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EFFECTIVITY METHOD OF CHROMATOGRAPHY TO ISOLATE COMPOUND OF A METABOLITE SECUNDER AT *PEPEROMIA PELLUCIDA L.* PLANT WITH METHANOL SOLVENT

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Abstract

The aim of this research to identify the efectivity method of chromatography to isolate compound of a metabolite secunder at Peperomia Pellucida L. plant With methanol solvent. As much as 200 gram simplisia plant macerated with the methanol solvent, n- hexan, and Ethyl acetate during 2x24 hours. Maserat obtained subsequently in distillation to produce condensed of methanol extracts of 40 grams. Extracts of condensed methanol subsequently analyzed using thin layer chromatography to see the existence of compounds in a sample. Results of chromatography thin retrieved the reflection of colors that indicate in plant secondary metabolite compounds there are messengers.

Keywords: *Peperomia pellucida L.*, Isolation, Simplisia, Secondary Metabolites.

1. Introduction

1.1. Background

Indonesia is very rich about biodiversity, especially flora that have many kinds of plants. One of plants that usually used as traditional medicine is *Peperomia pellucida L.* In General, society does not know the efficacy and benefits of the plant *Peperomia pellucida L. Peperomia pellucida L.* is a little plant that has the shallow root. It also is weeds that typically grows wild in humid places and huddle. In traditional, this plant is used to treat some diseases, such as gout, ulcers, acne, Dermatitis, kidney disease, and stomach pain (Hariana, 2006). Society in sulawesi is also used this plant to lowering blood cholesterol (Sitorus, Momuat, dan Katja, 2013).

According to the result of the research that have done by Nithiya Paramsothy, Yasmiwar Susilawati, and Supriyatna (2012), they found that secondary metabolite compounds contained in the *Peperomia pellucida L* plant among other classes of compounds, flavonoids, tannins, saponins, steroids, monoterpen, and sesquiterpen. Secondary metabolites are compounds found in plants is bioaktif substances related to the chemical content in plants, so most plants can be used as medicinal ingredients.

Secondary metabolites can spread throughout the body organs of plants such as leaves, stems, roots, flowers, bark, fruit, and tubers. Type and its content can be the same or different organs of the plants. In this research the chromatography will be used is the column

chromatography where after that will be tested by thin layer chromatography purity. Chromatography method was chosen because of its various advantages, including the method of rapid and selective separation. The equipment used is simple and easy to come by. A complex mixture can be separated easily. Another advantage is the footage that was used very little. Test compounds in chromatography can be performed over and over.

The process of isolation of a compound at secondary metabolites of plants needed a good solvent to extract the compound. Selection of solvents in the extraction process will provide high effectiveness in observing the solubility of compounds of natural materials in a solvent. Methanol is one of the frequently used either solvents to extract compounds. In General, the methanol solvent is the most widely used solvent in the process of isolation of organic compounds of natural materials because it can dissolve the secondary metabolites (Darwis, 2000; Anonim 1993).

Extraction is a method used to isolate one component from another component or components of a mixture. Extraction of a component can be performed using chromatography. Identification of secondary metabolite compounds used spectrophotometer UV-VIS and IR. Based on the background of the above researchers tried to do the isolation and identification of secondary metabolites are compounds found in the plant messengers *(Peperomia pellucida L.)* with solvent methanol that uses the methods of chromatography columns and tested purity with thin layer chromatography.

2. Method

2.1. Time and place of the research

This research is doing at Laboratorium Terpadu of University of Muhammadiyah Semarang during 4 months. This research used chromatography column method and tested purity with thin layer chromatography.

2.2. The research variables

In this research process isolation as a free variable, and compounds of secondary metabolites as a variable. The object of the research was *Peperomia pellucida L.* plant. Early methods used solvent extraction using maceration. The results extracted from the maceration will reuse the column chromatography method and tested purity with thin layer chromatography.

2.3. Tools and materials

The tools that used in this research are beaker, measuring cup, dropper drops, analytic, evaporator, balance eyedropper tool, a set of micro-column chromatography, thin

layer chromatography set of tools, UV lamps, funnels, funnel, onto a stand and clamps, spatula, test tubes, petri dish, UV-Vis Spectrophotometry, and infrared Spectrophotometry.

As for the plant materials that are used in this research is Peperomia pellucida l. were obtained from the area of Semarang. The chemicals used are aquades, methanol, ethyl acetate, n-heksan, silica gel, TLC.

2.4. Research design

a. The extraction process
    1. Wash and clean the roots, stems, and leaves of Peperomia pellucida l, cut small then dried and then extracted by means of maceration using methanol, n-heksan, and ethyl acetate.
    2. Maceration is performed during 2x24 hours, every 1x24 hours filtered extract and maceration again with a new solvent .
    3. Made one the extract so we can retrieved the extract.
    4. Doing the distillation using a tool so we can collect the extract.

b. Separation and purification

    Methanol extracts that obtained will be analysed using thin layer chromatography to see the existence of compounds in a sample. Methanol extracts as much as 2 grams separated by chromatography stationary phase column with silica gel and dielusi eluen. Isolates from methanol extracts of column chromatography results tested purity thin layer chromatography with 2 dimensions to see the same pattern of stains to merge. If the isolates remained single stain patterns shows, then we can say it's been pure isolates. Thin layer chromatography results isolates that have retention factor (Rf) of the same combined and evaporated and tested phytochemical.

c. Identification of compounds

    The result of Separation and purification isolates from the fraction of methanol has been in thin layer chromatography, and identified using spectrophotometer UV-Vis and IR spectrophotometer to know secondary metabolite compounds contained in the plant messengers.

3. Result

3.1. Sample preparation

The contingent consists of plants stems and roots are cleaned by means of washed up clean, then dried in the open air until the rest of water dry. Once dry then cut

with small size and dried again under way at an open place that is not exposed to sunlight. Subsequently weighed and retrieved as many as 600 grams dried simplisia.

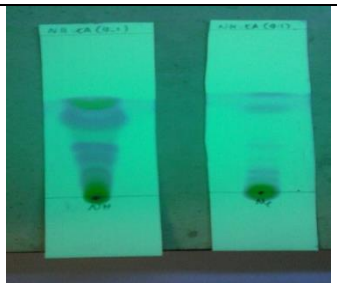
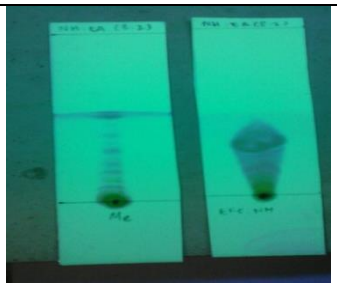
3.2. Extraction

Every 200 grams of dried plant simplisia Messenger extracted by means of maceration using methanol solvent, n-heksan, and ethyl acetate. Maceration is carried out during 2x24, where each 1 x 24 hour extract is filtered and maceration again with a new solvent but the same types. The filtrate is obtained then merged and distilled with distillation tools to extract thick formed. Condensed extracts obtained as much as 20% of the weight of dry each simplisia maceration is about 40 grams and bluish green. The most viscous extract obtained was of solvent methanol.

3.3. The separation

Extracts of condensed methanol and n-heksan that obtained are analyzed by thin layer chromatography (TLC). TLC performed using phase of motion in the form of eluen with n-eluen comparison heksan: ethyl acetate (8:2) and (9:1). This is done to see the presence of compounds that are present in the sample through patches of stain and the different colors. In addition to knowing the right eluen comparison.

The result of TLC can be seen below :

	
N-heksan : Ethyl Acetat 9:1	N-heksan : Ethyl Acetat 8:2

4. Discussion

4.1. Why *Peperomia pellucida L.*?

Peperomia pellucida L. is a little plant and shallow rooted. The Peperomia pellucida l plant is a weed that normally grows wild in humid places and huddle. It has traditionally been used in treating some diseases, such as gout, ulcers, acne, dermatitis, kidney disease, and stomach pain (Hariana, 2006). Community in North Sulawesi have also utilize this plant for lowering blood cholesterol (Sitorus, Momuat, and Katja, 2013). However, the public at large, not to know the efficacy and benefits of the *Peperomia pellucida L*. Based on the problems researchers will isolate a compound found in that plant.

4.2. Why should be cleaned and dried before maceration?

The plant messengers before maceration is done in advance, cleaned and dried. The purpose of these activities is to eliminate the pollutant substances and decrease water levels at the plant *Peperomia pellucida L.* so that it will not interfere with the time of process isolation.

4.3. Why should maceration?

Among the various types of methods of separation, extraction solvent or water extraction is also called the method of separation is the best and popular. The principle of this method is based on the distribution of dissolved substances with specific comparisons between the two are not mutually mixed solvents, such as instructor, karbontertraklorida or chloroform. Their boundaries are dissolved substances can be transferred in different amounts in the second phase of the solvent. Extraction solvent used researchers is maceration. Maceration is a way of extracting material with it does not use solvent soaking water for a certain time (Pharmacopoeia Indonesia, 1995). Maceration method generally uses the solvent water or solvent is non a non polar. In theory when simplisia steeped in solvents then it will happen so that substances concentration difference arises, a solution of diffusion style terpekat will be urged towards the exit. The solution of trying to achieve a balance of concentration. This process will be stopped after a concentration balance or saturation. In this case, the extraction process was declared finished.

4.4. Why Used Methanol Solvent, N-Hexsan, And Ethyl Acetat?

Selection of solvents in the extraction process will provide high effectiveness in observing the solubility of compounds of natural materials in a solvent. Methanol is one of the frequently used either solvents to extract compounds. In General, the methanol

solvent is the most widely used solvent in the process of isolation of organic compounds of natural materials because it can dissolve the secondary metabolites, (Darwis, 2000; Anonim 1993).

Extraction of three solvents gives the yield varies for each solvent used. Of the three extracts obtained can be seen that the methanol extracts, extract, is the most numerous in number. This obviously shows that the content of organic compounds are relatively large polarnya, followed in succession by ethyl acetate extract (semi polar) and n-heksan (non-polar).

4.5. Why Should Destilation?

Distillation separation in principle is a method of separation based due to the difference between the boiling point components will be separated. Compounds are secondary metabolites is a compound that is not resistant to heat, light, and other chemicals. However, in the process of distillation is done indirectly. Researchers used a water bath so that the extract untouched by fire.

4.6. Why in TLC?

TLC is one of chromatography. Chromatography method was chosen because of its various advantages, including the method of rapid and selective separation. The equipment used is simple and easy to come by. A complex mixture can be separated easily. Another advantage is the footage that was used very little. Test compounds in chromatography can be performed over and over.

5. Conclusion

Based on the result research, it concluded that thick extract of the plant messengers *(Peperomia pellucida L.)* contains several compounds are secondary metabolites. As for the stages in the research starting with sample preparation, as many as 200 grams of dried plant sample agent dimaserasi during 2x24 hours with solvent methanol, n-heksan, and ethyl acetate. The filtrate is obtained then merged and distilled with distillation tools to extract thick formed. Condensed extracts obtained as much as 20% of the weight of dry each simplisia maceration is about 40 grams and bluish green. The most viscous extract obtained was of solvent methanol. TLC test results showed a secondary metabolite compounds found in the plant messengers views through the reflection of color.

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