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Extraction and Purification of Piperine from Black Pepper

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Abstrak

Experiments were carried out on the extraction and purification of piperine from black pepper using the Soxhlet method, with the aim of understanding how liquid-solid extraction works using a Soxhlet extractor, testing the purity of piperine using thin layer chromatography (TLC), and identifying alkaloid class compounds based on qualitative tests. Piperine is an alkaloid compound derived from pepper that has antioxidant and anti-inflammatory properties. Piperine is yellow with needle-shaped crystals that are poorly soluble in water but readily soluble in organic solvents. Piperine can be extracted from black pepper by the soxhletation method, which is a repeated extraction with dichloromethane (DCM) solvent using a 2-circulation process of vapor-liquid changes of the solvent with the help of heating. The piperine extract obtained was then separated from the DCM solvent by evaporation to obtain piperine extract weighing 2.5000 grams with a yield of 8.333% when compared to the initial mass of black pepper of 30 grams. The TLC test with ethanol:water eluent gives RF 0.60, so that the extract can be said to be pure, and qualitative tests with Dragendorff reagent give an orange color, indicating the positive presence of alkaloid compounds in the extract in the form of piperine.
Keywords : *Piperine, alkaloid, dan soxhlet*

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INTRODUCTION

Piperine, a potent alkaloid found primarily in black pepper and other Piper species, stands out as a compound of immense interest in both scientific and traditional medicinal spheres. With its distinct chemical structure and multifaceted pharmacological effects, piperine has emerged as a subject of extensive research and exploration. This bioactive compound not only contributes to the characteristic pungency and flavor of black pepper but also exhibits a wide range of potential health benefits. From its role in improving nutrient absorption to its antioxidant and anti-inflammatory properties, piperine's versatility underscores its importance in a wide range of fields of study and application. The concentration of piperine in pepper is around 5-9%. Piperine has a yellow color with needle-like crystals, is heat resistant due to its high boiling point, and is slightly soluble in water but easily soluble in ethanol, ether and chloroform (Hamdani, 2024). The structure of Piperine according to Tiwari et al 2020 :

Extraction is a technique of separating a compound from another unwanted compound based on the difference in solubility (Pratiwi et al, 2023). The purpose of extraction is to attract chemical components found in natural materials. This extraction is based on the principle of mass transfer of the components of the substance into the solvent, which begins to occur in the interface layer and then diffuses into the solvent (Meigaria et al, 2016). There are two types of extraction, namely solid-liquid extraction or leaching and liquid - liquid extraction or solvent/separating extraction. In solid - liquid extraction, there are 3 types, namely maceration, percolation, and soxhletation. Because the concentration of piperine is very small in black pepper, an effective extraction and purification method is needed so that it can produce piperine extract from black pepper as much as possible. Of the many extraction and purification methods available, the Soxhlet method is very suitable for piperine extraction and purification.

The Soxhlet extraction method utilizes a device called the Soxhlet apparatus, which is a repetitive extraction instrument employing the same solvent. It utilizes a continuous circulation

technique to alter the solvent's vapor-liquid phase through the application of heat. Initially introduced in 1879 by Franz von Soxhlet, the Soxhlet extractor was constructed using materials such as thimble, a F-shaped tube, and a siphon. However, to conduct the extraction process with this apparatus, additional components are necessary, including a condenser, a round-bottom flask, and a heating mantle. The thimble serves as a reservoir for the sample, while the F-shaped tube provides a pathway for the heated solvent vapors. Below the thimble, the siphon acts as a mechanism for cycle enumeration, considering each complete solution transfer from the siphon into the round-bottom flask as one cycle. Conversely, the condenser acts as a cooling apparatus to facilitate the condensation procedure, the round-bottom flask functions as a container for the solvent and extraction results, and the heating mantle serves as the heat source throughout the entirety of the extraction process (Leba, 2017).

The Soxhlet extraction method is the best method for extracting piperine because the piperine compound is thermostable and can be extracted more effectively by heating using the Soxhlet method. The Soxhlet extraction method is an extraction using a solvent that undergoes a cycle that is carried out specifically so that continuous extraction occurs with a relatively constant amount of solvent with the presence of a condenser. The soxhlet extraction method also made the biological activity of the sample stays when it is heated with soxhlet method. Hence, the sochlet method can be used in obtaining drug essences (Heinrich, 2004). The Soxhlet exctraction method is a heating method, the solvent used will undergoe circulation or cycle, which is why compared to other methods, the Soxhlet extraction method provides higher extract yields (Soemiati, A. 2013).

METHODOLOGY

Ingredients and Materials

The tools used in this experiment were a set of Soxhlet apparatus, electromantel, glass funnel, chromatography vessel, measuring cup with a volume of 5 mL, pencil, petri dish, filter paper boiling stone, analytical balance, beaker, rotary vacuum evaporator, watch glass, capillary tube, ruler, Thin Layer Chromatography plate, and tweezers.

The materials used in this experiment were coarse black pepper powder, dichloromethane (DCM) solvent, ethanol, water, and Dragendorff's reagent.

Method

The first step carried out in this experiment was soxhletation. First, weigh 30 grams of the black pepper sample flakes, then wrap it in filter paper and tie it tightly with string to minimize the extraction product being contaminated by the sample flakes. The next step is to fill 3/4 of the round bottom flask with DCM (dichloromethane) solvent and enough boiling stones to prevent bumping. DCM is used because it has a low boiling point, namely 40°C, so it evaporates easily and speeds up extraction and solvent evaporation. Then the sample is put into the thimble, then the soxhlet extractor is assembled from bottom to top, namely the heating mantle, round bottom flask, soxhlet, and condenser. Next, a water hose can be installed on the condenser with water flowing from bottom to top so that there are no air voids remaining and condensation can occur effectively.

Once the tool is fully installed, extraction can begin by turning on the water flow and heating the mantle. Extraction is carried out in 2 cycles, with the mechanism for each cycle as follows: the heated solvent will produce steam which will rise to the condenser through pipe F and be cooled by the condenser so that it forms dew and falls to wet the sample in the thimble. Over time, this fluid will flood the sample, and if its height exceeds the siphon, it will escape and flow back into the round-bottom flask. This is called a cycle. After two cycles, the heating pad and tap can be closed to stop the extraction process, and the tool can be removed from the assembly. From this stage, an extract is obtained which is mixed with the solvent.

The second step in this experiment is to evaporate the DCM solvent from the piperine extract using a rotary vacuum evaporator. First, the support on the rotary vacuum evaporator is raised, then the round bottom flask containing the extract and solvent is attached to the clamp of the rotary vacuum evaporator which has been smeared with petroleum jelly so that it does not get stuck. Once the round bottom flask is confirmed to be installed perfectly, the support can be lowered so that the round bottom flask is submerged in a heating bath containing distilled water. After that, a set of rotary vacuum evaporators can be turned on by connecting the cooler, heating bath, rotary evaporator, and vacuum pump to a power source. First, the cooler can be turned on by pressing the on button, then

setting the rotation speed of the round bottom flask by 10. Next, the heating bath temperature is set at 35°C, and the pressure in the vacuum pump is set at 280°C. It should be noted that during evaporation bumping can occur due to the high temperature and the pressure being too high or too low, so that if a bump occurs, the temperature of the heating tank can be lowered or the pressure can be increased. However, if the evaporation process is not running, which is indicated by the absence of condensed solvent, then the pressure can be lowered. The evaporation process is carried out until the solvent in the extract runs out; only then can the rotary vacuum evaporator set be turned off. The recovered solvent can be removed from the condenser and returned to its place, while the round bottom flask containing the extract can be removed by pressing the rotary knob so that the neck of the flask does not break. The resulting extract was then weighed and 2,5000 grams were obtained.

The next step in this practical is purification by sublimation (recrystallization) which aims to obtain pure piperine crystals. The process is carried out by heating the extract in a petri dish on a hot plate covered with filter paper and placing a beaker filled with water on top so that the extract evaporates and forms crystals on the filter paper. As a result, yellow piperine crystals with a needle shape were obtained.

The experiment continued with testing the purity of piperine resulting from extraction and evaporation using thin layer chromatography (TLC) and Dragendorff reagent. Purity testing using TLC is the separation of solutes based on differences in migration in a system consisting of a stationary phase (TLC plate) and a mobile phase (eluent).

Initially, a 10 ml eluent system was made in a beaker, namely ethanol: water = 1:1. Then cover with a watch glass to reduce evaporation. Then the extract was spotted with a capillary tube on a TLC plate which had been marked 1 cm from the bottom and ½ cm from the top. Next, the TLC plate can be inserted into the chromatography vessel containing the eluent in an upright position and stop the TLC process when the elution has reached the upper limit mark. Then the spots from the TLC results can be observed under a UV lamp at a wavelength of 254 nm and show that the extract is not pure because the spots produced are not only 1. The distance the eluent travels is 7 cm while the distance the analyte travels is 4.2 cm.

The final step of this practical is to spray the TLC plate with Dragendorff reagent to qualitatively test the presence of an alkaloid compound in the form of piperine, which is indicated by the formation of an orange color due to the interaction between the alkaloid compound and trinitro-Bismuth (III) ($Bi(NO_3)_3$) in Dragendorff. From the experiment it can be seen that the plate produces an orange color. This means that a piperine-alkaloid compound was detected.

Set of Tools Used

Picture 1. Soxhlet Apparatus

Picture 2. Rotary Vacuum Evaporator Apparatus

Picture 3. TLC

RESULT

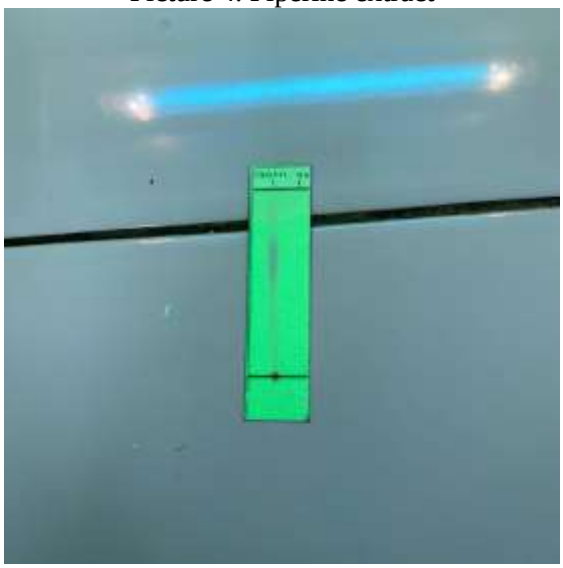
Piperine Yield From Black Pepper

= (extract piperine mass/sample mass) x 100%
= (2,5000 gram/30 gram) x 100%
= 8,333%

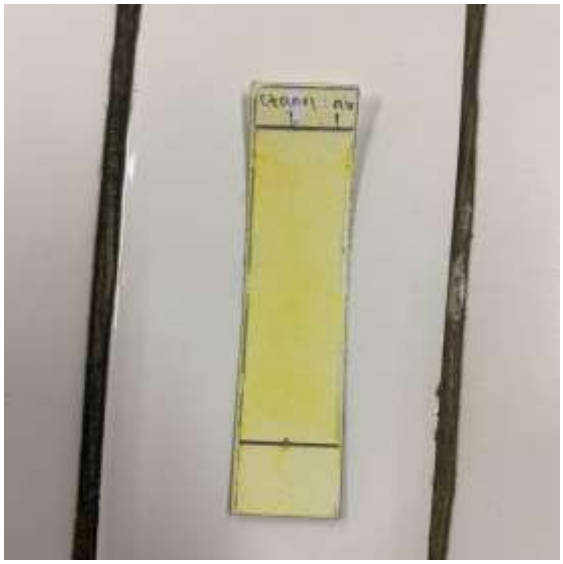
Nilai RF Piperine

= distant traveled by dyes/distant traveled by solvent
= 4,2/7
= 0,60

Picture 4. Piperine extract



Picture 5. TLC result



Picture 6. Dragendorff Result

DISCUSSION

Piperine is an alkaloid compound derived from pepper that has antioxidant and anti-inflammatory property. Piperine is a yellow-needle shaped crystals that has low solubility in water, but highly soluble in organic solvents. Piperine can be extracted from black peppers by with soxhletation method, which is a repeated extraction with dichloromethane (DCM) solvent using a 2-circulation process of vapor liquid changes of the solvent with the help of heating.

In the soxhlet apparatus, the heated solvent will produce steam which will rise to the condenser through pipe F, and then the condenser will cool it so that it would forms dew and falls, wetting the sample in the thimble. Over time, this fluid will flood the sample, and when the height exceeds the siphon, it will escape and flow back into the round bottom flask. These counts as one cycle. After two cycles, the extraction can then be stopped and the soxhlet apparatus can be dismantled. From the extract results obtained, it can be seen that the extract has a yellowish color with a few black powders. This is caused by the pepper flakes being mixed into the extract in the soxhletation process.

After going through the soxhletation stage, the resulting piperine extract mixed with DCM can be evaporated to separate the piperine extract from the DCM solvent. As a result, 2.5000 grams of piperine extract was obtained with a yield of 8.333%. This yield value is a good value for extracting natural materials because natural materials do not contain that much extract material.

The RF value was also calculated and an RF value of 0.60 was obtained by dividing distant traveled by dyes by distant traveled by solvent from TLC. The RF results obtained are still in the range 0.2-0.8 so the extract results can be considered as pure.

The obtained extract were then recrystallized so the yellow colored needle shaped crystals of piperine were obtained. This repeated recrystallization process are done to increase the purity of the crude product.

Next, the Dragendorff test was carried out with the aim of determining whether there was an alkaloid group in the form of piperine in the extract product. The Dragendorff test was carried out on a TLC plate which had previously undergone a TLC test by spraying it on it and an orange color was formed due to the interaction between the alkaloid compound and trinitro-Bismuth (III) ($Bi(NO_3)_3$) in Dragendorff. This means that piperine-alkaloid compounds were detected.

CONCLUSION

From the practicum that has been carried out, it can be concluded that:

1. How solid-liquid extraction works using a Soxhlet extractor is carried out by repeatedly heating the solvent to produce steam, which flows through the pipe, which will be cooled by the condenser and form dew. Then, solvent moisture falls, bathes the sample on the lead, and dissolves the analyte. If the height exceeds the siphon, the solvent containing the analyte will fall into the round bottom flask, and this is called a cycle.
2. The purity of organic compounds can be tested using thin-layer chromatography (TLC) using different eluent ratios as the mobile phase and TLC plates as the stationary phase to obtain the Rf value, which also varies depending on the polarity of the eluent. Pure compounds are marked with 1 spot, while impure compounds are marked with trace spot, as the results in this experiment.
3. Qualitative identification of alkaloid compounds can be done by spraying Dragendorff's reagent on the TLC plate. A positive test for the presence of alkaloids in the form of piperine is characterized by the formation of an orange color.

REFERENCES

Cinelli, M. A., & Jones, A.D. 2021. *Alkalines Of The Genus Datura: Review Of A Richresources For Natural Product Discovery*. Molecules, 26 (9), 2629

Hamdani, Ridwan. *Studi Pembentukan Dan Karakterisasi Kompleks Inklusi Piperin Dari Lada (Piper Nigrum) Dengan B-Siklodekstrin Dan Garam Mono-6-Amonium B-Siklodekstrin Klorida*. Diss. Universitas Pendidikan Ganesha, 2024.

Indarto Et Al. 2017. *Aktivitas Antibakteri Ekstrak Daun Binahong Terhadap Propionibacterium Acner*. Jurnal Tadris Biologi, 10(1). 67-68.

Khairunnisa, K., & Ridwanto, R. 2023. *Uji Aktivitas Anti Oksidan Dari Ekstrak Etanol Dari Daun Sidaguri (Sida Rhoumbifolia L.) Dengan Metode Ppph 0,1-Difenil-2-Pikrilhidrazil.* Jurnal Kesehatan, 1(3), 510-522.

Leba, Maria L.A. U. 2017. *Ekstraksi Dan Real Kromatografi*. Yogyakarta: Deepublish.

Mariana Et Al. 2018. *Validan Metode Penetapan Kuantitatif Metanol Dalam Urin Menggunakan Gas Chromatography-Flame Lonization Detector.* Indo J. Chem. Sci, 713), 277 - 289.

Mugaria Et Al. 2016. *Strining Fitokimia Dan Uji Aktivitas Antioksidan Ekstrak Aseton Daun Kelor.* Jurnal Wanana Matematika Dan Sains, 10(2).

Meigaria, K. M, Mudianta I. W., & Martiningsih, N. W (2016). Skrining Fitokimia Dan Uji Aktivitas Antioksidan Ekstrak Aseton Daun Kelor (Moringa Oleifera). Jurnal: Wahana Matematika Dan Sains, Vol. 10, No. 2

Pratiwi Et Al. 2023. *Ekstraksi Pektin Dari Kulit Kakao Menggunakan Pelarut Asam Sitrat*. Jurnal Tekkim, 315), 693-703.

Sugiarto Et Al. 2023. *Prediksi Data Lesetimbangan Cair-Cair Pada Sistem Terner Air + Asam Laktat + Pelarut Menggunakan Model Unifac-Dmd.* Prosiding Semnas Teknik Kimia: Pengembangan Tetnologi Kimia Untuk Pengolahan Sda.

Tiwari Et Al. 2020. *Piperine: A Comprehensive Review Of Method Of Isolation, Purification, And Biological Properties*. Medicine In Drug Discovery, 7.