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DETERMINATION OF FLAVONOID CAPACITY BY USING ETHANOL EXTRACT OF CIPLUKAN LEAVES (*Physalis angulata* L.) USING UV-VIS SPECTROFOTOMETRY METHODS

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Abstract:
Ciplukan leaves (*Physalis angulata* L.) have a composition of main phytochemical compounds including alkoloids, phenolics, tannins, flavonoids that can function as antioxidants, antibacterial, antiviral, anti-inflammatory, antiallergic, and anticancer. The purpose of this study was to identify and determine the levels of flavonoid compounds contained in the Ethanol Extract of Ciplukan Leaf (*Physalis angulata* L.) Ethanol extraction of Ciplukan Leaves (*Physalis angulata* L.) was carried out using the maceration method using 96% ethanol solvent, a flavonoid identification test was carried out on the Ethanol Extract of Ciplukan Leaves with the addition of Mg metal powder, concentrated HCI, and 10% NaOH, then determining Flavonoid levels using the UV-Vis spectrophotometric method using Quercetin as a comparative raw material. A qualitative test of Ethanol Extraction of Ciplukan Leaves (*Physalis angulata* L.) containing flavonoids has been carried out. Determination of flavonoid levels using UV-vis spectrophotometry obtained 0.0235%.
Keywords: Ciplukan (*Physali angulata* L), Flavonoids, UV-VIS Spectrophotometry

Introduction

Indonesia is a region known as a producer of medicinal raw materials that can be used to treat various diseases. Plant species that have medicinal properties, and are also used as traditional medicine. In this traditional medicine itself, most of the concoctions come from plants in the form of roots, fruits, flowers, leaves and seeds (Yasir, et al., 2018). Ciplukan contains active compounds including saponins (in buds), flavonoids (leaves and buds), polyphenols and fisalin in (fruit) (Ramesh, *et al*., 2014).

Herba ciplukan (*Physalis angulata* L.) is one of the plants that has potential as a traditional medicine. This plant is generally found as a wild plant, the content found in this plant is polyphenol and flavonoid compounds (Ali *et al*., 2012). Because of the importance of ciplukan in medicine, the quality of its safety and usefulness must be improved through research and development. To improve the quality of safety and usefulness of ciplukan as an Indonesian natural medicine, it is necessary to standardize the raw materials, both in the form of simplisia and in the form of extracts or galenic preparations. Based on the description above, the

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researcher is interested in conducting research by developing ethanol extract of ciplukan leaves (*Physalis angulata* L.) by determining flavonoid levels using the Uv-Vis spectrophotometric method.

Methods

A. Tools and Materials

The tools used were test tubes (Pyrex), glass jars (Pyrex), erlemeyers (Pyrex), volumetric flasks (Pyrex), drop pipettes, measuring cups, (Pyrex), vaporizer cups and masks, gloves, analytical scales (Shimadzu), flannel cloth, a set of rotary evaporator (Biobase), spatels, dark glass vessels, burettes, and Uv-Vis spectrophotometry (Genesys 10S Uv-Vis). The materials used were Ciplukan (Physalis angulata L) leaves, distilled water (PT. Promedika Mitra Utama), 96% ethanol (Merck), 10% aluminum (III) chloride (Merck), 1M sodium acetate (Merck0 and quercetin (Merck).

B. Sample Preparation

1. Raw Material Collection
   Collection of ciplukan (*Physalis angulata* L.) leaf raw materials from Ketahun sub-district, regency, North Bengkulu.
2. Wet Sorting
   Samples of ciplukan (*Physalis angulata* L.) leaves are then separated or sorted when the plant is still fresh free from the remains of foreign substances, twigs, different flowers or other plants and soil attached to the plant.
3. Washing
   Washing is carried out using clean water, namely tap water or running water, which is not polluted by waste and other chemicals, so that the samples used are clean from attached dirt.
4. Sharpening
   Sharpening is done using a sharp knife that is not blunt so that the rust substance does not stick to the sample to be used. This sharpening is done to expand the surface of the raw material so that it is easy to dry in the drying process.
5. Drying and dry sorting
   Drying is done by aerating at room temperature 15-30 ° C and dry sorting is done to separate foreign objects that are still left in the simplisia after the drying process.
6. Storage
   Storage of dried simplisia is stored in a tightly closed container so that the quality of the simplisia is maintained and not mixed with other simplisia.

C. Preparation of Ciplukan Leaf Extract (*Physalis angulata* L.)

In the maceration process, all 200 grams of simplisia powder were put into a closed container and immersed in 2000 ml of the first solvent, left for 6 hours while occasionally shaking, then allowed to stand for 18 hours at room temperature. The filtrate and residue were separated then the residue was macerated again with 2000 ml of solvent using the same process. This process was carried out continuously until the color of the filtrate was constant. The filtrate is collected and evaporated using a rotary evaporator until it becomes a thick extract (Novi Fajar Utami, et al., 2020).

D. Extract Characteristics

1. Organoleptical

The organoleptical test was carried out with the aim of knowing the smell, color, consistency of the ciplukan leaf extract (*Physalis angulata* L.) This examination was carried out visually by observing the shape, color, and smell. (Ministry of Health, 2000).

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2. Yield

The purpose of the yield is to determine the ratio between the extract obtained and the initial simplisia. (Ministry of Health, 2000).

E. Qualitative Analysis of Flavonoid Content

1. Flavonoid test with 10% NaOH by inserting 2 drops of thick ethanol extract of ciplukan leaves (*Physalis angulata* L.) into a test tube, added with 2-4 drops of 10% NaOH solution (Asih, 2009), the color change is observed until it becomes yellow to yellow-brown.
2. Flavonoid test by adding 5 drops of hydrochloric acid and magnesium metal powder if the solution produces yellow, orange and red colors then the material is positive for Flavonid (Nugraha et al, 2016).

F. Determination of total flavonoid content

Weigh carefully approximately 2 g of powdered simplisia, put into a 10 mL volumetric flask, then add methanol to the limit mark then homogenize and filter. Then pipet 0.5 ml of sample solution of 2 g dry extract add 1.5 ml of methanol then add 0.1 ml of aluminum chloride solution 10, then add 0.1 ml of sodium acetate 1M and 2.8 ml of distilled water. Allow to stand for 30 minutes and put into cuvette. Measure the absorbance at the maximum wavelength of quercetin by Uv-Vis spectrophotometry. Flavonoid compound levels were determined with the regression equation of the calibration curve. The results obtained were calculated with a dilution factor so as to obtain the concentration of flavonoids contained in the dry extract of ciplukan herb (*Physalis angulata* L.) (Krisyanella, et al., 2013) then calculate the percentage of total flavonoids as quercetin in the extract with a standard curve.

G. Data Analysis

To calculate the flavonoid content contained in the ethanol extract of ciplukan leaves (*Physalis angulata* L.) can be calculated based on the calibration curve of the reading results from the UV-Vis spectrophotometer, and the linear regression equation using the Lambert-Beer law.

Result and Discussion

1. Extraction Result

The samples used were ciplukan leaves (*Physalis angulata* L) taken from Ketahun District, North Bengkulu Regency. The ciplukan taken is young because it still contains many active compounds and the collection is carried out in the morning before photosynthesis, this is done in order to homogenize the harvest time, after harvesting, wet sorting is carried out, washing with running water and drying. The sample used for this test is ciplukan herb (*Physalis angulata* L) which has been tested at the faculty of mathematics and natural sciences biology laboratory, Bengkulu University. After that, it is continued with the characterization of simplisia which aims to obtain good quality simplisia and meet the standardization of Materia Medika Indonesia (1978). Extraction was carried out by maceration, namely by soaking simplisia as much as 200 grams of ciplukan leaf powder (*Physalis angulata* L) into 96% ethanol until submerged. Maceration was carried out in a dark bottle for 3-5 days with occasional shaking then the extract was filtered to obtain a liquid extract. The extract obtained is evaporated with a rotary evaporator at a temperature of 40ºC. this aims to keep the extract from being damaged so that a thick extract is obtained. So that the results obtained from refluxing as much as 200 g of sample is 34.069 g.

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2. Organoleptical Test Results

In the organoleptical test of ciplukan leaf extract (*Physalis angulata* L) obtained from the consistency results in the form of a thick blackish green liquid, the smell of the extract is typical.

3. Yield Test Results

The yield test serves to determine the level of secondary metabolites carried by the solvent but cannot determine the type of compound carried by the solvent (Aminah, 2017). The yield value of Ciplukan leaf extract is 17.034%, where the greater the yield produced, the more efficient the treatment applied by not ruling out other properties, (Nurhayati, 2009 deck).

4. Qualitative Test Results

Identification of flavonoid compounds in ciplukan leaf extract (*Physalis angulate* L.) is added to magnesium powder and then add a few drops of concentrated hydrochloric acid (HCI) to form a yellow color. The purpose of adding magnesium powder is to produce complex compounds and the addition of concentrated HCI is used to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. Glycosyl will be replaced by H from the acid due to its electrophilic nature. This reduction of magnesium and concentrated HCL will produce complex compounds by causing a yellow color (Pratiwi, 2010). After conducting qualitative tests on ciplukan leaf extract (*Physalis angulate* L.), quantitative testing was then carried out to determine how much flavonoid levels in ciplukan leaf extract (*Physalis angulate* L.) using the Uv-Vis spectrophotometric method.

5. Flavonoid Content Determination Test Results

Determination of flavonoid levels from ethanol extract of ciplukan leaves (*Physalis angulata* L.) by UV-Vis spectrophotometric method, the compound used as a standard solution in the determination of flavonoid levels is quercetin, because quercetin is a flavonoid flavonol group that has a keto group at atom C-4 and also a hydroxyl group at neighboring atoms C-3 and C-5 (Dyah Nur Azizah, et al. 2014). Flavonol is known as a flavonoid characterizing compound because of its widespread presence in plants. In addition, most medicinal plants show high quercetin content activity (Hayatus, et al. 2017).

Determination of the maximum wavelength is 431 nm. in research (Rega, et al 2018) also obtained a maximum wavelength of 431 nm for kursetin. After obtaining the maximum wavelength routine, a calibration curve is carried out with concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm.

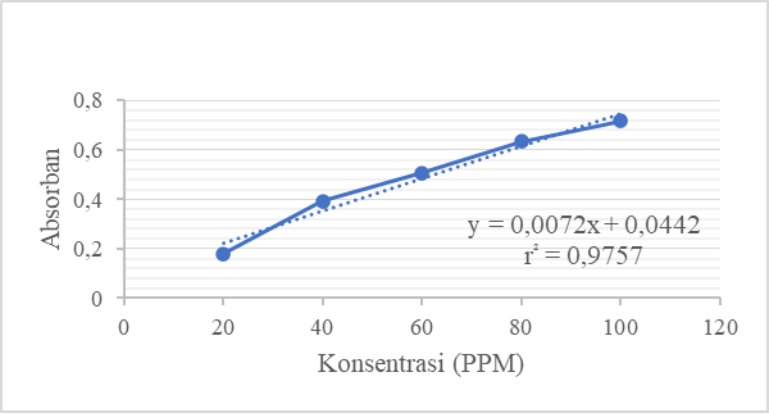

Figure 1. Quercetin Calibration Curve

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The purpose of making this standard curve is to help determine the levels of flavonoid compounds in the sample through the linear regression equation of the routine calibration curve. So that the regression equation y=0.0072x + 0.0442 is obtained with a correlation coefficient of 0.9757. The value of r which is close to one proves that the regression equation is linear (Harmita, 2004). The measurement of flavonoid content using UV-Vis spectrophotometer showed that the content of flavonoid compounds in ciplukan (*Physalis angulate* L.) leaf extract was 0.0235%.

Conclusion

Based on the results of the research that has been carried out, it can be concluded that:

1. Ethanol extract of ciplukan leaves (*Physalis angulata* L.) is positive for flavonoids.
2. Flavonoid content of ethanol extract of ciplukan leaves (*Physalis angulata* L.) by UV-Vis spectrophotometric method with an average value of 0.0235%.

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