CHAPTER I
INTRODUCTION

1.1 Background

Chromatography is a mixture separation technique based on the speed of propagation of the components in a certain medium. The description of chromatography was first described by Michael Tswett, a Russian biotanist who worked at the University of Warsaw. At that time, Michael Tsweet separated chlorophyll from other pigments from plant extracts using column chromatography filled with calcium carbonate (Wulandari, 2011). The rapid development of several types of chromatography systems includes paper chromatography, thin layer chromatography, gas chromatography, and high performance liquid chromatography (Alen dkk., 2017).

In Thin Layer Chromatography (TLC), there is a thin layer (0.1-2 mm thick) consisting of a solid material superimposed on a flat support surface (plate), which is usually made of glass, but can also be made of polymers or metals. The coating is attached to the surface with the aid of a binder, usually calcium sulfate. Thin layer chromatography can be used for a wide variety of purposes in separations, such as tofu, tempe, isoflavone powder which have many benefits (Wulandari, 2011).

In this practicum, (sample name) is used as a medium for isolating pigments which will be tested by thin layer chromatography. (sample name) is used because (explain the reasons for using the sample, at least 2 sentences).

1.2 Experiment Objectives

1 Students can isolate chlorophyll pigments.

2 Students can separate the mixture into its components using thin layer chromatography.

1.3 Experiment Benefits

1. Students can find out how to isolate chlorophyll pigments
2. Students can separate the mixture into its components by thin layer chromatography.

CHAPTER II
LITERATURE REVIEW

2.1 Chromatography Definition

Chromatography has been defined primarily as a separation process used to separate mixtures that are essentially molecular. Chromatography relies on the re-splitting of mixed molecules between two or more phases. Chromatography types include adsorbs chromatography, liquid partition chromatography, and ion exchange. The main systems used in partition chromatography are gas partitioning, liquid partitioning using a fixed base (e.g. column chromatography), paper chromatography and thin layers (Underwood and Day, 1983).

Chromatography is used to separate mixed substances into their components, for example flavonoids and isoflavonoids found in tofu, soybean powder, and tauco as well as *Scoparia dulcis, Lindernia anagalis, and Torenia violacea*, which have many benefits. Some of the advantages of isoflavone compounds that are potential for human health, including as antioxidants, anti-tumor / anti-cancer, anti-cholesterol, antiviral, allergy, and can prevent osteoporosis (Meyer, 2010).

In chromatography, the components to be separated are between two phases, namely the stationary phase and the mobile phase. The stationary phase is the phase that will hold the components of the mixture while the mobile phase is the phase that will dissolve the components of the mixture. Components that are easily held in the stationary phase will be left behind or not move, while components that are easily dissolved in the mobile phase will move faster (Wulandari, 2011). The mobile phase known as the developer solvent will move along the stationary phase due to the influence of the capillaries on ascending expansion or due to the effect of descending expansion (Meyer, 2010). Furthermore, there are some applications of chromatography, namely:

1. Thin Layer Chromatography

   Analysis using TLC can be used to identify sample whose chemical content groups are known. The chemical content groups include alkaloids, antra glycosides, arbutin, cardiac glycosides, bitter substances, flavonoids, saponins, essential oils, coumarins, and phenol carboxylic acids (Underwood and Day, 1983).

2. High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is a type of chromatography system with a high performance and very efficient method, which is capable of producing good separations in a short time. HPLC instruments can be assemblages of modular or individual elements, but can also be designed as a single instrument. The modular concept in this case is much more flexible than the failure of a single component. HPLC instruments at least have elements or devices such as a solvent reservoir, a transfer line with frits, a high pressure pump, a sample injection device, a column, a detector, and data acquisition which usually coincides with data evaluation (Meyer, 2010).

3. Gas Chromatography

The same like HPLC, gas chromatography is also a type of chromatography system with a high performance method. The most basic difference between the two is that GC can only deal with volatile substances or can be completely vaporized at high temperatures and from which volatile derivatives can be obtained reliably. Only about 20% of organic compounds can be identified by gas chromatography (GC) without any prior treatment where for liquid chromatography, the sample must be dissolved in first (Meyer, 2010).

2.2 Thin Layer Chromatography

Thin layer chromatography (TLC) is one type of chromatography that is most often performed for the analysis and separation of an organic component. Thin layer chromatography (TLC) was developed by Izmailoff and Schariber in 1938. TLC is a form of planar chromatography, apart from paper chromatography and electrophoresis. In contrast to column chromatography in which the stationary phase is filled or packaged in it, in thin layer chromatography, the stationary phase is a uniform layer (uniform) on a flat surface supported by a glass plate, aluminum plate or plastic plate. Nonetheless, this planar chromatography can be said to be the open form of column chromatography (Underwood and Day, 1983).

The working principle of TLC is to separate samples based on differences in polarity between the sample and the solvent used. This separation uses the principle of adsorption and partition which is determined by the stationary phase (adsorbent) and mobile phase (eluent). The stationary phase is used in the form of a silica plate

and the mobile phase is adjusted according to the type of sample to be separated. The solution or solution mixture used is called an eluent. The chemical component (analyte) moves upward following the mobile phase because the adsorbent's absorption capacity of the chemical components is not the same so that the chemical component can move with different distances based on the degree of polarity. The closer the polarity between the samples to the eluent, the more the sample will be carried away by the mobile phase (Alen dkk., 2017).

There are several advantages, such as simple, inexpensive equipment, fast analysis time, and excellent resolution, which keep this method popular. Chromatography is roughly composed of a mobile phase and a stationary phase. In TLC, the stationary phase is silica gel GF254 while the mobile phase is an organic solvent. In its implementation, thin layer chromatography is easier and cheaper than column chromatography. Likewise the equipment used. In thin layer chromatography, the equipment used is simpler and it can be said that almost any laboratory can carry out any time quickly (Alen dkk., 2017).

2.3 Retardation Factor (RF)

Retardation factor (Rf) is a parameter used to describe the migration of compounds in TLC. The value of Rf is a parameter that states the position of the stain in the stationary phase after eluting. Determination of the Rf value of the analyte, namely comparing the migration distance of the analyte stain with the migration distance of the mobile / eluent phase (Wulandari, 2011).

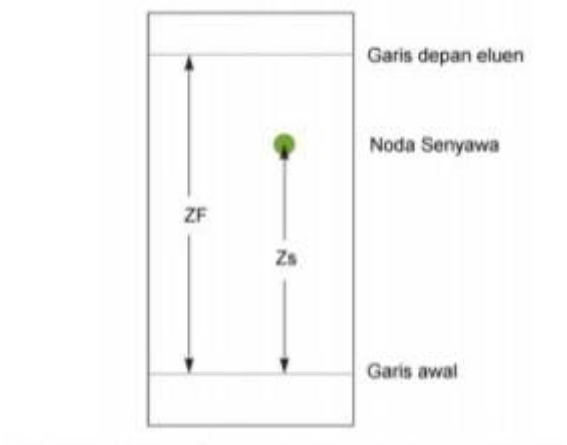

Figure 2.1 Illustration of the migration of analytes and eluents on the TLC plate

The retardation factor can be calculated as a ratio of:

$$\text{Rf} = \frac{\textbf{\textit{Analite Migration Distance}}}{\textbf{\textit{Eluen Migration Distance}}} = \frac{\textbf{\textit{Zs}}}{\textbf{\textit{Zf}}}$$

(2.1)

The Rf values ranged between 0 and 1 and the best Rf values were between 0.2-0.8 for UV detection and 0.2-0.9 for visible detection and 20-80 for the relative Rf for UV detection. At Rf less 0.2, there is no equilibrium between the components of the compound with the stationary and mobile phases so that the shape of the stain is usually less symmetrical. At Rf above 0.8 the analyte stain will be disturbed by the absorbance of the stationary plate impurity observed on visualization with a UV lamp. Visible detection Rf can be higher than UV detection because the stationary phase impurities do not react with the appearance of the stain so that the stains at Rf 0.2 - 0.9 can still be observed properly by controlling the development conditions such as chamber saturation, constant composition of solvent mixtures, constant temperature etc. gives a reproducible Rf value (Alen dkk., 2017).

2.4 Chlorophyll

Chlorophyll is the substance that gives plants their green color. Chlorophyll is useful for plants for photosynthesis, which allows light to be absorbed by plants. Chlorophyll is rich in nutrients such as minerals, vitamins, proteins, nutrients, and micronutrients. Chlorophyll itself is a molecule obtained from plant extracts and can function as an energy source (Meyer, 2010).

2.5 Extraction Process

Extraction is a method of separating a substance based on the degree of solubility in the solvent. The solvent used can be either organic or inorganic. If organic substances are to be produced, the solvent used is also organic. Vice versa for inorganic. If the selection of the solvent is not suitable, then the results obtained will be little or not at all because the solvent is not correct (Wulandari, 2011). The kinds of extraction processes are divided into two:

1. Solid - liquid extraction
2. Liquid - liquid extraction

The extraction at least consists of two stages, namely:

1. Intensive mixing of the extraction material with a solvent, namely after being shaken and then allowed to stand, the substance dissolves and distributes itself into the two solvents (Wulandari, 2011).
2. The separation of the two liquid phases is as perfect as possible, namely when the mixing occurs mass transfer occurs, namely the extract leaves the first solvent (carrier medium) and enters the second solvent (extraction medium) (Wulandari, 2011).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Materials and Instruments

3.1.1 Materials

1. Sample
2. Ethanol 96%
3. Anhydrous sodium sulfate
4. N-Hexane
5. Ethyl acetate
6. Aquadest
7. Chlorophome

3.1.2 Instruments

1. Volume pipette
2. Separation funnel
3. Capillary pipe
4. Dropper pipette
5. Glass funnel
6. Beaker glass
7. Porcelain exchange
8. Scales
9. TLC plate
10. Erlenmeyer

3.1.2 Instrument's Illustration

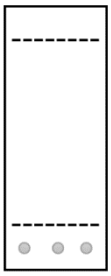

Figure 3.1 TLC plate

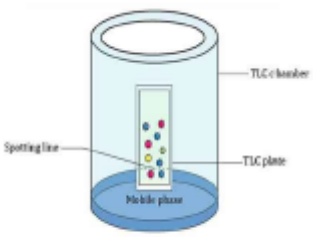

Figure 3.2 Set of Tools

3.2 Experimental Methods

3.2.1 Isolation of leaf chlorophyll pigments

1. Grind fresh leaves using a porcelain currency
2. Transfer the crushed product to a beaker, add 96% ethanol solvent, then wait 1 hour. The beaker glass is covered with a watch glass to prevent the evaporation of ethanol.
3. After 1 hour, filter the solution using filter paper and a glass funnel.
4. Extract the filtrate with chloroform solvent in a separating funnel. Shake until an emulsion forms. Add distilled water to the chloroform layer if two layers haven't been formed (if two layers are not formed, add anhydrous sodium sulphate).
5. Separate the two phases obtained, take the chloroform phase (lower layer).

3.2.2 Analysis of isolated components by TLC

1. Drop the isolated green leaf solution on the TLC plate that has been provided.
2. The streak is done at a distance of 1 cm from the bottom edge of the TLC plate. Allow it to dry, then elute it with a solvent mixture of n-hexane: ethyl acetate (7: 3).
3. After reaching 0.5 cm from the upper limit, take the plate, dry it, and observe for spots that appear in visible light.
4. Photo of TLC plate as evidence. In general, you will see coloured spots, such as orange (carotenoid), green blue (chlorophyll-a), green (chlorophyll-b), and yellow (xanthophyll).

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