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EXTRACTION OF THE HEARTWOOD OF *Artocarpus lakoocha*: THE EFFECTS OF METHOD AND MATERIAL-SOLVENT RATIO TO YIELD OF EXTRACTION OF THE CRUDE EXTRACTS

EKSTRAKSI KAYU *Artocarpus lakoocha:* PENGARUH METODE DAN RASIO BAHAN TUMBUHAN-PENYARI TERHADAP RENDEMEN EKSTRAKSI

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ABSTRACT

Artocarpus lakoocha Roxb. (Ma-haad) is a medicinal plant commonly used in Thai Traditional Medicine (TTM) for a wide array of indications. In this study, we compared yield of extraction and the Thin Layer Chromatography (TLC) profile of the heartwood of *A. lakoocha* extracted with the different methods and different material-solvent ratios. Extractions were carried out by maceration and reflux extraction with ethanol as the solvent, in material-solvent ratio of 1:5 and 1:3. TLC profile was obtained from the separation of the extracts with methylene chloride/methanol (85:15) on silica gel F_{254} plate. The result exhibited that reflux extraction produced the highest yield of extraction and the material-solvent ratio of 1:3 generated more yield than that of 1:5. Nevertheless, the profile of TLC chromatogram of those extracts was similar.

Key words: *Artocarpus lakoocha*, extraction methods, material-solvent ratio, TLC profile, yield of extraction.

Introduction

Methods for extraction and isolation of natural products are well-established nowadays. The information on chemical and physical nature of the compound(s) to be isolated, also the outcomes desired from the process, are needed to design the extraction method. However, for unknown natural products, sometimes it may be necessary to try out pilot extraction methods to find out the best possible method. At the time of choosing a method, one should appreciate and weigh up the advantages and disadvantages of all available methods, particularly focusing on their efficiency and the total cost involved (Sarker and Nahar, 2012).

Maceration and reflux extraction are two of conventional extraction methods. Both are popular and easy to perform to extract bioactive compounds from medicinal plants. In general, these traditional extraction methods have drawbacks such as the use high temperature, consumption of large amount of solvent, long extraction time, the need to evaporate of huge amount of solvent and low yield (Sahne et al., 2016). These drawbacks are also applied for maceration and reflux extraction. Although maceration is suitable for both initial and bulk extraction of plant materials, it can be quite time-consuming and consume large volumes of solvent for bulk extraction. The main disadvantage of extraction under reflux is that thermolabile components risk being degrade (Seidel, 2012).

Artocarpus lakoocha Roxb. (Ma-haad) is a medicinal plant found widely in South and Southeast Asia. Its ethnopharmacology uses, pharmacology activities, and phytochemistry studies have been reported, mainly those grown in Thailand and India. Previous studies showed that it possessed a wide spectrum antibacterial activity (Kumar et al., 2010; Pandey and Bhatnagar, 2009), antioxidant activity with IC_{50} value less than 100 ppm (Borah et al., 2017; Kumar et al., 2010), anthelminthic activity against Indian earthworm, *Schistosoma mansoni*, and *Fasciola gigantica* (Kumar et al., 2010; Preyavichyapugdee et al., 2006; Saowakon et al., 2009), insectisidal activity (Kumar et al., 2010), and also showed a wound healing properties (Shila et al., 2015). The phytochemical studies reported that it contain tannins,

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alkaloids, benzofurans, stilbenoids, terpenoid, glycosydes, saponins, and also flavonoids (Kumar et al., 2010; Namdaung et al., 2018; Pandey and Bhatnagar, 2009). There were reports on isolation of secondary metabolites from *A. lakoocha*, such as prenylflavone, prenylated stilbene, and 2-arylbenzofuran derivates (Maneechai et al., 2012; Namdaung et al., 2018; Sritularak et al., 2010). Isolation of a peroxidase with a wound healing properties was also reported previously (Shila et al., 2015). Those metabolites were considered responsible for the pharmacological activity of *A. lakoocha*.

In this study, the heartwood of *A. lakoocha* was extracted with the different methods and different material-solvent ratios. The yield of extraction and Thin Layer Chromatography (TLC) profile of the extracts were compared.

Method

Material

The plant material used in this study was coarse dried powder of heartwood of *A. lakoocha* obtained from Khon Kaen, Thailand. Ethanol was used as the solvent, while methylene chloride and methanol were used as the mobile phase in TLC analysis.

Maceration

Each 45 g of heartwood of *A. lakoocha* was macerated with 135 and 225 mL of ethanol to obtain plant material-solvent ratios of 1:3 and 1:5, respectively, for 7 days. The residual plant material was separated from the solvent by a filtration using Whatman #1 filter paper. The yield of extraction was calculated by comparing the weight of obtained extract to the weight of the powder of the extracted heartwood of *A. lakoocha*. The basic protocol was according to standard method (Seidel, 2012).

Reflux Extraction

Forty five g of heartwood of *A. lakoocha* was immersed in 225 mL of ethanol to obtain plant material-solvent ratio of 1:5, and the flask was connected directly to a reflux condenser for 10 hours in a reflux apparatus. The residual plant material was separated from the solvent by a filtration using Whatman #1 filter paper. The yield of extraction was calculated as calculation of yield of extraction of extracts obtained from maceration. The basic protocol was according to standard method (Seidel, 2012).

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TLC Analysis of the Extracts

The extracts were spotted on a Silica gel 60 F_{254} and developed in a mobile phase consisted of methylene chloride/methanol (85:15). The detection was conducted in a UV lamp with the wavelength of 254 and 366 nm (Maneechai et al., 2009).

Results and Discussion

In this study, the coarsely milled heartwood of *A. lakoocha* was used. The size of particle of the extracted material did not affect the efficiency of extraction, as previous reported that particle size of powder of *Andrographis paniculata* did not significantly affect the yield of extraction of andrographolide (Wongkittipong et al., 2004).

There were two parameters studied: the method and the ratio of plant material-solvent used. Table 1 shows the yield of extraction obtained from 3 extractions. In the fixed material-solvent ratio of 1:5, reflux extraction produced extract 15 times higher than that of maceration. Those two methods are differed by the temperature and time of extraction (Figure 1). The higher temperature used in an extraction process generally will increase the yield of extraction, as the use of thermal energy will increase solubilization of metabolites in the plant materials and disrupt cellular structures of the plant material. This condition leads to improving of the efficiency of the extraction (Mustafa and Turner, 2011). The positive effects of the use of the heat for improving the efficiency of extraction have been reported in extraction of polyphenols from *Thymus serpyllum* (Jovanović et al., 2017), andrographolide from *A. paniculata* (Wongkittipong et al., 2004), and also volatile fractions from *Lonicera macranthoides* (Wu et al., 2015).

Nevertheles, the higher the temperature used in extraction, the bigger chance there will be bioactive metabolites degradation. This phenomenon has been reported in the extraction of curcumin from *Curcuma longa* (Sahne et al., 2016). This especially applies to plant containing thermolabile metabolites. Main constituents of the sample extracted are prenylflavones and oxyresveratrol (Maneechai et al., 2012, 2009). There is a potency of degradation of prenylflavones as a study previously reported a different profile of total flavonoids extracted from *Citrus limon*

with and without heat treatment (Ledesma-Escobar et al., 2016).

Table 1. The yield of the extraction

Samples	Method	Material-solvent ratio	Yield of extraction (%)
I	Maceration	1:3	0.56
II	Maceration	1:5	0.13
III	Reflux	1:5	2.09

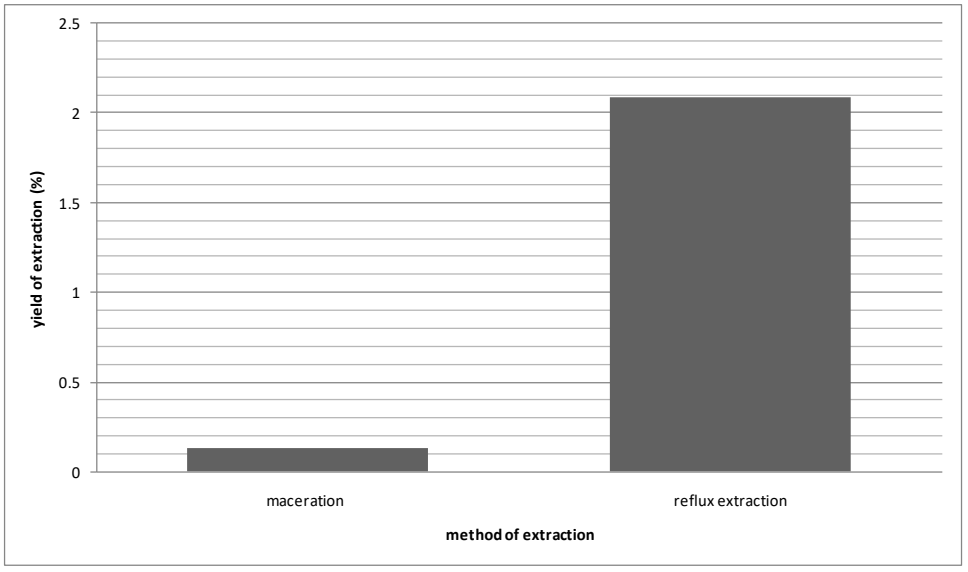

Figure 1. The profile of yield of extraction obtained from maceration and reflux extraction in fixed material-solvent ratio of 1:5.

Our study only calculated the yield of extraction of crude extract of *A. lakoocha* and not necessarily yields of extraction of prenylflavones or oxyresveratrol, so we can't discuss whether degradation of those compounds was taken place when reflux extraction was performed. In this study, maceration is performed in such a longer time (16.8 times longer) than that of reflux extraction, somehow the yield of extraction from maceration is lower. It indicated that increasing the time of extraction does not necessarily increase the efficiency of the extraction. This result was similar to a study

reported that time of extraction was not statistically important factor for the extraction of polyphenols from *Aronia melanocarpa* dried fruit (Ćujić et al., 2016). Our result highlights the drawback of maceration as a steady state extraction. The extraction process will stop once the amount of metabolites extracted in the solvent is in equilibrium with that inside the plant materials (Singh, 2008).

The profile of plant material:solvent ratio to extraction efficiency is depicted in Figure 2. The ratio of 1:3 and 1:5 produced yield of extraction of crude extract of 0.56 and 0.13%, respectively. Both treatments exhibited low yield of extractions because the ratios were too high, and the ratio was too close. During extraction, the amount of the solvent was too little, so the equilibrium of the concentration of the metabolites in the solvent and in the plant materials is reached and hence stop the extraction process (Singh, 2008). To obtain a better extraction efficacy, the ratio of plant material:solvent should be decreased. Extraction of phenolics and flavonoids from *Rosa canina, Hippophae rhamnoides,* and *Crataegus monogyna* with maceration provided plant material-solvent ratio of 1:10 (w:v) as the highest concentrations of both targeted compounds (Predescu et al., 2016). Optimation of parameters of extraction of total phenolics and total anthocyanins contents from *A. melanocarpa* using maseration resulted in 1:20 as the best ratio of plant material:solvent (Ćujić et al., 2016). The similar manner was also shown in maceration of polyphenolic compounds from *Malus domestica* pomace (Rezaei et al., 2013).

The TLC profiles of 3 extracts are similar and shown in Figure 3. Under UV lamp at wavelength of 254 nm, they are separated into 3 spots with Rf value of 0, 0.22, and 0.95, respectively. The spot with Rf of 0.95 gives a bright flourosense under UV lamp at wavelength of 366 nm. We did not do this analysis quantitatively, so we can discuss it any further. Nevertheles, we proposed those extract might not contain oxyresveratrol as there were no spots observed at Rf value of 0.59. Oxyresveratrol of several extracts of heartwood of *A. lakoocha* and Puag-Haad was identified at that specified Rf when they were separated under the same TLC system in this study (Maneechai et al., 2009).

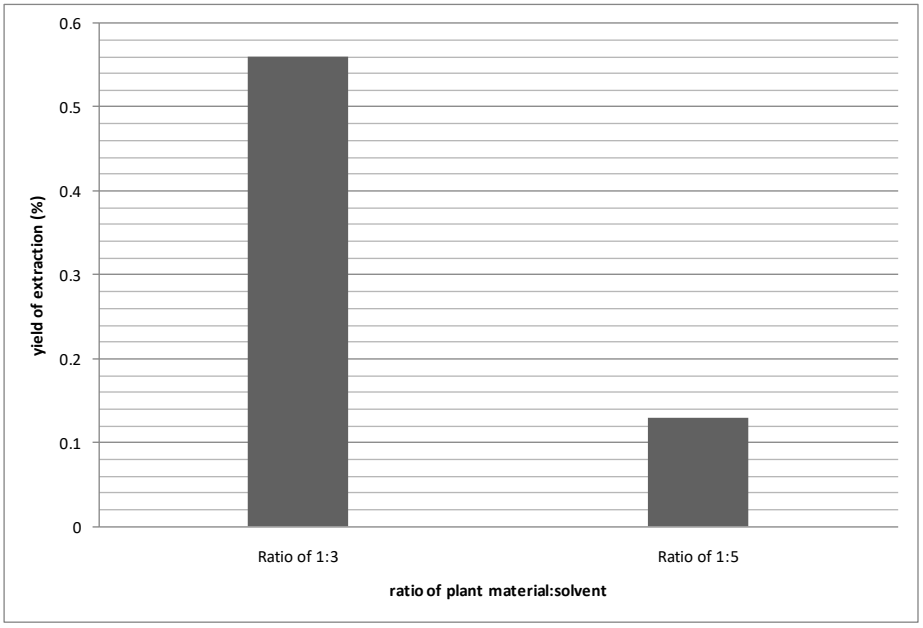

Figure 2. The profile of yield of extraction obtained from macerations with ratio of plant material-solvent of 1:3 and 1:5.

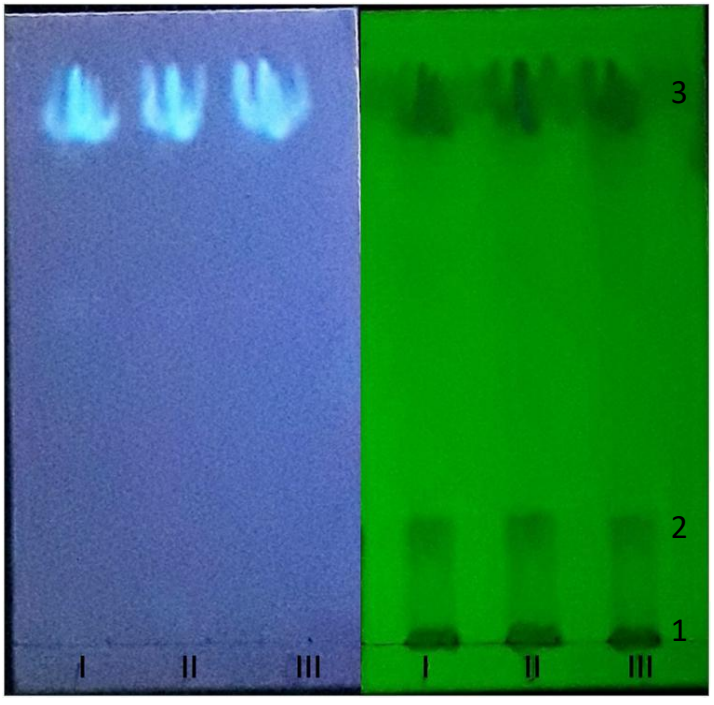

Figure 1. TLC profile of extract A. lakoocha visualized at 366 nm (left) and 254 nm (right). (I) extract obtained from maceration with plant material-solvent ratio of 1:3, (II) extract obtained from maceration with plant material-solvent ratio of 1:5, (III) extract obtained from reflux extraction with plant material-solvent ratio of 1:3

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Conclusion

Reflux extraction produced the highest yield of extraction, while maceration in both plant material-solvent ratio (1:3 and 1:5) generated a much lower yield of extraction. The TLC profile of those extract was similar, with 3 main spots observed at Rf value of 0, 0.22, and 0.95, respectively.

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