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by Herman Herman

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Herman^{1,2}, Arsyik Ibrahim², Bakti Puji Rahayu², M Arifuddin¹, Yuspian Nur¹, Wisnu Cahyo Prabowo¹, Maryono³, Neneng Siti Silfi Ambarwati⁴, Laode Rijai¹, Islamudin Ahmad^{1,2,*}

Herman^{1,2}, Arsyik Ibrahim², Bakti Puji Rahayu², M Arifuddin¹, Yuspian Nur¹, Wisnu Cahyo Prabowo¹, Maryono³, Neneng Siti Silfi Ambarwati⁴, Laode Rijai¹, Islamudin Ahmad^{1,2,*}

¹Laboratory of Pharmaceutical Research and Development of TROPICAL PHARMACA, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, 75119 East Kalimantan, INDONESIA.

²Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, 75119 East Kalimantan, INDONESIA.

³Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Makassar, 90222 South Sulawesi, INDONESIA.

⁴Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, East Jakarta, 13220 DKI Jakarta, INDONESIA.

Correspondence

Islamudin Ahmad

Laboratory of Pharmaceutical Research and Development of Tropical Pharmaca, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, 75119 East Kalimantan, INDONESIA.

Phone no: +6281342205060

E-mail: islamudinahmad@farmasi.unmul.ac.id

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ABSTRACT

Applying green chemistry principles in the exploration of an active secondary metabolite from natural products has continued to experience a significant increase in the last decade, mainly natural deep eutectic solvent (NADES) usage. *Mitragyna speciosa* (Korth.) Havil (*Rubiaceae* family) is a native of East Kalimantan, which is traditionally used as an herb to treat headaches and inflammation by drinking boiled water a few leaves. On the other hand, this plant has an addictive effect. This study aims to determine the single factor effect of NADES citric acid-glucose-based microwave-assisted extraction (MAE) to obtain the yield of total polyphenols content (TPC) from *M. speciosa* leaves. Dried powder of *M. speciosa* leaves was extracted using NADES citric acid-glucose based MAE method with some different conditions such as NADES (citric acid-glucose) ratio (4:1, 5:1, 6:1 g/g), solid-liquid ratio (10:1, 15:1, 20:1 mL/g), microwave power (90, 270, 450 Watts), and extraction time (15, 20, 25 min). The determination of TPC was performed using Folin-Ciocalteu reagent and gallic acid as a standard with different concentration levels. The absorbance measured using spectrophotometer UV-VIS at 746 nm to obtain a regression formula of $Y = 0.0022X - 0.00095$, where $R^2 = 0.9977$. Based on the result, the best of single factor condition effect was obtained the TPC value including NADES ratio of 248.69 mg GAE/g sample (5:1 g/g citric acid-glucose), a solid-liquid ratio of 146.93 mg GAE/g sample (15:1 mL/g solvent-sample), microwave power of 192.20 mg GAE/g sample (270 Watts), and extraction time of 358.59 mg GAE/g sample (15 min). The single factor effect of NADES citric acid-glucose-based MAE shows a difference in TPC value based on various conditions of this method.

Key words: Citric acid-glucose, *Mitragyna speciosa* (Korth.) Havil, Microwave-assisted extraction, Natural deep eutectic solvent, Total polyphenols content.

INTRODUCTION

In the last decade, natural deep eutectic solvents (NADES) have emerged as potential alternative solvents to replace conventional organic solvents, toxic, volatile, and flammable. NADES has been described as a product of hydrogen bonds of two different types of compounds. The appropriate concentration ratio can cause an intense depression at the melting point compared with the one of a single component state.¹⁻⁴ NADES composition is derived from natural secondary metabolites such as amino acids, organic acids, amines, sugar alcohols, and sugars. It can also be combined with water with a certain number of molar ratios. NADES is formed based on the number and position of hydrogen bonds consisting of recipients of hydrogen bonds (HBA) and hydrogen bond donors (HBD). Both positions are very influential on the stability of NADES that are formed. In addition, the addition of water can affect properties such as biocompatibility, viscosity, conductivity, toxicity, and stability of the solvent.^{1,3,5-7}

A combination of citric acid and glucose is one type of NADES composition and successfully utilized as a green solvent to extract the target secondary metabolites from natural products, mainly from plants.^{8,9} Some studies have reported applying this solvent type, such as extraction of total polyphenols

and caffeine from coffee beans,¹⁰ extractions and determination of phenolics in *Cajanus cajan* leaves,¹¹ and extraction and separation of main bioactive flavonoids from *Radix Scutellariae*.⁸ The use of NADES combined with non-conventional extraction methods has proven to be more effective than using conventional solvents because of its non-flammable, non-volatile, non-toxic, biodegradability, environmentally friendly, and edible.^{2,3,12}

Mitragyna speciosa Korth. Havil (*Rubiaceae* family) is a native plant in Southeast Asia, mainly in Indonesia, Malaysia, Thailand, and Vietnam.¹³ In East Kalimantan, the local community uses this plant as an herb to treat headaches and inflammation by drinking boiled water a few leaves. Some studies of *M. speciosa* extract on rats are known to have an active anti-inflammatory of 200 mg/kg. This plant contains several types of glycosides, rich in polyphenols and terpenoids.^{14,15,16} Besides, this plant has activities that can affect the central nervous system due to contains secondary metabolites that have addictive effects, such as mitragynine, 7-hydroxy-mitragynine, painantein, speciesiin, and speciosiliatin.^{17,18} Application of NADES bases microwave-assisted extraction has not been reported.

In the present study, a single factor effect of NADES based microwave-assisted extraction on total polyphenols content extraction from *M. speciosa*

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was performed using different combination factors to obtain the best extraction condition. This study aims to determine the single factor effect of NADES citric acid-glucose-based microwave-assisted extraction to obtain the yield of total polyphenols content from this plant.

MATERIALS AND METHODS

Materials and equipment

M. speciosa leaves were collected from Melak, West Kutai, East Kalimantan, Indonesia. The voucher specimen was identified at the Laboratory of Dendrology Faculty of Forestry, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. Citric acid and sucrose were purchased from CV. Chlorogreen Bandung, West Java, Indonesia. Folin-Ciocalteu reagent, gallic acid standard, and sodium carbonate were purchased from Sigma Aldrich USA (via PT. Elo Karsa Utama, Indonesia). Meanwhile, the equipment was used, including Microwave domestic 900 Watts (Modena, USA), spectrophotometer UV-Vis, micropipette, and other glassware.

EXTRACTION PROCESS

NADES Preparation

In this study, a combination of citric acid and glucose as NADES (with three different ratios of 4:1, 5:1, and 6:1 g/g, respectively) were used according to the previous studies,^{10,19,20} with slight modification. Briefly, citric acid and glucose were weighed based on each different ratio. Both materials were melted at a specific temperature using a magnetic stirrer. After melting, aqua demineralization was added and homogenized. Subsequently, the obtained NADES solution was cooled at room temperature and filtered to obtain a homogeneous solution.

NADES Based Microwave-Assisted Extraction

A NADES citric acid-glucose-based microwave-assisted extraction was applied to obtain total polyphenols content (TPC) value from *M. speciosa* leaf using different condition factors based on some literature.^{9,10,19-24} Briefly, dried powder of *M. speciosa* leaf (5 g) was extracted using NADES citric acid-glucose base microwave-assisted extraction method using different conditions (including NADES ratio, solid-liquid ratio, microwave power, and extraction time) as can be seen in Table 1. Then, the extract solution and residue were separated using the Buchner funnel. The obtained extract solution was stored at room temperature and until ready to analyze.

Determination of Total Polyphenols Content

The total polyphenols content (TPC) value was determined using Folin-Ciocalteu reagent according to some literature.^{25,26,27,28,29,30,31} The absorbance was measured using spectrophotometer UV-Vis at 746 nm with a bit of adjustment. Briefly, the standard and sample solution (1 mL) were mixed with 5 mL of distilled water and 0.5 mL Folin-ciocalteu reagents, then homogenized for 5 min. Subsequently, a 2 mL NaCO₃ solution was added and homogenized, and incubated for 30 min until ready to analyze. A standard solution of gallic acid with various concentrations from 12.5 to 200 µg/mL was used to obtain the

linear regression formula of $Y = 0.0022X - 0.00095$, where $R^2 = 0.9977$. The TPC value of extract samples was determined using this formula.

RESULTS AND DISCUSSION

Single Factor Experiment on Process

In this study, an extraction method-based microwave-assisted extraction (MAE) was developed using a combination of citric acid and glucose as natural deep eutectic solvent (NADES) to extract the total polyphenol content from *M. speciosa* leaves. Based on studies that have been reported previously,^{8,10} some extraction condition factors (Table 1) that affect the ability to separate the target of secondary metabolite contained in the sample matrix. The extraction time and the microwave power can be adjusted to the modified domestic microwave device by adding a condenser to prevent the solvent from evaporating and reducing the pressure in the microwave during the extraction process. At the same time, the NADES ratio and the solid-liquid ratio were obtained from the combination of the two types of NADES and the comparison between samples and solvents. The use of NADES as an alternative solvent to replace conventional organic solvents because of its non-toxic nature, environmentally friendly, and inexpensive.^{3,32} The combination of the MAE method with NADES is very suitable for extracting polyphenol compounds in natural product plants.

Single Factor Effect of Natural Deep Eutectic Solvent-based Microwave-Assisted Extraction on Total Polyphenols Content

Effect of citric acid-glucose combination

In the present study, citric acid-glucose with the ratio of 6:1, 5:1, and 4:1 g/g were selected according to previous research,¹⁰ where both NADES components act as hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), respectively.^{33,34,35} The distilled water was added to accelerate NADES preparation and reduce viscosity. Meanwhile, other condition factors, such as solid-liquid ratio, microwave power, and extraction time, are carried out in constant form (10:1 mL/g, 450 watts, and 10 min, respectively).

Table 2 shows the measurement results of total polyphenol levels from each extract obtained in the range of 200 to 250 mg GAE/g sample. The three variations of the NADES concentration show that the 5:1 NADES ratio has a higher TPC value than the other ratios (Figure 1). It shows that the 5:1 g/g NADES ratio was the most optimum ratio, and these results were in accordance with studies that have been previously reported by Ahmad *et al.*, 2018¹⁰ and Wei *et al.*, 2015.¹¹

Effect of Solid-Liquid Ratio

The solid-liquid ratio is the one-factor ratio between the number of solid samples (dried simplicia) and the solvent. This factor has an essential role during the extraction process and is related to the contact area between the sample (solid) and solvent (liquid) that affects extraction efficiency. The contact area can reach optimum conditions when the liquid phase is saturated to solid. The use of large quantities of solvents can cause improvidence and much waste, while the use of small amounts results in an incomplete extraction process.^{36,37}

In this study, the ratio of 10:1, 15:1, and 20:1 mL/g with other factors in a constant state such as NADES ratio of 5:1 g/g, extraction time of 10 min, and microwave power of 450 Watts. As can be seen in Table 2 shows that the TPC value varies based on the difference in solid-liquid ratio. Optimum conditions were obtained at a solid-liquid ratio of 15:1 mL/g (Figure 2).

Table 1: Design experimental of single factor condition of NADES based microwave-assisted extraction on TPC value from *M. speciosa* leaves.

Factor variable	Unit	Range and level		
		Low	Medium	High
NADES Ratio	g/g	4	5	6
Solid - liquid ratio	mL/g	10	15	20
Microwave Power	Watts	90	270	450
Extraction Time	Minutes	10	15	20

Table 2: Effect of Citric acid – glucose ratio on TPC value.

Citric Acid-Glucose ratio (g/g)	Absorbance	TPC (mgGAE/g)	Average TPC (mgGAE/g)	Deviation Standard
4:1	0.075	228.08	232.07	4.57
	0.076	231.08		
	0.078	237.06		
5:1	0.082	261.30	248.69	10.92
	0.076	242.39		
	0.069	199.42		
6:1	0.071	205.13	203.23	2.29
	0.071	205.13		

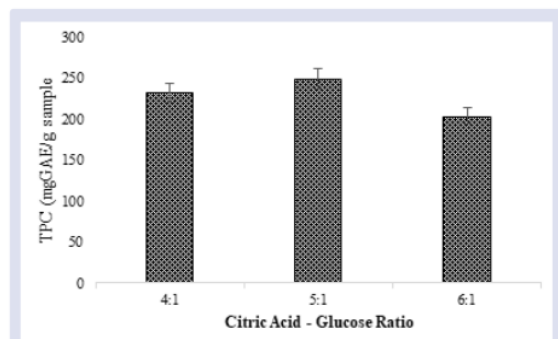


Figure 1: The optimum single factor of NADES composition ratio on polyphenols extraction from *M. speciosa* leaves.

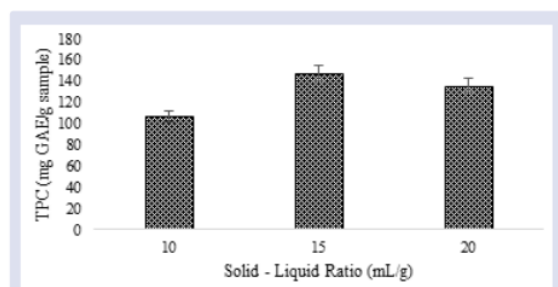


Figure 2: The optimum single factor condition of solid-liquid ratio on polyphenol extraction from *M. speciosa* leaves.

Effect of Microwave Power

The microwave power from the microwave-assisted extraction method can affect the rate of equilibrium and interactions between solvents and solutes (secondary metabolites) in the sample matrix and control the partition of solutes between the sample and the extraction phase.³³ To determine the effect of microwave power on the extraction efficiency of TPC, extraction was carried out at 90, 270, and 450 Watts, respectively, with an of 10 min extraction time, 5:1 g/g NADES ratio, and the solid-liquid ratio of 10:1 mL/g sample.

The results obtained (Table 4) show that the microwave power from 90 to 270 watts has an increased TPC value. This is due to the separation of the secondary metabolite constituents from the sample matrix due to the increase in temperature and pressure due to the microwave power. In contrast, the microwave power of 270 to 450 Watts tends to decrease due to the possibility of secondary metabolite compounds being

damaged due to excessive temperature and pressure. Figure 3 shows the optimum TPC value at 270 watts of microwave power because, in these conditions, there is an equilibrium between temperature and microwave pressure with the extraction process.

Effect of Extraction Time

In Table 5, the effect of extraction time was performed using different the time include 10, 15, and 20 min. In range 10-15 minutes of the efficiency of extraction time shows a tendency to increase in TPC value. The phenomenon indicates that an increase in extraction time can cause an acceleration of the mass transfer of the analyte and a decrease in the viscosity of the extraction solvent.^{38,39} Whereas, if the extraction time was extended by more than 15 min (precisely for 20 min), the TPC value has decreased. This was due to the high microwave power, and the longer extraction time can damage the structure of the components of the target secondary metabolite.⁴⁰ Therefore, According to Figure 4 shows that the optimum extraction time is obtained at the 15th min.

Based on this study's result, the single factor effect of NADES citric acid-glucose-based microwave-assisted extraction with different conditions includes NADES ratio, solid-liquid ratio, microwave power, and extraction time. These results are preliminary data to optimize the

Table 3: Effect of Solid-Liquid Ratio on TPC value.

Solid-Liquid ratio (mL/g)	Absorbance	TPC (mgGAE/g)	Average TPC (mgGAE/g)	Deviation Standard
10:1	0.057	107.25	106.63	1.071
	0.056	105.39		
	0.057	107.25		
15:1	0.035	158.70	146.93	11.113
	0.030	136.62		
	0.044	145.45		
20:1	0.023	145.59	135.457	12.657
	0.019	121.27		
	0.055	139.51		

Table 4: Effect of different microwave power on TPC value.

Microwave Power (Watt)	Absorbance	TPC (mgGAE/g)	Average TPC (mgGAE/g)	Deviation Standard
90	0.051	175.95	156.76	17.383
	0.041	142.08		
	0.053	152.24		
	0.065	194.16		
270	0.063	188.28	192.20	3.399
	0.065	194.16		
	0.059	188.84		
450	0.048	154.20	185.69	30.048
	0.058	214.04		

Table 5: Effect of different extraction time on TPC value.

Extraction Time (Minutes)	Absorbance	TPC (mgGAE/g)	Average TPC (mgGAE/g)	Deviation Standard
10	0.043	194.09	176.42	15.922
	0.036	163.18		
	0.038	172.01		
	0.085	371.56		
15	0.080	349.94	358.59	11.438
	0.081	354.26		
	0.039	194.09		
20	0.037	163.18	173.48	17.847
	0.036	173.261		

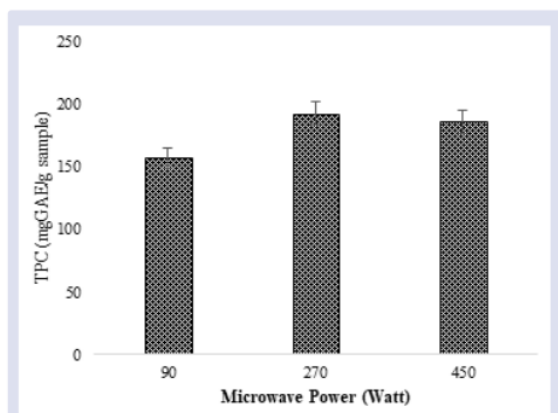


Figure 3: The optimum single factor condition of microwave power on polyphenols extraction from *M. speciosa*.

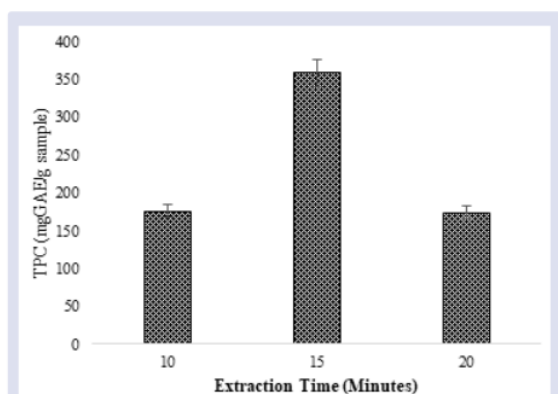


Figure 4: The optimum single factor of extraction time on polyphenols extraction from *M. speciosa*.

extraction method using the NADES based on microwave-assisted extraction method to obtain the optimum target secondary metabolite from *M. speciosa* leaves quickly, easily, efficiently, and environmentally friendly.

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CONFLICTS OF INTEREST

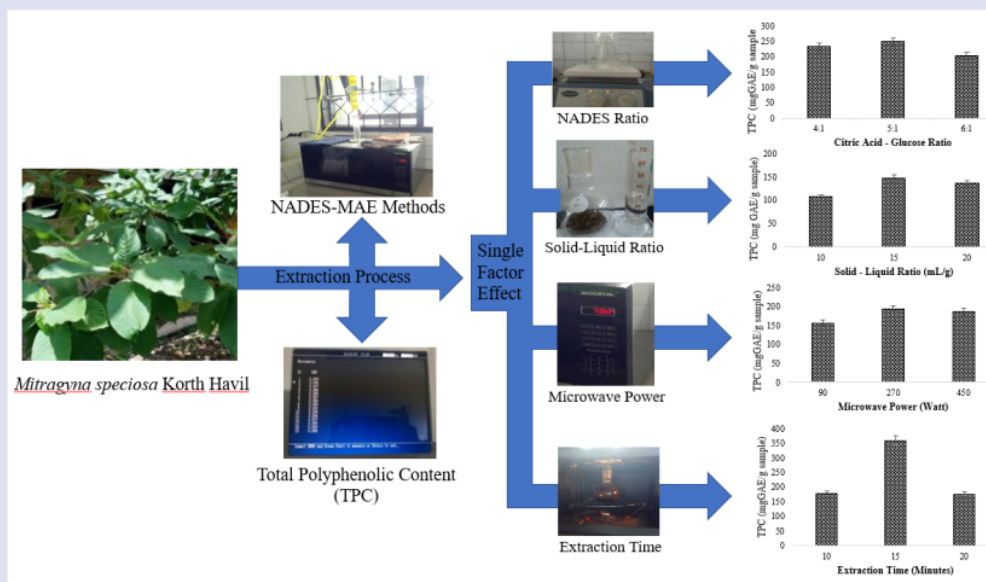
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GRAPHICAL ABSTRACT



SUMMARY

1. The best of single factor condition effect of NADES-MAE method was obtained the TPC value including:
 - NADES ratio of 248.69 mg GAE/g sample (5:1 g/g citric acid-glucose)
 - A solid-liquid ratio of 146.93 mg GAE/g sample (15:1 mL/g solvent-sample)
 - Microwave power of 192.20 mg GAE/g sample (270 Watts)
 - Extraction time of 358.59 mg GAE/g sample (15 min).
2. The single factor effect of NADES citric acid-glucose-based MAE shows a difference in TPC value based on various conditions of the NADES-MAE method.

ABOUT AUTHORS



Dr. Herman, a lecturer, and researcher at Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. Research interest in medicinal chemistry, Pharmaceutical Analysis, and natural product chemistry.



Arsyik Ibrahim, a doctoral candidate at Faculty of Pharmacy, Universitas Airlangga, Surabaya, East Java, Indonesia. As a lecturer and researcher at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Mulawarman University, East Kalimantan, Indonesia, he has experience in Pharmacognosy and Natural Products, working to search and discover drugs from nature, identification, and screening of anti-cancer and active antimicrobial.



Bakti Puji Rahayu, an undergraduate student at the Faculty of Pharmacy, Universitas Mulawarman. Research interest in natural product chemistry.



M. Arifuddin, a lecturer and researcher at Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. He has an interest in natural products and microbiology, especially isolation compound of endofit fungi from plants.



Yuspian Nur, a doctoral candidate at the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Bandung, West Java, Indonesia. As a lecturer and researcher at the Faculty of Pharmacy, Universitas Mulawarman. Research interest in the development of sensor and biosensors, also focusing on analytical chemistry.



Wisnu Cahyo Prabowo, a doctoral student at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Padjadjaran, Bandung, West Java, Indonesia. As a lecturer and researcher at Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan Indonesia. Research interest in Natural Products.



Maryono, a doctoral candidate at Postgraduate Program, Faculty of Pharmacy, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia. As a lecturer and researcher at the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar. Research interest in Natural Products Chemistry



Dr. Neneng Siti Silfi Ambarwati, a Lecturer and Researcher at Cosmetology Department, Faculty of Engineering, Universitas Negeri Jakarta, East Jakarta, Indonesia. The research focused on natural products for drug and cosmetic discovery and development, extraction technology, and cosmetic ingredients (cosmeceuticals).



Prof. Dr. Laode Rijai, Professor at Department of Pharmaceutical Sciences and Dean at Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. He has experience in the area of Natural products and Organic Chemistry.



Dr. Islamudin Ahmad, Associate Professor at Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, East Kalimantan, Indonesia. He has experience in Pharmacognosy and Natural Product Chemistry, working in drug discovery of natural products, green extraction engineering, isolation and identification of active compounds, screening activity, and mainly degenerative diseases

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