

# Effects Of Solvents On The Amount Of Anthraquinone

Nurul Ain Jumri

Faculty of Chemical Engineering and Natural Resources  
Universiti Malaysia Pahang  
Gambang, Malaysia  
nurulain.jumri@gmail.com

Mimi Sakinah Abdul Munaim

Faculty of Engineering Technology  
Universiti Malaysia Pahang  
Gambang, Malaysia  
mimi@ump.edu.my

Zularisam Ab Wahid

Faculty of Engineering Technology  
Universiti Malaysia Pahang  
Gambang, Malaysia  
zularisam@ump.edu.my

*Abstract*—Dyes derived from natural sources have appeared as a significant alternative to synthetic dyes. Recently, the scientific community has begun to show interest in *Morinda citrifolia* as it is one of the natural sources. The roots of these plants were reported to be a good source of important compounds, known as anthraquinones, which have been proved an important naturally occurring pigments that are widely distributed in nature to produce yellow or red dyes. However, it is not known which method and types of solvent will obtain the highest anthraquinone extract from *Morinda citrifolia*. In order to tackle this problem, this research was carried out to study effects of solvents on the concentration of anthraquinone using solvent extraction method at room temperature for 10 hour. Ethanol, acetone and acetonitrile were types of solvent used. The anthraquinone extract was analyzed using UV-Vis Spectrophotometer afterwards. It was observed that the acetone is the best solvent to extract the highest anthraquinone concentration followed by ethanol and acetonitrile respectively. Acetone gave anthraquinone concentration about 0.0844g/L, while ethanol and acetonitrile was 0.0440g/L and 0.0355g/L correspondingly. It also shows that acetone gave the best color in which brick red color. The results suggested that the solvent extraction method able to yield colorants from *Morinda citrifolia* which can work out commercially, but the color varies based on the types of solvent used.

*Keywords*—natural; extraction; *Morinda Citrifolia*; UV-Vis Spectrophotometer

## 1. INTRODUCTION

Dyes is known as substances in which, provide color when applied to a substrate by a process that alters any crystal structure of the colored substances. Many industries such as textile, pharmaceutical, plastics, photographic, cosmetics, food, and paper industries are widely employed those substances with considerable coloring capacity [9]. Usually, dyes is classified based on their application and chemical structure which are composed of a group of atoms that is known as chromophores. These

chromophore is responsible for the dye color. It containing centers that are based on diverse functional groups, such as azo, arilmethane, carbonyl, anthraquinone, methine, nitro, and others. In addition, electrons donating or withdrawing substituents so as to generate or intensify the color of the chromophores are denominated as auxochromes. Common auxochromes are amine, carboxyl, sulfonate and hydroxyl [4].

There are over 10,000 different dyes are used industrially and about  $7 \times 10^5$  tons of synthetic dyes are annually produced worldwide mainly in the textile industry [25] which is present the largest and most versatile group of all dyestuffs consumed [6]. However, the textile plant's wastewater is classified as the extremely polluting of all the industrial sectors, taking into account the volume generated. The increased demand for textile products as well as their increase in production, and the use of synthetic dyes have contributed to dye wastewater substantial sources of severe pollution problems in present times [1]. Synthetic dyes are known to be hazardous as it produces skin allergy, toxic wastes and other harmfulness to the human body [2]. Thereby, the dye industry is obligatory to minimize toxic effluents and to stop the production of potentially hazardous dyes or pigments [13]. Due to the outcome of the firm environmental standards imposed by several countries in response to the allergic reactions and toxic associated with synthetic dyes, a revival curiosity in the use of natural dyes in textile coloration has been growing these days. The reasons were natural dyes are friendlier to the environment and can demonstrate preferable biodegradability [10].

Natural dyes derived from flora and fauna are believed to be safe because of their non-toxic, non-carcinogenic and biodegradable nature. Instead of play particular roles, different parts of plants likes stem, leaves, flowers, roots and others part can possess a wide variety of colors [16]. In this research, *Morinda citrifolia* (Mengkudu) is used as the source of natural dye. This plant always drew much attention to worldwide not only because of it purposes on food and medicinal for over 2000 years but also as the source of natural dye. Traditionally, the roots of *Morinda citrifolia* plants were found to be useful to produce yellow and red dye [26]. It also be known as Noni and were originated in tropical Asia or Polynesia. Nowadays, increasing number of studies was focusing on finding competent methods for production and extraction of anthraquinones from these plants. Reference [7] also supported that anthraquinones are of fundamental importance both in industry and medicine. In current years, several novel extraction techniques have been developed for the extraction of nutraceuticals from plants, including ultrasound-assisted extraction, microwave-assisted extraction, accelerated solvent extraction and supercritical fluid extraction [23]. The selection of the method to extract active components with maximum yield mostly depends on the nature of compounds and nature of raw material to be processed [20] and according to [18], extraction of anthraquinones from plant roots is widely conducted and is conventionally performed by solvent extraction. The traditional techniques of solvent extraction are mostly based on the correct choice of solvents and/or the use of agitation to increase the solubility of the desired compounds and improve the mass transfer [14].

Yet, not all pigments from plants can be used as dyes even though the pigments yield a broad range of colors. This is because the pigments are believed to be dependent on their solubility characteristic which is it might not dissolve in water or certain solvent. Sometimes, the solubility of plant pigments also depend on their molecular structure. In simple words, the rule for solubility is, "like dissolves like" which is polar solvents will dissolve other substances that are polar while non-polar solvents will dissolve substances that are non-polar and the other way round. There are many factors affecting solubility which are temperature, polarity, pressure and molecular size. Basically, solubility increases with temperature. However in this study, it was not focused on the temperature. It was found that solutes only can dissolve in solvents that have a similar polarity and this study ignored the pressure factor as it was found that pressure does not affect solubility. Another factor that affect the solubility is molecular size. It is difficult for solvent molecules to surround bigger molecules. A general rule can be found that larger particles are generally less soluble. Therefore, in that case, if the pigments has the same polarity, the one with smaller particles will be more soluble. However, there were very few publications in scientific journals do exist related to this area. Hence, this research was conducted to determine the effect of solvents on anthraquinone extract and the overall objective of this paper was to highlight the unique capabilities, advantages and use of certain solvent thus enabling the readers to predict its promising future.

## 2. MATERIAL

### A. Materials

*Morinda citrifolia* that was used in this study were grown locally in Malaysia. This plant was identified at the herbarium under the registration numbers KLU 22480. The samples were washed using running tap water and separated before being chopped into pieces [26]. Standard Alizarin was used in this study. All chemicals were purchased from Sigma Sdn Bhd and used as received or otherwise stated.

### 3. METHOD

#### A. Preparation Of Standard Curve

In this study, a calibration curves of Alizarin solutions [3] in various solvent was used as reference in order to quantify the amount of anthraquinone in the sample [17]. Preparation of different dilution of Alizarin solutions was performed by mixing the Alizarin with various solvents (ethanol, acetone, acetonitrile) in the test tube. Alizarin range used were 0 to 1.0g/L and the final volume in each test tubes are 5ml [5].

#### B. Preparation Of Sample

The roots of *Morinda citrifolia* were harvested, washed, and then oven dried at 50<sup>0</sup>C for 2 days. The dried sample was then ground to small size using mortar and pestle to an average size of 0.2 mm in diameter to reduce surface area [17]. The ground samples stored in dry place until use [21].

#### C. Solvents Extraction Process

For extraction, 2 g of root sample was placed into a beaker with 200 ml of solvent (ethanol, acetone, acetonitrile). In order to study the types of solvent, 80% composition of solvent was used which is 80% is the percent of the solvent while, the rest is the percent of distilled water as well. The extraction was performed at the temperature of 25<sup>0</sup>C [18] and carried out for up to 10 hour [15]. Then, the extract was filtered with a filter paper (Whatman, no.1, USA) and the concentration of anthraquinones was measured by a spectrophotometer afterwards [18]. The extraction was repeated three times.

#### D. Determination Concentration Of Anthraquinone

For detection of dyes, UV-visible spectrophotometer has traditionally been the most frequently used method [11]. Thus, UV-Vis spectrophotometer model Hitachi U-1800 was used in this study in order to determine the concentration of anthraquinone in the sample. The concentrations of anthraquinones extracts were analyzed by measuring the absorbance at 435nm, following the spectroscopic method [17]. Various type of solvents were used as a blank. Preparation of standard curve was necessary by plotting the average blank corrected 435 nm reading for each standard versus its concentration in g/L. The anthraquinone concentration of the unknown sample was determined using standard curve.

### 4. EXPERIMENTAL RESULTS

#### A. Effects of Types of Solvents On The Amount Of Anthraquinone

There was a large volume of published studies especially in the case of extraction from plant tissues mentioning the presence of desired compounds or the group of compounds in various cells in different parts of the raw material. In this paper, it focused on the extraction of anthraquinone from roots of *Morinda citrifolia* using solvent extraction methods. Reference [12] highlighted that in order to extract the desired compounds from the raw material, the solvent have to reach and dissolve them. In purpose to reach the desired compounds, the solvent will attack the cell wall of raw material and penetrates it. There were numerous studies have attempted to explain that a correct choice of solvent was core for obtaining the best extraction process. On account of this, types of solvents being the parameter studied in this paper, and the solvents used was chosen based on their polarity relative to anthraquinones. Polarity was acknowledged to be the most common classification of solvent. Reference [22] discovered that polar solvent was defined as the one that will dissolve and stabilize dipolar or charged solutes.

On the part of determining which one of the solvents in meet for the optimum anthraquinone extract, the extraction was performed at room temperature (25<sup>0</sup>C) for 10 hours. The polar solvents were chosen due to the fact that anthraquinone was polar and the solvents were ethanol, acetone and acetonitrile. Among these solvents, ethanol and acetone were conventional for practical use as it were in adherent with good manufacturing practice (GMP). On the other hand, even though acetonitrile was considered as toxic solvent, it provide an insight into the effect of solvent properties in extraction [17]. The effect of different types of solvent on the absorbance and concentration of anthraquinone was shown in Tab. 1 below. Tab. 1 indicates that optical density of anthraquinone was directly proportional to the concentration of anthraquinone and these results supported by Beer-Lambert Law shown from (1). In this equation, it was observed that the optical density was in linear form to the concentration of solution. During the measurement of concentration, those three type of solvents were used as a blank and the preparation of standard curve was performed by plotting the average blank corrected 435 nm reading for each standard versus its concentration in g/L. The anthraquinone concentration of the sample was determined using standard curve.

$$\text{Beer's Law : } A = \epsilon cl \quad (1)$$

where,  $\epsilon$  is the absorbance or optical density,  $c$  is concentration of solution and  $l$  is path length.

Table 1: Effect of types of solvent on absorbance and concentration of anthraquinone

Types of solvent	Optical Density (abs)	Concentration (g/L)
Ethanol	1.013	0.0440
Acetone	1.994	0.0844
Acetonitrile	0.767	0.0355

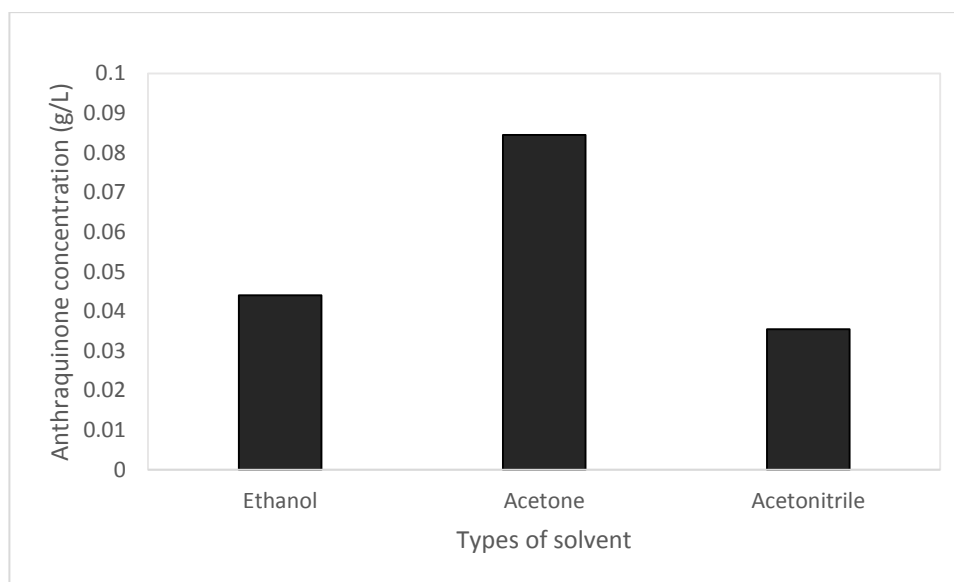
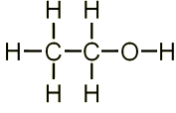
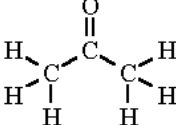
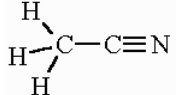


Figure 1: Effect of anthraquinone on types of solvent at room temperature

From the data from Fig. 1, it was apparent that at room temperature, acetone extract highest anthraquinone about 0.0844g/L while the lowest one was acetonitrile about 0.0355g/L. This results was meeting to the previous study which according to [17] acetone shared the most structural similarity with anthraquinone and this fact was also pointed out by another study which said that the similarity of carbonyl functional group (C=O) in acetone to that of anthraquinone play part in the upgrading of anthraquinone extraction efficiency [24]. For further explanation, the molecular and structural formula of acetone was provided in the Tab. 2 and the structural formula of anthraquinone was shown in Fig. 2. In the Tab. 2, it was observed that acetone has molecular formula  $(\text{CH}_3)_2\text{C}=\text{O}$  which was a planar and highly polar molecule with a large dipole ( $\mu = 2.88$ ) and large dielectric constant ( $\epsilon = 20.7$ ). Reference [24] reported that the significant role of acetone in boosting the effect of anthraquinone extraction efficiency could also be enlightened in term of higher mass transfer coefficient that allowed the better access of acetone to the anthraquinone reserve in each cell of *Morinda*. Moreover, acetone was found has the lowest viscosity in the Tab. 2, which allows it to diffuse into the root matrix easily while acetonitrile, on the other hand has the low viscosity compared to the ethanol, but gave the lowest extraction efficiency. This might be due to the fact that the molecular structure of acetonitrile was most different from that of anthraquinones resulting in the lowest solubility compared with the other solvents [17].

Instead of just focusing on the optical density and concentration of anthraquinone, this paper also has shown an interest in the color extracted from the roots of *Morinda citrifolia*. It was evident that a wide variety of colors ranging from light pomegranate, light brown and brick red could be obtained from the dye extracted from *Morinda citrifolia* using different types of solvent. The color extracted from the roots of *Morinda citrifolia* was demonstrated in Fig. 3 below. Among the solvent, acetone extracted the best colors of dyes which were a brick red color. This results must be due the highest concentration it achieved when compared to other solvents. While in contrast, lower concentration gave pale and dull colors. The color component isolated from the roots of *Morinda citrifolia* was an alizarin type of compound containing an anthraquinone group. Alizarin belongs to the anthraquinone class of dyes which was commonly used in textile industry. Alizarin has alike chemical structures of anthraquinone (Fig. 4) and known as 1,2-dihydroxyanthraquinone. The fact of which solvent used to extract the color was important because a true dye must have a suitable color, be able to attach itself to the material or be capable of being fixed on it and be fast to light and washing when fixed. From Fig. 3, water was also being tested in order to confirm the fact that [8] discovered which alizarin was almost insoluble in water. In that case, water would not be able to extract the best color and the result was only light pomegranate. Actually, depends on the strengths of alkali or acid used, natural alizarin can vary from scarlet to pink to red with a bluish tint. A strong alkali will create a violet-blue color whereas a diluted alkali will create a violet red. In other hands, a strong acid will give rise to a yellowish red and the alcoholic and aqueous solutions are a rose in color while the ethereal ones give golden-yellow [8].

Table 2: Properties of solvents used in solvent extraction

Types of solvent	Molecular formula	Structural formula	Viscosity at 25 <sup>o</sup> C (cP)
Ethanol	CH <sub>3</sub> CH <sub>2</sub> OH		1.07
Acetone	(CH <sub>3</sub> ) <sub>2</sub> C=O		0.31
Acetonitrile	CH <sub>3</sub> CN		0.37

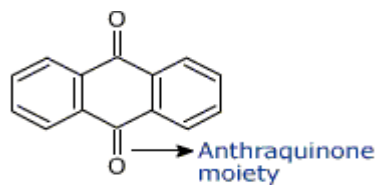


Figure 2: Molecular structure of Anthraquinone

Table 3: Effect of types on solvent on yield of color

Types of solvent	Color
Ethanol	Light brown
Acetone	Brick red
Acetonitrile	Light brown



Figure 3: Color extracted based on types of solvent

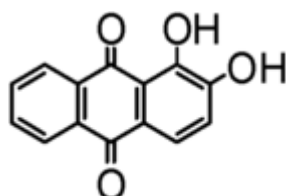


Figure 4: Molecular structure of Alizarin

In this paper, in order to study the types of solvent, 80% composition of solvent was used which was 80% is the percent of the solvent while, the rest is the percent of distilled water as well. Reference [24] revealed that the roles of water in the enhancement of extraction efficiency were prominent in every case of anthraquinone extraction with several types of solvent. This might be described by a greater electrostatic force of water relative to other molecules. Therefore, the extraction with solvents other than water might also require a participating role of water to some point to increase the extraction efficiency. Such finding corresponded to the experimental results for 80% acetone which was 2.6 times higher than 0.025 mg/ml for 100% acetone. The swelling of *Morinda citrifolia* root cells increased the surface area and assisted the extraction efficiency by the solvents.

## 5. CONCLUSION

Solvent extraction method is a process designed for isolated bioactive component by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). This method has been extensively used to extract bioactive components from many plants where in this study, this method was successfully extracted anthraquinone. Either used as single or in a combination with aqueous, the common solvents used for extraction were ethanol and acetone. This study proved that different solvents gave the different results to the extraction of anthraquinone in which, in this case of study, acetone has been found as the best solvent among ethanol and acetonitrile. It was concluded that the solvent extraction method that were used was the applicable method for the extraction of anthraquinone from *Morinda citrifolia* roots.

## ACKNOWLEDGMENT

I gratefully acknowledge the financial support of this study by MyBrain 15 and generosity of University Malaysia Pahang for access of lab facilities, without which the study could not have been completed.

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