 3.

According to the study's findings, various extracts of the T. *crispa* stem have the potential to reduce uric acid better than allopurinol. The results of the uric acid test for samples with treatments A, B, and C had relatively the same value (52%-53%), while the value for treatment D was smaller than the other three treatments, which was about 47%. The yields for treatments A and B have a value of 4-5%. While for treatment C, the yield was 8.57%. Based on this information, the optimum condition was treatment C, with an extraction time of 45 minutes and an amplitude of 60%.

Polyphenols and flavonoids have been shown to lower uric acid levels by acting as antioxidants and inhibiting free radicals, as well as inhibiting several enzymes, including xanthine oxidase, cyclooxygenase and lipoxygenase.[47] Polyphenols are xanthine oxidase substrates. Because xanthine oxidase preferentially oxidizes polyphenols rather than xanthine, the presence of polyphenols reduces uric acid production.[48]

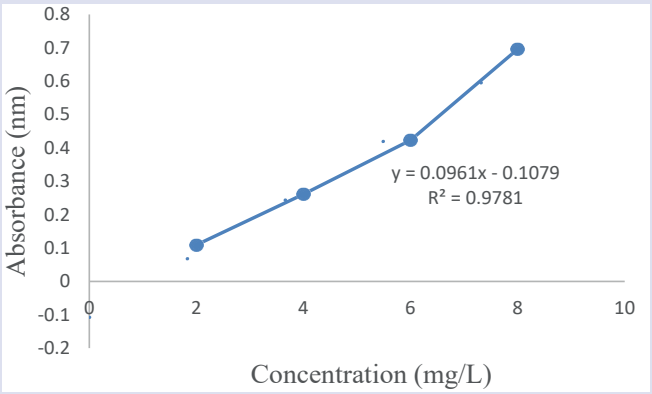

Figure 2: Curve for Gallic acid used as a standard.

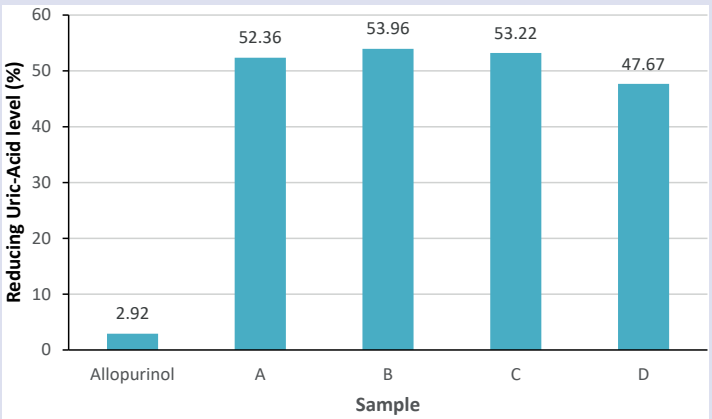

Figure 3: Graph of the reducing uric acid level (%) of Allopurinol and various of *Tinospora crispa* stem extract where (A) with 30 minutes extraction time- 60% of amplitude, (B) with 35 minutes extraction time- 65% of amplitude, (C) with 45 minutes extraction time- 60% of amplitude, (D) with 45 minutes extraction time- 65% of amplitude.

Table 3: Total phenolic content of ethanolic extract of *Tinospora crispa* stem.

Sample	Total Phenolic Content (mg GAE/g extract)
A (30 minutes - 60%)	1029.92 ± 0.3
B (35 minutes - 65%)	1023.20 ± 0.3
C (45 minutes - 60%)	981.37 ± 0.7
D (45 minutes - 65%)	1008.64 ± 0.6

Table 4: The reduction of uric acid (%) after incubated with allopurinol and various of *Tinospora crispa* stem 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	0.4854 ± 0.0004	0.01462 ± 0.0004	2.92 ± 0.09
A (30 minutes - 60%)	0.0070	0.2618 ± 0.0006	0.2382 ± 0.0006	52.36 ± 0.1
B (35 minutes - 65%)	0.0057	0.2698 ± 0.001	0.2302 ± 0.001	53.96 ± 0.2
C (45 minutes - 60%)	0.0056	0.2661 ± 0.0005	0.2339 ± 0.0005	53.22 ± 0.1
D (45 minutes - 65%)	0.0064	0.2383 ± 0.0009	0.2616 ± 0.0009	47.67 ± 0.2

CONCLUSION

Based on the results of the study, it can be concluded that the phytochemical test results of *Tinospora crispa* stem extract using UAE contain saponins, tannins and alkaloids. The total phenolic content is high and has a high potential for reducing uric acid. The optimum extraction of extract C was treated for 45 minutes and had an amplitude of 60% with a total phenolic content of 981.37 ± 0.7 mg/L and a reduction of uric acid level of 53.22 ± 0.1%.

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. 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.
2. Chen YZ. The prevalence of gout in mainland China from 2000 to 2016: a systematic review and meta-analysis. J Public Health. 2017;25:521-529.
3. Kim JW. Prevalence and incidence of gout in Korea: data from the national health claims database 2007-2015. Rheumatol Int. 2017;37(9):1499-1506.
4. Chen C, Lu J-M, Yao Q. Hyperuricemia-Related Diseases and Xanthine Oxidoreductase (XOR) Inhibitors: An Overview. Medical Science Monitor: International Medical Journal of Experimental and Clinical Research. 2016;22:2501.
5. Maiuolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of Uric Acid Metabolism and Excretion. International Journal of Cardiology. 2016;213:8-14.
6. Kubota Y, Demarco MM, Folsom AR. Serum Uric Acid, Gout and Venous Thromboembolism: The Atherosclerosis Risk in Communities Study. Thromb Res. 2016;144:144-148.
7. Liu S, Perez-Ruiz F, Miner JN. Patients with Gout Differ from Healthy Subjects in Renal Response to Changes in Serum Uric Acid. Joint Bone Spine. 2016;84(2):183.
8. Ren YC, Jin TB, Sun XD, Geng TT, Zhang MX, Wang L, *et al*. PDK2 and ABCG2 Genes Polymorphisms Are Correlated with Blood Glucose Levels and Uric Acid in Tibetan Gout Patients. Genet Molec Res. 2016;15(1).
9. Prasad M, Matteson EL, Herrmann J, Gulati R, Rihal CS, Lerman LO, *et al*. Uric Acid Is Associated with Inflammation, Coronary Microvascular Dysfunction, and Adverse Outcomes in Postmenopausal Women. Hypertension. 2017;69(2):236-242.
10. Ling X, Bochu W. A Review of Phytotherapy of Gout: The Perspective of New Pharmacological Treatments. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2014;69(4):243-56.
11. Aitken MJ. Developments in Gout Treatment: New Approaches to an Old Disease. 2017.
12. Wang Y, Zhang G, Pan J, Gong D. Novel in-Sights into the Inhibitory mechanism of Kaempferol on Xanthine Oxidase. J Agric Food Chem. 2015;63(2):526-534.
13. Darmawan J, Valkenburg H, Muirden K, Wigley R. The Epidemiology of Gout and Hyperuricemia in a Rural Population of Java. Journal of Rheumatology. 1992;19(10):1595-9.
14. Sinku R, Sinha MR. Preliminary phytochemical screening and physiochemical analysis of *Tinospora cordifolia* Miers. Journal of Medicinal Plants Studies. 2018;6(1):177-180.
15. Ahmad W, Jantan I, Bukhari SN. *Tinospora crispa* (L.) Hook. f. & Thomson: A review of its ethnobotanical, phytochemical, and pharmacological aspects. Frontiers in Pharmacology. 2016;7:59.
16. Agustina W, Nurhamidah N, Handayani D. Skrining fitokimia dan aktivitas antioksidan beberapa fraksi dari kulit batang jarak (*Ricinus communis* L.). Alotrop. 1(2).
17. 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.
18. Zhang QW, Lin GL, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine. 2018;13:20.
19. 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.
20. Wang L, Weller CL. Recent Advances in Extraction of Nutraceuticals from Plants, Trends in Food Science & Technology. 2006;17:300-312.
21. 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. 2020;2020:1-38.

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

23. 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.

24. Rostagno MA, Palma M, Barroso CG. Ultrasound Assisted Extraction of Soy Isoflavones, Journal of Chromatography A. 2003;1012(2):119-128.

25. Chemat F, Rombaut N, Sicaire A-G, Meullemiestre A, Tixier A-S-V, Vian MA. Ultrasonics Sonochemistry. 2017;34:540-560.

26. Rostagno MA, Palma M, Barroso CG. Ultrasound Assisted Extraction of Soy Isoflflavones, Journal of Chromatography A. 2003;1012(2):119-128.

27. Shirsath SR, Sonawane SH, Gogate PR. Intensification of extraction of natural products using ultrasonic irradiations-A review of current status. Chemical Engineering and Processing. 2012;53:10-23.

28. Li H, Pordesimo L, Weiss J. High Intensity Ultrasound-Assisted Extraction of Oil from Soybeans. Food Research International. 2004;37(7):731-738.

29. Dias ALB, Aguiara AC, Rostagno MA. Extraction of natural products using supercritical fluids and pressurized liquids assisted by ultrasound: Current status and trends. Ultrasonics Sonochemistry. 2021;74:1-20

30. 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.

31. Ciulei I. Practical Manuals on the Industrial Utilization of Chemical and Aromatic Plants: Methodology for Analysis of Vegetable drugs. Ministry of Chemical Industry, Bucharest (1st edition). 1982.

32. 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.

33. 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.

34. 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.

35. Oladunmoye MK. Immunomodulatory Effects of Ethanolic Extract of *Tridax procumbens* on Swiss Albino Rats Orogastrically Dosed with *Pseudomonas aeruginosa* (NCIB 950). Int J Trop Med. 2006;1(4):152-155.

36. Kang MH, Lee MS, Choi MK, Min KS, Shibamot T. Hypoglycemic Activity of *Gymnema sylvestre* Extracts on Oxidative Stress and Antioxidant Status in Diabetic Rats. J Agric Food Chem. 2012;60(10):2517-2524.

37. Jayashree P, Shalini M, Meenambiga SS, Suganya V. Phytochemical Screening and GC-MS Analysis of *Epiphyllum oxypetalum* Flower Extracts. Research J Pharm Tech. 2020;13(12):5893-5897.

38. Brand-Williams W, Cuvelier ME, Berset C. Use of a Free Radical Method to Evaluate Antioxidant Activity. LWT-Food Science and Technology. 1995;28(1):25-30.

39. 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.

40. Sundang M, Nasir S, Sipaut C, Othman H, Antioxidant Activity, Phenolic, Flavonoid, and Tannin Content of Piper betle and Leucosyke capitella. Malaysian Journal of Fundamental & Applied Sciences. 2012;8(1):1-6.

41. Sanchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velasquez DA. The Folin-Ciocalteu Assay Revisited: Improvement of its Specificity for Total Phenolic Content Determination, Analytical Methods. 2013;5(21):5990-5999.

42. Romadanu R, Hanggita S, Lestari S. Pengujian aktivitas antioksidan ekstrak bunga lotus (*Nelumbo nucifera*). Jurnal FishtecH. 2014;3(1):1-7.

43. Huang D, Ou B, Prior RL. The Chemistry Behind Antioxidant Capacity Assays, Journal of Agricultural and Food Chemistry. 2005;53:1841-1856.

44. Mathew S, Abraham TE, Zakaria ZA. Reactivity of Phenolic Compounds Towards Free Radicals Under *in vitro* Conditions, Journal of Food Science and Technology. 2015;52(9):5790-5798.

45. Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. 2015;30(1):11-26.

46. 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.

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

48. 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

Tinospora crispa Stem

Simplicia Set Up and Extraction with UAE Methods using parameters variation of time and amplitude

Sample	The Various parameter of Time (Minutes)-Amplitude (%)	Sample Weight (g)	Crude Extract Weight (g)	Yield (%)
A	30-60	104.4742	4.6868	4.49
B	35-65	104.5621	5.8558	5.60
C	45-60	104.5742	8.9568	8.57
D	45-65	104.6879	11.0933	10.60

Phytochemical Screening

Total Phenolic

Anti-Uric Acid Potential Activity Test

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

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

Sample	Absorbance	Concentration of Uric Acid (mg/L) react with sample	Concentration of Uric Acid (mg/L) the rest of reaction	Reducing Uric Acid Level (%)
Uric Acid Standard (0.5 mg/L)	0.0602		0.5000	
Allopurinol (0.5 mg/L)	0.0584	0.4854 ± 0.0004	0.01462 ± 0.0004	2.92 ± 0.09
A (30 minutes - 60%)	0.0070	0.2618 ± 0.0006	0.2382 ± 0.0006	52.36 ± 0.1
B (35 minutes - 65%)	0.0057	0.2698 ± 0.001	0.2302 ± 0.001	53.96 ± 0.2
C (45 minutes - 60%)	0.0056	0.2661 ± 0.0005	0.2339 ± 0.0005	53.22 ± 0.1
D (45 minutes - 65%)	0.0064	0.2383 ± 0.0009	0.2616 ± 0.0009	47.67 ± 0.2

Sample	Total Phenolic Content (mg GAE/g extract)
A (30 minutes - 60%)	1029.92 ± 0.3
B (35 minutes - 65%)	1023.20 ± 0.3
C (45 minutes - 60%)	981.37 ± 0.7
D (45 minutes - 65%)	1008.64 ± 0.6

The phytochemical test results of Tinospora crispa stem extract using UAE contain saponins, tannins, and alkaloids. The total phenolic content is high and has a high potential for reducing uric acid. The optimum extraction of extract C was treated for 45 minutes and had an amplitude of 60% with a total phenolic content

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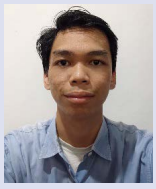

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Cite this article: Sukiman M, Irawan C, Ismail, Putri ID, Utami A, Dewanta A, *et al*. Optimization of Ultrasound-Assisted Extraction of *Tinospora crispa* Stem, Phytochemical Screening, Total Phenolic Content and Anti Gout Potential Activity. Pharmacogn J. 2022;14(2): 308-314.