PROFILE OF THIN-LAYER CHROMATOGRAPHY AND UV-VIS SPECTROPHOTOMETRY OF AKAR KUNING STEM EXTRACT (*Arcangelisia flava*)

***[1]Mohammad Rizki Fadhil Pratama, [2]Suratno, & [1]Evi Mulyani**

[1]*Department of Pharmacy, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya*, RTA. Milono St. Km. 1.5 Palangka Raya, Indonesia
[2]*Department of Medical Laboratory Technology, Faculty of Health Science, Universitas Muhammadiyah Palangkaraya*, RTA. Milono St. Km. 1.5 Palangka Raya, Indonesia

*e-mail: m.rizkifadhil@umpalangkaraya.ac.id

ABSTRACT

This study aims to obtain the profile of Thin-Layer Chromatography (TLC) and Ultraviolet-Visible (UV-Vis) spectrophotometry from ethanol extract of akar kuning stems (Arcangelisia flava) from Central Kalimantan. The TLC method is used with the orientation phase of the combination of polar-non-polar solvents resulting from orientation, while ethanol is used as the solvent for UV-Vis spectrophotometers. TLC results showed the formation of 3 stains on a combination of polar solvents chloroform : methanol : water while in a non-polar solvent combination n-hexane : ethyl acetate did not show any stains. Comparison of retention factor (Rf) values shows the best combination of polar solvents to separate stains at a ratio of 5 : 2 : 1, respectively. Separation in 2-dimensional TLC with polar solvents showed a similar pattern with 1-dimensional separation in the form of 3 stains. UV-Vis spectrophotometer results showed 4 main peaks with wavelength 227.2; 267.4; 345.2; and 425.3 nm, respectively. The profile of the peak formed is very similar to that shown by berberine, one of the main metabolites of akar kuning. TLC and UV-Vis spectrophotometers profiles obtained are expected to support further research using akar kuning stems, especially those from Central Kalimantan.

Keywords: Akar kuning, TLC, UV-Vis Spectrophotometry

INTRODUCTION

Kalimantan as one of the islands with the largest forest area in Indonesia promises the potential for resource wealth of native medicinal plants that seem to be unlimited. Until now, there are many medicinal plants from Kalimantan that have not been investigated for the efficacy and active metabolites (Setyowati, 2010). Among the medicinal plants from Kalimantan that have been known are akar kuning (*Arcangelisia flava*), plants with round wood-shaped stems that propagate on tall trees in the forests of Kalimantan. Akar kuning can be found in almost all forest areas of Kalimantan, including in Central Kalimantan (Wahyuono *et al*., 2006). These plants can also be found in other regions in Indonesia with different names such as in Sumatra, Sulawesi, Java, and Papua known as kayu kuning (Widi & Indriati, 2007). The part of akar kuning that is most commonly used is stem, although other parts such as roots and fruit are also occasionally used (Subiandono & Heriyanto, 2009).

The use of akar kuning (*Arcangelisia flava*) in Dayak tribes from Kalimantan has been carried out from generation to generation to treat various diseases, some of which are antibiotics, analgesics, and antipyretics (Fahrianoor *et al*., 2013; Haug, 2014). Not only traditionally, the use of akar kuning stems has also begun to be used semi-modernly by making pharmaceutical forms of active ingredients from akar kuning stem extract (Maryani *et al*., 2013). However, the lack of information on the content of compounds from akar kuning stems and their phytochemical profiles is a challenge, especially in extracting nutritious chemicals. One of the efficacious chemical compounds of the akar kuning stem that is known is berberine, an alkaloid with a wide range of pharmacological activities (Pratama, 2017).

Information about berberine itself has been known in full, including among others identities such as Thin-Layer Chromatography (TLC) profiles and Ultraviolet-visible (UV-Vis) spectrophotometers (Wang *et al*., 2018). The TLC profile will provide an overview of the number of compounds, polarity properties, physical properties, and characteristics of the separation of compounds from an extract (Coskun, 2016). While the UV-Vis spectrophotometer profile will provide information on the number, shape, and wavelength at the peak which is typical for certain compounds (Kaijanen *et al*., 2015). This study aims to obtain the TLC and UV-Vis spectrophotometer profile from akar kuning stem extract. The results obtained will confirm the presence of nutritious compounds such as berberine in the akar kuning stems. In addition, the profile obtained will be the basis for further research, especially those that use akar kuning as active ingredients.

MATERIAL AND METHODS

Tools and Materials

The materials used in this study were akar kuning stems, 96% ethanol, n-hexane pro analysis (p.a.), ethyl acetate p.a., chloroform p.a., methanol p.a., water, and silica gel plate for TLC. Akar kuning stems are collected from the area around of Palangka Raya, Central Kalimantan. Akar kuning themselves are known to grow in forest areas around the City (Pratama, 2016). The equipment used includes maceration chamber, rotary evaporator, TLC chambers, capillary tubes, and UV-Vis spectrophotometer.

Methods

This study was divided into 3 stages, namely extraction, TLC, and UV-Vis spectrophotometer. The extraction process is done by maceration method, considering the ease in the process (Azwanida, 2015). The solvent used is ethanol which is known to dissolve both polar and non-polar compounds. The extraction process is repeated 3 times to ensure all chemical compounds in the sample can be absorbed by the solvent used. The liquid extract obtained is then concentrated using a rotary evaporator to obtain a thick extract (Abarca-Vargas *et al*., 2016).

The thick extract obtained was then diluted with a small amount of 96% ethanol to be spotted on a 1 x 10 cm TLC using a capillary tube. The amount of spotted extract should not be too much because it can interfere with the elution process, but also not too little so that the stain formed is easy to observe. From the formed stain, the retention factor (Rf) is calculated to be compared to the stain in the different eluent comparisons. The Rf value itself is calculated based on the ratio between the stain distance to the eluent or the mobile phase distance (Bele & Khale, 2011). In addition, to clarify the observation process, 2-dimensional TLC is used using 2 eluent comparisons which provide the best stain separation (Coskun, 2016).

UV-Vis spectrophotometer profile was carried out using a small amount of ethanol extract of akar kuning stems. The extract used was diluted using the same solvent until the absorbance obtained did not exceed 0.8 Å. Too much absorbance can affect readings, especially on UV-Vis spectrophotometers with low resolution (Behera *et al*., 2012). Spectrum obtained will show the number of main peaks with a range of wavelengths from each peak.

RESULTS AND DISCUSSION

The collected akar kuning stems are dried and chopped until rough powder is obtained. The shape of the powder is rather coarse which makes it easier when the filtering process is done so that no powder parts are taken together with the liquid extract (Deshmukh & Gaikwad, 2014). As much as 1000 g of coarse powder is then weighed and put into the maceration chamber for further filling with a solvent to soak the entire powder surface. Besides being a universal solvent that can dissolve various polar and non-polar compounds, ethanol is also relatively inexpensive with low toxicity (Dai & Mumper, 2010). The liquid extract obtained was then concentrated using a rotary evaporator and dried over a Waterbath with a temperature of 40 °Celsius until the weight remained. The amount of thick extract obtained was 82.384 g, so that the yield of ethanol extract from akar kuning stem was obtained:

$$\frac{82.384\ g}{1000\ g} \times 100\% = 8.24\%$$

The amount of extract obtained is relatively large considering the part of the plant used is a stem which is usually relatively not too much to contain secondary metabolites. The yield of almost 10% shows that the number of secondary metabolites found in the akar kuning stems is very large. The large number of secondary metabolites will facilitate the subsequent research process due to the availability of sufficient extracts to carry out various tests and several series of tests (Guerriero *et al*., 2018)

TLC Profile

TLC was carried out to determine the separation profile of ethanol extract of akar kuning stems against various types of solvents used. The TLC process was carried out on extracts using 2 solvent systems, namely n-hexane : ethyl acetate as a combination of non-polar and chloroform : methanol : water as a combination of polar solvents (Matysik *et al*., 2016). The results of TLC showed unsatisfactory results, especially because in the combination of non-polar solvents there were no stains that appeared in the overall solvent ratio. While in the combination of polar solvents, there are 3 stains on all combinations of solvents with different Rf values. The stains that appear also form 'tailings' which make it difficult in the observation process, although it can still be observed. Visualization of TLC results from each eluent combination is presented in Figures 1 and 2, while the Rf values obtained in each solvent are presented in Table 1.

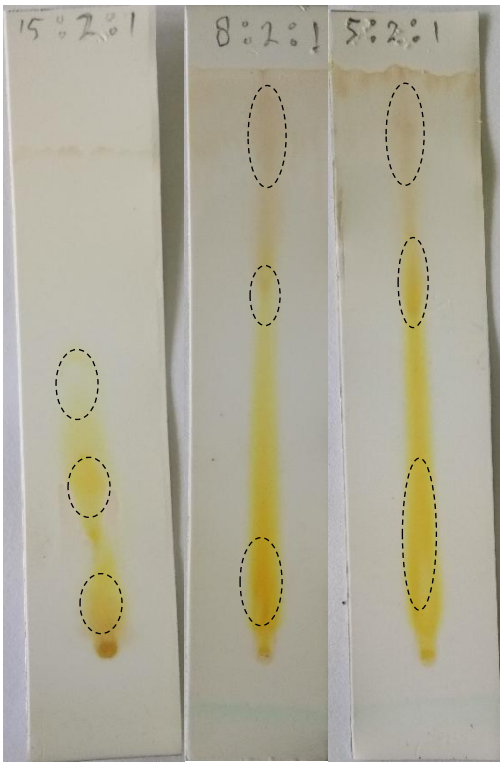
The separation of the stains that occur in the combination of polar eluents shows stagnant results, where the separation on the eluent with the ratio of 8 : 2 : 1 and 5 : 2 : 1 gives a Rf value that is not much different. However, the separation shown in the 5: 2: 1 eluent combination gives the difference between the largest Rf values. In other words, the resolution of the eluent is greatest compared

to other eluents and provides the best separation (Stoll *et al*., 2011).

After knowing the eluent combination that gives the best separation, in this case is chloroform : methanol : water with a ratio of 5 : 2 : 1, then 2-dimensional TLC was conducted. A 2-dimensional TLC is performed to see more clearly the separation indicated from each stain in the stationary phase which has a longer trajectory. 2-dimensional TLC uses 2 types of solvents which differ in polarity but separate compounds in the sample to be studied (Matysik *et al*., 2016). The solvent combination used was chloroform : methanol : water with a ratio of 8 : 2 : 1 for the first separation and a ratio of 5 : 2 : 1 for the second separation. At first glance, the three stains have characteristics and the Rf value is almost the same as the separation in 1-dimensional TLC. The interesting thing is that the first stain looks the clearest with the largest area, showing the most concentration compared to other compounds (Kagan & Flythe, 2014). The results of 2-dimensional TLC show 3 stains as shown in Figure 3.

(a) (b) (c)

Figure 1. TLC results from ethanol extract of akar kuning with non-polar solvent *n*-hexane : ethyl acetate by ratio 8 : 2 (a), 7 : 3 (b), and 6 : 4 (c), respectively.



(a) (b) (c)

Figure 2. TLC results from ethanol extract of akar kuning with polar solvent chloroform : methanol : water by ratio 15 : 2 : 1 (a), 8 : 2 : 1 (b), and 5 : 2 : 1 (c), respectively.

Table 1. Rf values of TLC akar kuning ethanolic extract

Parameter	Solvent combination					
	n-hexane : ethyl acetate			chloroform : methanol : water		
	8 : 2	7 : 3	6 : 4	15 : 2 : 1	8 : 2 : 1	5 : 2 : 1
Rf	-	-	-	0.52 0.37 0.11	0.93 0.65 0.16	0.94 0.68 0.26

Figure 3. 2-dimensional TLC results from ethanol extract of akar kuning with polar solvent chloroform : methanol : water by ratio 15 : 2 : 1 and 5 : 2 : 1

UV-Vis Spectrophotometer

UV-Vis spectrophotometry is performed to see the number and characteristics of the peaks in the sample extract. The number of peaks that appear in each extract is often linearly proportional to the number of compounds in the extract. The more number of peaks shows the increasing number of compounds, although in some conditions a compound can show peaks at several wavelengths. The peak characteristics are mainly shown in compounds which have more than one pharmacophore function group (Pratama & Pratomo, 2017). The measurements themselves are carried out both at ultraviolet light (200-400 nm) and visible (400-700 nm) wavelengths (Kolb *et al*., 2001). The results of the ethanol extract spectrum of akar kuning stems are presented in Figure 4.

The spectrum of ethanol extract of akar kuning stems shows at least 4 main peaks. Each peak is in the UV wavelength region respectively 227.2; 267.4; and 345.2 nm, and those in the visible wavelength region are 425.3 nm. Of the four peaks it has a character very similar to berberine, an alkaloid which is known to be found in various types of plants, one of which is the akar kuning (Pratama & Pratomo, 2017). Interestingly, the literature shows that standard berberine compounds are also known to show 4 peaks at wavelengths that are very close to the wavelength obtained in the results, which are respectively 228; 263; 345; and 420 nm (Koide *et al*., 2001). The number of peaks in berberine is quite a lot, one of which is due to the number of pharmacophore from berberine itself which is more than one. Berberine itself is known to have a bright yellow color, very much in accordance with the uptake that appears in the 420 nm area which is a complementary area of the yellow spectrum (Pundarikakshudu & Dave, 2010).

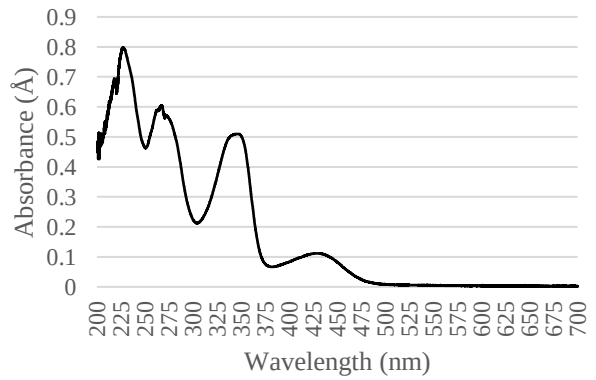


Figure 4. UV-Vis spectrum from ethanol extract of akar kuning

CONCLUSION

This study has successfully provided the profile of TLC and UV-Vis spectrophotometer from ethanol extract of akar kuning stems, where the results of TLC showed the formation of 3 stains on a combination of polar eluents with 4 main peaks in the UV-Vis spectrum. Even though the stains shown were not very clear due to the influence of 'tailing', the resolution of each stain that appeared to be quite well separated and distinguished from one another. Further enhancement of eluent polarity is predicted to be able to separate stains that appear even better. The appearance of peaks in the UV-Vis spectrum itself indicates the content of berberine compounds and their variants in large quantities, although further separation is needed to identify the extract components. However, this study provides a good basis for further research, especially those using akar kuning stem extract.

ACKNOWLEDGEMENT

This work was supported by a grant from Direktorat Riset dan Pengabdian Masyarakat Kementerian Riset Teknologi dan Pendidikan Tinggi Republik Indonesia (Directorate of Research and Community Service Ministry of Research Technology and Higher Education Republic of Indonesia) through the scheme Penelitian Dosen Pemula with grant number 3/E/KPT/2018.

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