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Exposure to light during the extraction process affects UV-Vis spectrophotometer profile of ethanol extract of Akar Kuning (*Arcangelisia flava*)

Evi Mulyani [1], Suratno [2], Arthi Venkatesan [3], Mohammad Rizki Fadhil Pratama [1,*]

[1]Department of Pharmacy, Faculty of Health Sciences, Universitas Muhammadiyah Palangkaraya, 73111 Palangka Raya, Indonesia
[2]Department of Medical Laboratory Technology, Faculty of Health Sciences, Universitas Muhammadiyah Palangkaraya, 73111 Palangka Raya, Indonesia
[3]Department of Integrative Biology, School of Bio Sciences & Technology, Vellore Institute of Technology, 632014 Vellore, Tamil Nadu, India
*corresponding author e-mail address: ***m.rizkifadhil@umpalangkaraya.ac.id*** | 56925239400

ABSTRACT

Akar kuning (*Arcangelisia flava*) is known to have a variety of active metabolites which can be extracted with polar solvents including ethanol. The process of extracting various metabolites from the akar kuning needs to be maximized by optimizing various conditions that affect the extraction process, including exposure to light. One method for assessing the success of the extraction process is to assess the UV-Vis spectrum profile. This study aims to determine how the exposure of light from akar kuning stem ethanol extract affects the UV-Vis spectrum profile. Extraction is done by maceration with ethanol solvent, using the UV-Vis spectrophotometry method to analyze the resulting extract. The experimental conditions were divided into three groups, consisting of samples that were extracted by exposure to sunlight, exposed to incandescent light bulbs, and not exposed to light at all. The results obtained showed that the extracted akar kuning sample in a condition not exposed to light at all produced the UV-Vis profile with the highest absorbance, almost double that of other conditions. This indicates that the extracted metabolite has the most quantity compared to other conditions. Conversely, the UV-Vis profile in samples exposed to sunlight has a peak profile that is different from other conditions, indicating that UV exposure to sunlight affects the type of metabolites extracted. These results confirm that the most optimal condition for obtaining as many metabolites as possible from akar kuning is in conditions not at all exposed to light.

Keywords: *akar kuning; extraction; peak shift; light exposure; UV-Vis spectrophotometer.*

1. INTRODUCTION

The development of medicinal plants as an object for therapeutic treatment is now becoming increasingly popular to do, as more and more research on the pharmacological activities of various extracts and metabolites from medicinal plants. Various methods have been developed to utilize medicinal plants as raw materials for various pharmaceutical preparations, ranging from simple methods through immersion and boiling, to through modern processes with the extraction and isolation of active substances [1, 2]. Especially for the extraction process, which is always the initial process in various studies related to medicinal plants. The availability of various kinds of extraction solvents with varying degrees of polarity also makes it easier for researchers to take efficacious metabolites from medicinal plants as much as possible [3].

One of the significant obstacles in the use of medicinal plants as part of therapeutic treatment is to ensure that the body can absorb the active compounds contained in plants and provide pharmacological effects in a predetermined dose [4]. In this case, the choice of extraction solvent both single and combination plays an important role, where the selection of the appropriate solvent will be able to extract the active metabolites from medicinal plants as optimal as possible, with other undesirable metabolites in the minimum amount possible [5]. Besides, several other factors also determine the success of the extraction process, such as the duration, temperature, and volume of the solvent. However, although these factors are influential, they are still rarely considered carefully compared to the choice of extraction solvent. In general, these factors have been determined in advance based on other research, not through the orientation process first [6, 7].

One factor that is rarely taken into account but can be quite influential on the course of the extraction process is exposure to light. In this case, the light can be from artificial light sources or sunlight. Previous research has been done to observe the exposure of light at a particular wavelength (λ) to the physical growth of a plant, as well as to observe the content of specific metabolites as the plant grows [8, 9]. Not much research has focused on the presence of light exposure itself on the metabolite profile during the extraction process. These factors tend to be ignored in the extraction process, especially extraction with non-heating methods such as maceration and percolation. One reason is that the soaking process takes more than 24 hours, so these factors tend to be difficult to control [6]. Whereas on the other hand, exposure to light, especially from the sun is known to affect the type of metabolites produced by a plant, even though the plant has been processed into dried simplicia [10, 11]. Thus, differences in the provision of exposure to light both in terms of exposure time and the type of light source may also affect the type and amount of metabolites that have been successfully extracted [12].

Several methods can be used to determine the type and amount of metabolites that have been obtained from the extraction process, including chromatography and spectrophotometry methods [13]. Among these methods, spectrophotometry has several advantages, such as the process is quite fast, and the results obtained can show both the amount and quantity of metabolites obtained [3]. Among other spectrophotometric methods, UV-Vis

spectrophotometry is the most widely used. Apart from the relatively low cost needed, the process is also simple and does not require special treatment. Indeed, this method has several weaknesses, including the information offered is quite minimal compared to other methods that provide complete information. However, for preliminary studies such as comparison of metabolite profiles, the information obtained is sufficient to obtain conclusions [14].

The purpose of this study was to determine the effect of differences in light exposure on the UV-Vis spectrophotometer profile of medicinal plant extracts, in this case, ethanol extracts from akar kuning stems (*Arcangelisia flava*). Akar kuning is a medicinal plant from Kalimantan, Indonesia which is known to have a variety of pharmacological activities such as antimalarial, antibacterial, and anticancer. The presence of berberine alkaloids from akar kuning stem extracts is known to play an important role in various pharmacological activities. [15, 16]. The reason why akar kuning was chosen is that comparative data for its UV-Vis spectrophotometer profile have been obtained, and this medicinal plant is currently prevalent in Indonesia for use as a traditional medicine to treat various diseases [17]. The extraction method used was maceration with ethanol solvent, where the experimental conditions were divided into three treatments, consisting of exposure to direct sunlight, to light from a bulb lamp, and without being exposed to light at all.

2. MATERIALS AND METHODS

2.1. Tools and materials.

The tools needed in this study is quite simple, including analytical balance, maceration chambers, rotary evaporators, and UV-Vis spectrophotometer Genesys 10S. While the materials used include akar kuning stems as well as ethanol 96% pro analytic. For storage containers used include cabinets with UV lights, cabinets with light bulbs, and dark cabinets without any lighting. The bulb used is a type of incandescent bulb Philips brand 100 w. Cabinets with UV lights are only used for storing samples at night.

2.2. Preparation of dry simplicial.

The akar kuning stem used was obtained from the Kahayan Market, Palangka Raya, Indonesia. Samples that have been collected are then cleaned under running water and shredded by a shaved machine until a coarse powder is obtained. The powder is then dried in the morning sun until dry powder is obtained. Drying of simplicia powder using high temperature like using the oven is avoided because it can reduce and damage the metabolite content in medicinal plants [18].

2.3. Extraction process.

The sample was divided into three test groups with different treatments for exposure to light. The 500 g akar kuning powder was weighed with analytical balance and then transferred to the maceration chambers for each test group. Each chamber was then added as much as 1.5 L of ethanol 96% until all the akar kuning powder was completely submerged. Each chamber containing samples were then stored according to each test group. The first group was given exposure to direct sunlight in the morning to evening, then transferred to cabinets with UV lights at night. The second group was given exposure to light from morning to night by being placed in cabinets with light bulbs. While the third group was not exposed to light at all by being placed in cabinets without lights and not translucent. Each sample then immersed for 24 hours at room temperature while stirring every six hours.

After 24 hours, the filtering process was carried out for each sample using Whatman filter paper and fat-free cotton wool. The filtered liquid extract is then concentrated using a rotary evaporator at 50°C with 50 rpm. The thick extract is then taken and collected using a spatula.

2.4. UV-Vis spectrophotometer analysis.

The thick extract from each test group was weighed and then diluted with 96% pro analytic ethanol to obtain a concentration of 0.01% w/v. A total of 1 ml of each sample was then transferred to the cuvette and then screened using a UV-Vis spectrophotometer in the λ range of 200-700 nm. Screening at these wavelengths will provide peak absorption information for both colored and colorless compounds [14]. The spectrums of each sample are then compared with each other by taking into account the parameters of the peak number, absorbance, and maximum λ.

3. RESULTS

Visually, there was no difference in the physical appearance of extracts obtained from each test group. Both the observations of the color, odor, taste, and consistency of each extract showed identical results. The most prominent physical characteristic is the brownish-yellow color, where the extract produced has a distinctive odor, although not too sharp. The complete results of the organoleptic test are presented in Table 1.

The UV-Vis spectrophotometric profile of each test group showed different results from each other. Some interesting points can be observed from the comparison of the UV-Vis spectrum profile of each treatment. First, the absorbance of samples not exposed to light is much higher than that exposed to light, where the highest peak has an absorbance of 1,726 Å at a maximum λ of 229.2 nm, almost double that of other groups. In samples exposed to sunlight, the highest peak was only 0.954 Å at a maximum λ of 228.8 nm.

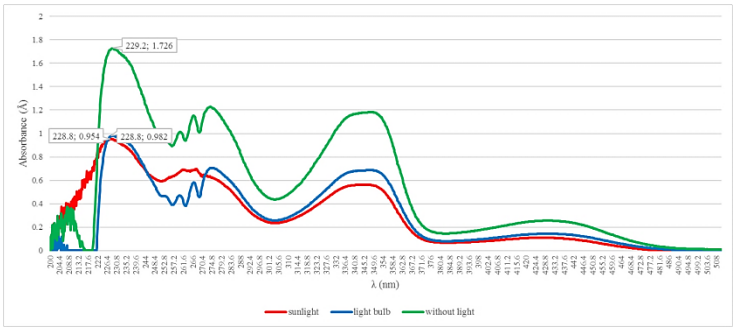

Figure 1. Comparison of the UV-Vis spectrum of each test group, values at the peak indicate absorbance at maximum λ.

Whereas the light bulb exposed samples showed the highest peak of 0.982 Å, also at a maximum λ of 228.8 nm. From these results, it can be concluded that the metabolites that were successfully extracted in the treatment were not exposed to light at

all much higher than those exposed to light. These results are practically different from the findings reported by Wang et al., which states that providing light exposure at specific wavelengths can increase the number of metabolites that have been successfully extracted [19]. Several factors can be the cause of these differences, including differences in the types of plants used. A comparison of the UV-Vis spectrum of each group is presented in Figure 1.

The second important point is the number of peaks that appear in each sample, where there are differences in the presence and shape of certain peaks. In general, each sample shows five peaks, with a range of λ 203.4-207.8 nm; 228.8-229.2 nm; 274,2-275.0 nm; 336.4-348.8 nm; and 427,6-432.2 nm. Of the five peaks, samples in the group that were exposed to direct sunlight experienced a peak shift in the λ 228.8-229.2 nm region, which resulted in the peak area of λ 203.4-207.8 nm experiencing noise and becoming invisible, as well as two minor peaks in the λ 260.8 region 266.8 nm becomes covered. The condition is most likely due to metabolic processes by enzymes from metabolites in the λ region due to the influence of sunlight. As we know, sunlight can stimulate certain enzymes in plant cells that can change a certain metabolite and produce products that have different physicochemical properties [12, 20, 21]. In this case, the metabolites in that area are likely to be metabolized into other compounds that have different chromophores and auxochrome from the parent compound, which causes a peak at λ that shifts from its original position [22]. On the other hand, samples that are exposed to light bulbs and those that are not exposed to light at all show very similar peak profiles. This confirms that the factor causing the peak shift is the UV rays found in sunlight and UV lights, not due to the presence of light in general.

The third important point that can be observed from Figure 1 is the distribution of peaks at a particular λ, where almost all major peaks are in the UV-C region, while there is only one peak in the UV-A region and one peak in the visible light region. This shows that most of the metabolites contained in the akar kuning stem extracts are relatively colorless because the absorbance of the majority is in the λ UV region. In the visible light λ region, the absorption only appears up to λ 507.6 nm, where the only peak appears in the λ area 427.6-432.2 nm. The area is an absorption area that is known to show complementary color to yellow, which is the color of berberine compounds [23]. In addition, berberines in pure form are also known to show peaks in the λ of 228; 263; 345; and 420 nm regions, which are close to the spectrum profile of the entire sample [24]. These results confirm that the presence of peaks that arise from each sample is an indication of the presence of berberines in the sample.

Overall, it can be concluded that to obtain as many metabolites (including berberines) from the akar kuning in the extraction process, the ideal conditions are not exposed to light at all. However, the type of metabolites extracted from each of these conditions needs to be re-identified in further research, because there is no certainty that the larger quantity of metabolites is linear with a larger quantity of berberine. In addition, this study also has limitations in which the exposure to the light used has not been determined by the magnitude of their λ. However, with the results that show that the most optimal conditions are without being exposed to light at all, research on the λ of the light used is no longer relevant to do.

Table 1. Organoleptic test of the viscous extract of akar kuning based on exposure to light.

Test Group	Taste	Odor	Color	Consistency
Samples exposed to sunlight	Bitter	Distinctive aroma	brownish-yellow	Sticky
Sample exposed to the light bulb	Bitter	Distinctive aroma	brownish-yellow	Sticky
Sample not exposed to light at all	Bitter	Distinctive aroma	brownish-yellow	Sticky

4. CONCLUSIONS

This research successfully confirms that the ideal conditions for extracting as many metabolites from akar kuning are in conditions not exposed to light at all. Conversely, exposure to sunlight should be avoided as much as possible because it can affect the type of metabolites extracted. However, this study has limitations because the exposure to light given is not determined by the magnitude of λ from the light given. Therefore, this research still needs to be developed further by paying attention to the λ of the light used. However, this research has become a reasonable practical basis for use in the akar kuning extraction process and does not rule out the possibility also applies to other types of medicinal plants.

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