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AVOCADO SEEDS (Persea americana Mill.): FORMULATIONS OF ELIXIR VARIED BY SOLVENTS COMPOSITION

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Abstract. Kidney stones disease is asignificant health problem in the world and Indonesia is no exception. Traditionally, avocado seeds can be used as a laxative medicine for kidney stones disease. Elixiris a clear and sweet hidroalkohol solution intended for oral use and a flavor is usually added to enhance the taste. This study was to determine theformula of the avocado seed elixir which has the best quality and the level of flavonoids.Elixir testing was performedusing several methods such as organoleptic, limpidity, viscosity, pH and specific weightas well as total flavonoids. The result showed that the best elixir formula was the Formula I which composed of 7%avocado seed extract, 5% ethanol, 10% propylene glycol, 40% sirupus simplex, 0.1%, essence mint and 100%aquadestilata ad. Furthermore, Elixirhad a brown color, mint aroma and pretty sweettaste. The test results showed that elixir had a viscosity of 7.92 cP; pH of 5.65; specific weight of 1.0413 g/mL andtotal flavonoids of 2.1020%.

Keywords: kidney stones; avocado seeds; elixir; flavonoids.

I. INTRODUCTION

Kidney stone disease is a significant health problem in the world and Indonesia is no exception. In 2013, the prevalence of patients which diagnosed kidney stonesby physician interviews in Indonesia amounted to 0.6 percent of the 250 million population **[1]**. Traditional treatment is oftenconsidered as an alternative ways, so that a lot of research is focused on potential plants which possibly contains certain chemical compounds that have a laxative effect on kidney stones. Laxative activity of flavonoids can help kidney to remove kidney stones through urine, while potassium can degrade calcium and bindswith oxalate so that its solubility in water increases (Nisma **[2]**).

Avocado (Persea americana Mill.) family Lauraceae is used in this study. Traditionally, avocado seeds can be used as a laxative medicine for kidney stones disease. A research by Adliah **[3]** showed that the water extract of avocado seedswith concentration of 1050 mg/100 ml is the highest dose to dissolve calcium oxalate in kidney stones with the highest potassium levels of 0.035%. On the other hand,the result of phytochemical test of avocado seeds extracts showed that the avocado seed contains polyphenols, flavonoids, triterpenoids, quinones, saponins, tannins and monoterpenoid as well as sesquiterpenoids (Zuhrotun **[4]**, 2007).

Avocado seeds extract is insoluble in water because it contains a quite high starch, approximately of 23% (Winarti **[5]**). Therefore, to improve its solubility, an elixir was provided. The purposes of the elixir preparation were to improve the solubility of important substances, to guarantee the homogeneity, to easily absorbed important substances in dissolved forms, to make the taste sweeter and have better aroma, andto easilyusedby people who havedifficulties to swallow drugs such as children andelderly. Elixir product is not widely distributed in the market. Therefore, there is a needs to make an elixir of herbalin order to facilitate peoples in the treatment of kidney stones. Elixir has the advantages that the solutions is clear and does not need to be shaken compared to suspension.

Solvents used in the preparation of the elixir were 70% ethanol, propylene glycol, and water which aimed to increase the solubility of the elixir. Anief **[6]** states that the concentration of solvents in the preparation of elixir is typically5 -10% of alcoholand 10 -25% of propylene glycol (Rowe **[7]**).

II. RESEARCH METHODS

Materials

Avocado seeds (*Persea americana* Mill) obtained from juice traders around Universitas Pakuan Bogor, 70% ethanol (Brataco Chemical), Propylene glycol (Brataco Chemical), essence mint (Brataco Chemical), aquadestilata (Brataco Chemical), Sodium Acetate 1M (Merck®), Quercetin (Sigma Aldrich®), $AlCl_3$ 10% (Merck®).

Produced of Avocado Seeds Dry Extract

Ripe avocado seeds were cleaned of dirt, washed and dried in an oven at 50°C. Once dried, seeds weregrinded and sieved using a sieve mesh 30. Extraction were prepared by using stew. A total of 100 grams of powder simplicia was

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put into a pot with 1 L of water, boiled for 15 minutes, startsafter the temperature reached 90^{o}C and stir up occasionally. Filter the water while hot through batiste, then put in a flask (initial treatment).Then, avocado seeds residue was added with 1 L of water, treat as same as the initial treatment. Finally, a filtrate is generated **[8]**. The liquid filtrate wasused to get dry extract using a vacuum dryer with a temperature of 60^{o}C.

Formulation of Elixir

Elixir was made with a concentration of 1.05 g/15 mL (one drink). Elixirwas made as much as 150mL(10.5g avocado seeds extract) according to the research of Adliah [3] which usesa laxative of kidney stones with a concentration of 1050 mg/100 mL. Elixir was made using3 formulas with different solvent composition.

The materials used in this study were weigh according to the formula. Avocado seeds extract were dissolved in 70% ethanol in a separate place,then propylene glycol was dissolved with water. Afterwards, both materials were mixed using a homogenizer. The mixture was filtered and put in a beaker and added with sirupus simplex. *Essence mint* in water solution was added. Finally, distilled water were add into the beaker and gently stir up the solution. Then put into a measuring cup of 200 mL and transferred into containers of 150 mL (Helmi **[9]**).

Elixir Testing

a. Organoleptic test

Organoleptic test was carried out in the elixir to directly assess the product which comprising of taste, color and aroma before packaged.

b. Limpidity test

Limpidity test was performed by using a centrifuge, which aimed to look for sediment in the elixir.

c. Viscosity and Flow test

Viscosity test was done by using a brookfield viscometer.

d. pH test

PH test was performed by using a pH meter calibrated with standard buffer pH 4 and pH 7. Measurements were done at room temperature with a pH condition of elixirbetween 5-7 (Connors **[10]**).

e. Specific Weight test

Measurement of specific weight was performed by using a pycnometer.

Determination of Total Flavonoids inAvocado Seeds

a. Determinationof Maximum Wavelength Quercetin

Absorbance at a wavelength of 380-780 nm was performed using a spectrophotometer (Helpida **[11]**).

b. Determination of Optimum Incubation Time

Absorbance was measured at the maximum wavelength with different incubation time of 5, 10, 15, 20, 25 and 30 minutesin order to get the optimum stable time **[11]**.

c. Preparation of Standard Curves quercetin

Elixir was shaken homogenously,left out during the optimum time, and then measured its absorbance at the maximum wavelength **[11]**.

d. Determination of Total Flavonoids

100 mg of avocado seeds extract was weighed and then diluted with methanol to 50 mL. Put 10 mL of the extract into a 50 mLflask using pipette and then added 1 mL of 10% aluminum chloride, 1 mL of 1M NaAcetate and distilled water. Afterwards, the solution was shaken homogenously, leftout during the optimum time, and absorbance was measured at the maximum wavelength. The absorbance data was inserted into the regression equation of the standard curve quercetin, then calculated the total flavonoid content **[11]**.

e. Determination of Total Flavonoids Elixir of Avocado Seed Extract

Elixir was weighed which equivalent to 100 mg of avocado seedsextract. Determination of flavonoids in elixir of avocado seed extract (*Persea americana* Mill) was carried out using the same method of determination of levels of flavonoids in the avocado seed extract (*Persea americana* Mill) **[11]**.

III. RESULTS AND DISCUSSION

Quality Test Results of Elixir

a. Organoleptic Test Results

Differences in taste were causedby the variation of solvent concentration. The higher the concentration of solvent, the bitterthe taste of elixir formula. Furthermore, bitter taste in the formula was possibily caused by the characteristics of dry avocado seed extract which had a bitter taste. So that, there was a need to addsweeteners in each elixir formula of the avocado seed.

b. Limpidity Test Results

Limpidity test was performed by measuring the absorbance of elixir using a spectrophotometer. The results showed that at a wavelength of 430 nm, the value of absorbance was 0.156A at Formula I, 0.222A at Formula II, and 0.238A atFormula III. In addition, the smallest value of the absorbance was the Formula I which had the clearestappearance thanFormula II and Formula III. Furthermore, the higher the concentration of solvent,the darker the appearance of the elixir. The darker appearance of the elixir affects the value of absorbance so that each formula produced different absorbance value. Spectrophotometer wasset at a wavelength of 430 nm, based on the resultsof flavonoids levels determination.

Centrifuge test was performed to determine sedimentation level. The results showed that each formula had different level of sedimentation as follows, Formula I was 0.3 cm, Formula II was 0.4 cm and Formula III was 0.5 cm. These results indicated that Formula I was the best formula due to its smallest amount of sediment than Formula II and Formula III.

c. Viscosity Test and Flow Characteristics Results

The results of viscosity test showed that elixir formula had an average viscosity value as follows,Formula I was 7.92 cP, Formula II was 11.56cP, and Formula III was 13.18 cP. The variation of solvent concentrationin each elixir

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formularesulteda different values of viscosity. Furthermore, the results of the flow characteristics showed that the Thixotropic flow was the most excellent flow characterdue to a combination of Plastis and Pseudoplastisflow. The flow characteristics obtained from thixotropic materials relies on rate of increasing and reducing the shearing stress as well as thetime of the sample experienced a rate of shear (Martin, **[12]**).

d. pH Test Results

pH measurement was done by using a pH meter calibrated with standard buffer pH 4 and pH 7at room temperature. The results showed that pH value of Formula I was 5.65, Formula II was 5.72 and Formula III was 5.73. Theseresults indicated that the pH of each elixir formula satisfied the pH requirements of elixir which should be range at pH 5 to 7. The pH of elixir should meets the pH of the intestinalbecause the elixir will be absorb in the intestine so that the pH should be at the same range of the intestinal pH which usuallyacidic to neutral at 5 to 7 [10]

e. Specific Weight Test Results

Specific weight is the ratio of the weight of the substance to thevolume of water that weighedat the same temperature **[8]**. The results showed that the average value of specific weight of the elixir was 1.0460 g / mL.

Analysis of Total Flavonoids of Dry Seeds Extract and Elixir Formula of Avocado Seed

The analysis was performed by using UV spectrophotometer at wavelengths420-440 nm. The quercetin standard was provided maximum absorption at a wavelength of 430 nm with the absorbance was 0.162.The optimum incubation time was done at 5, 10, 15, 20, 25, and 30minute. The results showed that the optimum incubation in standard quercetin was stable at20 minuteswith a absorbance value of 0.152. The relationship between the absorbance and concentration providedan equation of y = 0.0805x - 0.0039 with a correlation coefficient of r2 = 0.9998. The total flavonoids of dry avocado seed extract were calculated and the results showed that total flavonoids level of sample was 2.1023%,

The results of total flavonoids in elixir formula was:2.1020% in Formula I, 2.1017% in Formula II and 2.0986% in Formula III.In addition, the SD value of total flavonoids in elixir formula was 0.00188. Furthermore, the total flavonoid of elixir formula was lower than dry avocado seed extract, which each formula had different level of decreased amount as follows, Formula I was 0.0143%, Formula II was 0.0285%, Formula III was 0.1759%. These results indicated that the levels of flavonoids in the formula I had a little differences with dryavocado seedextract. The different levels of total flavonoids on each formula was caused by variation of solvent composition.

IV. CONCLUSION

1. Elixir formula of avocado seedthat have the best quality was Formula I which composed of7 %avocado seed extract, 5% ethanol, 10% propylene glycol, 40% sirupus simplex, 0.1%essence mint and 100% aquadestilata ad.
2. Total flavonoid of dry avocado seed extract was 2.1023% and elixir was 2.1020%.

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