# Enhanced Extraction of Total Polyphenols Content from Mitragyna Speciosa (Korth.) Havil Leaves using the Natural Deep Eutectic Solvent-based Microwave-assisted Extraction Method

*by* Herman Herman

# Enhanced Extraction of Total Polyphenols Content from *Mitragyna Speciosa* (Korth.) Havil Leaves using the Natural Deep Eutectic Solvent-based Microwave-assisted Extraction Method

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Abstract: Exploration of natural products is highly dependent on separation techniques, mainly solvent selection, one of which is using the green chemistry principle approach. *Mitragyna speciosa* (Koth.) Havil is an endemic plant of East Kalimantan which traditionally used for the treatment of various diseases. On the other hand, this plant has an addictive effect. The study aims to determine the impact of using natural deep eutectic solvent-based microwave-assisted extraction (NADES-MAE) on total polyphenol content (TPC) extraction from *M. speciosa*. Natural deep eutectic solvent (NADES) made by malting two combination types include citric acid-glucose; choline chloride-sorbitol; malic acid-glucose; and lactic acid-sucrose. The extraction process was carried out using microwave-assisted extraction (MAE), and the determination of TPC was analyzed using Folin-Ciocalteau's reagent and measured with a spectrophotometer type including 246.70 mg GAE/g sample (citric acid-glucose), 227.33 mg GAE/g sample (malic acid-glucose), 222.26 mg GAE/g sample (lactic acid-sucrose), and 219.02 mg GAE/g sample (choline chloride-sorbitol). According to the results, the NADES-MAE method shows differences in TPC based on the NADES types.

## 1 INTRODUCTION

*Mitragyna speciosa* [Korth.] Havil belongs to the family Rubiaceae, which is an endemic plant of Southeast Asia. It is spread in several countries such as Thailand, Vietnam, Malaysia, and Indonesia (Hassan et al., 2013). In Indonesia, this plant commonly found on Kalimantan island, mainly in East Kalimantan. Local community uses the leaves of M. speciosa for traditional medicine either with chewed up, smoked like cigarettes, and brewed like tea.

*M. speciosa* leaves are traditionally believed to have several medicinal properties such as a wound, fever, muscle aches, reduce appetite and diarrhea (Hassan et al., 2013; Raini, 2017). It has been scientifically proven to have pharmacological effects such as analgesic, stimulant, antidepressant, anti-inflammatory, antinociceptive, antioxidant, and antibacterial activities. (Mossadeq et al., 2009; Parthasarathy et al., 2009; Luliana et al., 2018).

Besides, this plant is an export commodity for farmers in East Kalimantan. Although most European countries have banned their use because of the addictive effects of the compounds they have, such as mitragynine, 7-hydroxy-mitragynine, painantein, speciesiin, and speciosiliatin (Horie et al., 2005; Chittrakarn, Penjamras and Keawpradub, 2012; Henningfield et al., 2018), this plant is also rich in polyphenols, terpenoids, and several types of glycosides (Takayama, 2004; Tohar et al., 2016; Chittrakarn, Penjamras, and Keawpradub, 2012; Brown, Lund, and Murch, 2017).

In some countries such as Malaysia, Thailand, Myanmar, and Australia, this plant is illegal. Meanwhile, New Zealand, Romania, Finland,



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Germany, and Denmark are controlled and included in the Schedule 1 drug category (Saingam et al., 2013; Henningfield et al., 2018).

The exploration of active compounds from natural products is very dependent on the separation technique. One way is the approach of green chemistry principles. Natural deep eutectic solvent (NADES) is a green solvent that can be an alternative solvent to replace conventional organic solvents. NADES has an advantage compared to conventional solvents because it has low toxicity, biodegradability, biocompatible with many media, and edible (Savi et al., 2018; Gomez and Espino, 2018).

Some study have reported on the use of NADES as an alternative solvent and combined with non-conventional extraction methods (such as microwave, supercritical, and ultrasonic) namely extraction of caffeine and chlorogenic acid from coffee beans (Ahmad et al., 2018), baicalin extraction from *Sturellaria baicalencis* Gergi (Wang et al., 2018), alpha-cellulose, holo-cellulose, and acid-insoluble-lignin (Pan et al., 2017), anthocyanins (Dai et al., 2016), phenols extraction from *Cajanus cajan* (Wei et al., 2012) and olive cake, onion seed, tomato and pear (agro-food industrial by-products (Fernández et al., 2017), anthocyanins from *Catharanthus roseus* (Dai et al., 2016), resveratrol from *Morus alba* (Alishlah, Mun'in, and Jufri, 2019) and peanut (Chen et al., 2018), and so on. However, the extraction of total polyphenolic content from *M. speciosa* leaves has not reported.

In the present study, the extraction of total polyphenolic content (TPC) was performed by using NADES with some combination type different and combined with microwave-assisted extraction (MAE). The study aims to determine the effect of using natural deep eutectic solvent-based microwave-assisted extraction (NADES-MAE) on total polyphenol content (TPC) extraction from *M. speciosa* leaves.

# 2 MATERIALS AND METHODS

## 2.1 Materials

The sample of *M. speciosa* leaf was obtained from Melak, Kutai Barat, East Kalimantan, Indonesia and were authenticated at Laboratory of Dendrology, Faculty of Forestry, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. The sample specimen was achieved at the Laboratory of Pharmaceutical Research and Development "FARMAKA TROPIS," Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. Citric acid, lactic acid, sucrose, glucose, malic acid, choline chloride, sorbitol were purchased from CV. Chlorogreen, Bandung, West Java, Indonesia. Gallic acid standard, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Sigma-Aldrich, USA (via PT. Elo Karsa Utama, Indonesia).

## 2.2 Extraction Process

### 2.2.1 Preparation of Natural Deep Eutectic Solvent (NADES)

In this study, for screening of NADES was used including citric acid–glucose (CAG), malic acid–glucose (MAG), lactic acid–sucrose (LAS), and choline chloride–sorbitol (CCS) with ratio of 4:1 g/g, 1:2 g/g, 1:1 g/g, and 1:2 g/g, respectively. The NADES component is weighed according to each ratio, then melted on a magnetic stirrer hotplate. Aqua demineralization is added according to the number of comparisons used. The mixture is stirred until homogeneous. The solution is stored in a closed bottle (Ahmad et al., 2018).

### 2.2.2 Extraction using NADES

A natural deep eutectic solvent-based microwave-assisted extraction (NADES-MAE) was performed to obtain total polyphenolic content (TPC) according to some literature (Z. F. Wei et al., 2015; Z. Wei et al., 2015; Dai et al., 2016; Ahmad et al., 2017b; Savi et al., 2018). Briefly, the powder simplicial of *M. speciosa* (5 gram) was extracted for 10 minutes (with 30% microwave power) using NADES-MAE method which some combination types of NADES. The sample residue and extract solution were separated using the Buchner funnel. The extract was deposited at a cold temperature and until ready to use. Whereas extraction using ethanol solvents was carried out by maceration. Samples are immersed in a solvent for 1 x 24 hours continuously, maceration is stopped when the solvent has begun to clear. The extract solution and the sample residue are separated using a separating funnel, then evaporated to obtain a dry extract.

## 2.3 Total Polyphenolic Content (TPC) Determination

The TPC was evaluated by using spectrophotometer UV-Vis method based on the literature (Bobo-García et al., 2014; Do et al., 2014; Ahmad et al., 2015) with slight modification. Briefly, the sample and standard solution (1 mL) was added to 5 mL aqua demineralization and 0.5 mL Folin-Ciocalteau reagent, homogenized and allowed for 5 minutes. Next, a 2 mL sodium carbonate solution was added, homogenized, and incubated for 30 minutes. The absorbance was measured using spectrophotometer UV-Vis with 770 nm. The standard solution of gallic acid (with a concentration of 12.5 ppm, 25 ppm, 50 ppm, 100 ppm, and 100 ppm, respectively) was used to obtain the regression formula: Y = a + bX, where X is concentration, and Y is absorbance.

# 3 RESULTS AND DISCUSSION

## 3.1 Extraction Process

The application of NADES to extract target secondary metabolite compounds from natural materials is expected to be an alternative solvent option to be able to replace conventional organic solvents. At this stage, it only focuses on the selection of NADES combination types that refer to previous studies (García et al., 2016; Fernández et al., 2017; Z. F. Wei et al., 2015; Dai et al., 2016; Wang et al., 2018; Ahmad et al., 2018; Alishlah, Mun'in, and Jufri, 2019; Yin-Leng and Suyin, 2019; Yuniarti, Saputri, and Mun'im, 2019). The NADES combination types were used in this study include citric acid–glucose (CAG), malic acid–glucose (MAG), lactic acid–sucrose (LAS), and choline chloride–sorbitol (CCS) with ratio of 4:1 g/g, 1:2 g/g, 1:1 g/g, and 1:2 g/g, respectively. While other factors such as extraction time, solvent and sample ratios, microwave power, and concentration of aqua demineralization were carried out under constant conditions.

In this study, the TPC extraction was performed by using the NADES-MAE method. The selection of the extraction method is based on the effectiveness of the use of the solvent, the extraction time, the cost efficiency, and the stability of the target compound. NADES-MAE was chosen because it is environmentally friendly, safe, inexpensive, low toxicity, and fast.

## 3.2 Total Polyphenolic Content (TPC)

The determination of the TPC was performed by using spectrophotometer UV-VIS at 746 nm with Folin-Ciocalteau reagent. The measurement results of standard gallic acid shown in Table 1. According to the results, it shows that the absorbance measurement results at each concentration are outstanding namely in the absorbance range of 0 up to 1 and following the literature (Bobo-García et al., 2014; Ahmad et al., 2017a).

Table 1: The absorbance results of the gallic acid standard.

| Concentration (ppm) | Absorbance | Average Absorbance | Standard Deviation |
|---|---|---|---|
| 12.5 | 0.024<br>0.025<br>0.025 | 0.024 | 0.0006 |
| 25 | 0.053<br>0.058<br>0.067 | 0.059 | 0.0071 |
| 50 | 0.092<br>0.106<br>0.107 | 0.101 | 0.0084 |
| 100 | 0.229<br>0.238<br>0.233 | 0.233 | 0.0045 |
| 200 | 0.430<br>0.445<br>0.446 | 0.440 | 0.0090 |

Based on the calculation results of the linear regression analysis shown in Figure 1, the equation obtained Y= 0.0022X-0.00095 with a correlation coefficient (R2) of 0.998 (Figure 1). Where Y is absorbance value, and X is the concentration of gallic acid standard. The equation formula was used to calculate the TPC from *M. speciosa* leaves by using different NADES combination types compared conventional organic solvent and extraction method.

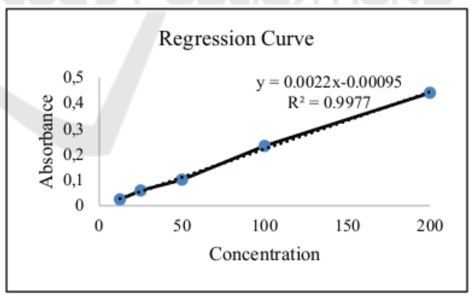


Figure 1: Regression curve of gallic acid standard.

According to the absorbance measurements for each extract (NADES combination type and ethanol), different absorbances were obtained and were in the absorbance range of 0 to 1 (at the concentration of the diluted sample) shown in Table 2. The TPC was calculated based on sample weight (mg GAE/g sample).

Table 2: Total polyphenolic content (TPC) of *M. speciosa* leaves based on the solvent type used.

| Solvent Types | Absorbance | Average Absorbance | Standard Deviation | TPC (mg GAE/g sample) |
|---|---|---|---|---|
| CAG | 0.929<br>0.845<br>0.853 | 0.875 | 0.0466 | 246.70 |
| MAG | 0.651<br>0.662<br>0.668 | 0.660 | 0.0084 | 227.33 |
| LAS | 0.850<br>0.861<br>0.921 | 0.877 | 0.0382 | 222.26 |
| CCS | 0.738<br>0.798<br>0.795 | 0.777 | 0.0338 | 219.02 |
| Ethanol | 0.560<br>0.526<br>0.528 | 0.538 | 0.0191 | 23.12 |

Based on the obtained TPC results (as can be seen in Figure 2), shows that the NADES combination type of CAG has a maximum yield of TPC (with a TPC value of 246.70 mg GAE/g sample) compared to other NADES combination types. But in general, it shows that the use of the NADE-MAE method is beneficial for extracting target secondary metabolites (mainly TPC) compared to conventional organic solvents.

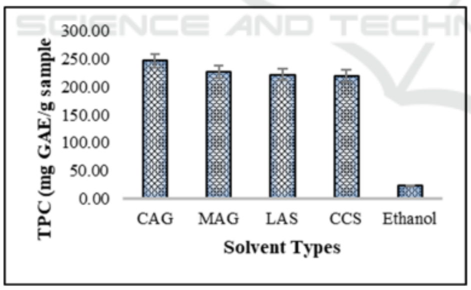


Figure 2: Efficiency extraction of TPC from *M. speciosa* leaves.

This research is the early step in developing an extraction method to obtain target secondary metabolites from *M. speciosa* leaves efficiently, easily, quickly, and safely. Furthermore, optimization of the NADES-MAE method will be carried out using the response surface methodology, identification of active secondary metabolites, and the scale-up extraction based on NADES-MAE.

# 4 CONCLUSIONS

According to the above results, the use of NADES-MAE method based on NADES combination type is beneficial to obtain TPC value compared with the other NADES combination type and conventional extraction method. The highest TPC value of 246.7; 227.33; 222.26; and 219.02 mg GAE/g sample was obtained by using citric acid-glucose (4:1 g/g); malic acid-glucose (1:2 g/g); lactic acid-sucrose (1:1 g/g), and choline chloride-sorbitol (1:2 g/g), respectively. This result was new data for the next study based on NADES-MAE methods efficiently, easily, quickly, and safely.

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