Determination Of Parameters Standardization Crude Drug And Extract Arabica Coffee Beans (*Coffea Arabica* L.)

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Abstract: Quality of crude drug extract of coffee bean (Coffeea arabica L.) determined by the value of crude drug and extract standard parameter. In this research, coffee bean derived from Pangalengan, Garut, and Tasikmalaya. Standard parameters determined consisted of non-specific parameters and specific parameters. The results of crude drug determination of non-specific parameters including the loss of dry ranged from 8,000%-10,667%;; water content ranged from 7,2%-8,0%; total ash content ranged from 7,2%-8,0%; and acid insoluble ash content ranged from 1,97%- 2,15%. The results of determination of specific parameters including organoleptic examination crude drug, oval shapes, green colour, typical coffee odour, bitter taste; water-soluble contents ranged between 12,30%-14,67%; levels ethanol-soluble contents ranged between 6,67%-8,50%. The results of extract determination of non-specific parameters including the specific gravity of the extracts ranged from 0,894-0,936; the loss of dry ranged from 18,1667%-21,83%; water content ranged from 5,0%-10%; total ash content ranged from 1,77%-3,18%; and acid insoluble ash content ranged from 0,56%- 0,69%. The results of determination of specific parameters including organoleptic examination extract, pasta shapes, green colour, typical extract odour, bitter taste; water-soluble contents ranged between 7,20%-7,73%; levels ethanol-soluble contents ranged between 32,000%-38,166%.

Keywords: Standard parameter, Crude drug, Extract, Coffee Bean, Coffea arabica L..

Introduction

Coffee is the most popular beverage in the world after water[6]. It is obtained from the processing of the fruits of coffee tree, whole plant of the genus Coffeea, Rubiaceae family[2]. They grow in more than 80 countries in tropical and subtropical regions, especially in Africa, Asia and Latin America[9]. One mainstay of Indonesian commodity is coffee that result ranks third after the rubber and pepper. In addition to having a distinctive flavor, coffee has its benefits as an antioxidant because it has polyphenols and stimulate the brain's performance. Coffee also has many shortcomings. The main problem of the consumption of coffee is the value of caffeine in coffee[10]. The coffee bean could provide benefits in cardiovascular and metabolic diseases. The anti-diabetic effect of extracts of Coffeea arabica was evaluated in diabetic rats. The aqueous extract of coffee green grain (63 and 93mg/kg) was administered once daily for fifteen days to alloxan-induced diabetic rats. The effect of aqueous extract on fasting blood glucose levels was measured. After 8 and 15 day of treatment, aqueous extract of coffee green grain administration showed significantly lower blood glucose levels compared to the diabetic control group[8].

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Coffee also has many drawbacks. The main problem of the consumption of coffee is the value of the caffeine contained in coffee[10]. Certainly caffeine is best known, however cafestol, kahweol and chlorogenic acid, are also compounds present in coffee with antioxidant properties attributed[10]. The total percentage of chlorogenic acid varies by state found in the coffee bean. Some authors reported that the chlorogenic acid in green beans of Coffeea arabica is 5.5 to 8.0% and in the roasted bean is 1.2 to 2.3%[1]. Robusta coffee has a caffeine content is almost twice as large, amounting to 2.4% compared with arabica coffee in the amount of 1.3% in dry conditions[12]. It can increase muscle tension, stimulates the heart, and increase gastric acid secretion due to caffeine is consumed in excess [7]. This leads to the need for the utilization of arabica coffee plants to be used more particularly in the health field.

Methods

Tools

Soxhlet, rotary evaporator (Buchi Rotavapor R-3000), water bath, analytical balance, bottles, porcelain bowls, plates silica, vessel (chamber) TLC, UV light (UV-betrachter Camag), aluminum foil, paper filter, oven, pycnometer, tool determination of water content of toluene distillation method, gas chromatography (GC), high performance liquid chromatography (HPLC) and various glass tools. Chemicals used is 96% ethanol, 70% ethanol, distilled water, toluene, reagent Mayer, Dragendorff reagent, Lieberman-Burchard reagent, chloroform, ammonia, 2N hydrochloric acid, 1% gelatin, iron (III) chloride, magnesium powders, amyl alcohol, ether, 10% vanillin in concentrated sulfuric acid and potassium hydroxide solution 5%, n-hexane, ethyl acetate.

Plant Materials

Plant material used is crude drug coffee beans obtained from three different areas, from Pangalengan, Garut and Tasikmalaya.

Procedures Specific

The research method involves the collection and determination of materials, examination of macroscopic and microscopic Arabica coffee beans, extraction, phytochemical screening, non-specific parameters, specific parameters and analysis of chemical compounds arabica coffee bean extract by means of thin layer chromatography and high performance liquid chromatography.

Specific parameters

Level water-soluble content

The dried sample were weighed for 5 g, further inserted into the flask swear and added 100 mL of water saturated chloroform. Whipped many times during the first 6 hours and left for 18 hours. Filtered and the filtrate was evaporated to 20 mL dry flat grounded in shallow dish preheated 105C and ditara, heat the remaining at a temperature of 105C until the weight remained. The concentration was calculated in % water soluble extract.

Level ethanol-soluble content

The sample (5 g) that has been dried in the air weighed. Inserted into the flask swear and added 100 mL of water saturated chloroform. Whipped many times during the first 6 hours and left for 18 hours. Filtered and the filtrate was evaporated to 20 mL dry flat grounded in shallow dish preheated 105C and ditara, heat the remaining at a temperature of 105C until the weight remained. The concentration was calculated in % water soluble extract.

Non-specific parameters

Loss of dry

Test material weighed for 1 to 2 gram in the shallow weighing bottle with a lid that has previously been heated at a temperature determination. Materials leveled the weighing bottle by shaking the bottle, until a thick layer of approximately 5 to 10 mm, put in a drying chamber, the lid is opened, and then dried at a temperature of determination to keep the weight. Before any drying, leave the bottle in a closed state in eksikator cooled to room temperature.

Water content

To determine the water content of the test material is as follows: 500 mL flask connected with reverse flow through tool coolant reservoir is equipped with a receiver tube 5 mL 0.1 mL scale. Heated using electric heaters where the temperature can be set or oil. The top of the pumpkin tube connector should wrap with asbest.

Total ash content

The way to determine the total ash content is to weighed 2 to 3 g of the test materials have been refined and incorporated into the crucible silicate incandescent slowly until exhausted charcoal, cooled and weighed. If in this way could not be eliminated charcoal, add hot water, stirred and filtered through ash-free filter paper. Incandescent filter paper along with the rest of the filtering in the same crucible. Filtrate inserted into the crucible, evaporated and incandescent to fixed weights. Total ash content is calculated on the weight of the test, expressed in% w / w.

Acid insoluble ash content

Boil ash obtained in the determination of total ash with 25 ml of dilute hydrochloric acid for 5 min LP. The part that is not soluble in the acid collected and filtered through ash-free filter paper, washed with hot water, incandescent in the crucible until the weight remained. Ash content that does not dissolve in acid calculated on the weight of the test, expressed in% w / w

Chemical Test Ingredients

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was performed with silica gel GF254 stationary phase and the mobile phase or developers with ethyl acetate: methanol: water (100: 13.5: 10) Determination of Caffein and Chlorogenic acid content by HPLC Caffein and chlorogeninc acid as biomarker compounds from coffee bean was analyzed by High Performance Liquid Chromatography (HPLC) by using Reverse Phase methods with a non polar column of ODS (250mmx4,6mm), 1 ml/minute of flow rate, UV detector at 320 nm, injection volume 20 mL. Mobile phase was used methanol: 1% acetic acid in water (40:60).

Result

Tabel 1. Results of Determination of Non-Specific Parameters Crude drug Arabica Coffee Beans

Parameters	Result (% b/b)			Range (%)
	Pangalengan	Tasikmalaya	Garut	
Water content (% v/b)	7,20	7,73	7,73	7,20-7,73
Total ash content (% b/b)	7,28	8,24	8,14	7,28-8,24
Acid insoluble ash content (%b/b)	0,144	0,176	0,162	0,144-0,176
Loss of dry (% b/b)	10,667	7,833	8,000	7,833-10,667

Tabel 2. Results of Determination of Non-Specific Parameters Extract Arabica Coffee Beans

Parameters	Result (% b/b)			Range (%)
	Pangalengan	Tasikmalaya	Garut	
Water content (% v/b)	5.833	5.833	6.667	5.833-6.667
Total ash content (% b/b)	1.77	3.18	2.26	1.77-3.28
Acid insoluble ash content (%b/b)	0.0098	0,0210	0,0142	0,0098-0,0210
Loss of dry (% b/b)	21.830	21.667	18.167	18.167-21.830
Specific gravity (m/v)	0.911	0.891	0.928	0.891-0.928

Tabel 3. Results of Determination of Specific Parameters Crude drug Arabica Coffee Beans

Parameters	Result (% b/b)			Range (%)
	Pangalengan	Tasikmalaya	Garut	
levels water-soluble contents	10,67	7,83	8,00	7,83-10,67
levels ethanol-soluble contents	6,67	8,50	8,00	6,67-8,50

Tabel 4. Results of Determination of Specific Parameters Extract Arabica Coffee Beans

Parameter	Result (% b/b)			Range (%)
	Pangalengan	Tasikmalaya	Garut	
levels water-soluble contents	32,5	29,5	32,5	29,5-32,5
levels ethanol-soluble contents	35,330	32,000	38,166	32,000-38,166

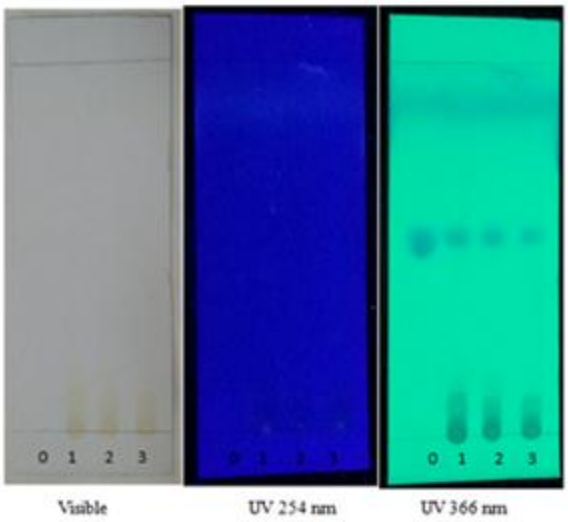

Figure 1. TLC profile of crude drug arabica coffee bean

Tabel 5. TLC spot and Rf value of crude drug arabica coffee bean

Plant Origin	Spot	Rf	Colour spot		
			Visible	UV 254	UV 366
Pangalengan	1	0,0250	Yellow	Dark blue	Gray
	2	0,0937	Yellow	Dark blue	Gray
	3	0,1625	Yellow	Dark blue	Gray
	4	0,5500	-	Mauve	Purple
Tasikmalaya	1	0,0312	Yellow	Dark blue	Gray
	2	0,0937	Yellow	Dark blue	Gray
	3	0,1625	Yellow	Dark blue	Gray
	4	0,5562	-	Mauve	Purple
Garut	1	0,0312	Yellow	Darkblue	Gray
	2	0,0812	Yellow	Dark blue	Gray
	3	0,1562	Yellow	Dark blue	Gray
	4	0,5562	-	Mauve	Purple

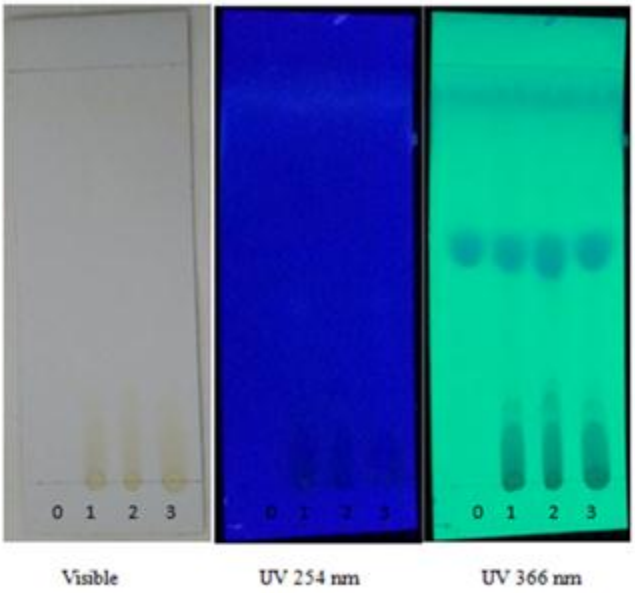

Figure 2. TLC profile of extract arabica coffee bean

Tabel 6. TLC spot and Rf value of extract arabica coffee bean

Plant Origin	Spot	Rf	Spot Colour		
			Visible	UV 254	UV 366
Pangalengan	1	0,0375	Yellow	Dark blue	Gray
	2	0,0750	Yellow	Dark blue	Gray
	3	0,1125	Yellow	Dark blue	Gray
	4	0,1437	Yellow	Dark blue	Gray
	5	0,1812	Yellow	Dark blue	Gray
	6	0,2375	-	Mauve	Purple
Tasikmalaya	1	0,0375	Yellow	Dark blue	Gray
	2	0,0687	Yellow	Dark blue	Gray
	3	0,1000	Yellow	Dark blue	Gray
	4	0,1312	Yellow	Dark blue	Gray
	5	0,1375	Yellow	Dark blue	Gray
	6	0,2375	-	Mauve	Purple
Garut	1	0,0375	Yellow	Dark blue	Gray
	2	0,0625	Yellow	Dark blue	Gray
	3	0,0875	Yellow	Dark blue	Gray
	4	0,1250	Yellow	Dark blue	Gray
	5	0,1750	Yellow	Dark blue	Gray
	6	0,20625	-	Mauve	Purple

Tabel 7. Retention time and AUC Standard by HPLC

Name	Retention time (min)	AUC	Concentration (ppm)
Caffeine	4.29	4.479	400
Chlorogenic acid	6.29	19.407	400
Ferulic Acid	12.91	66.523	400

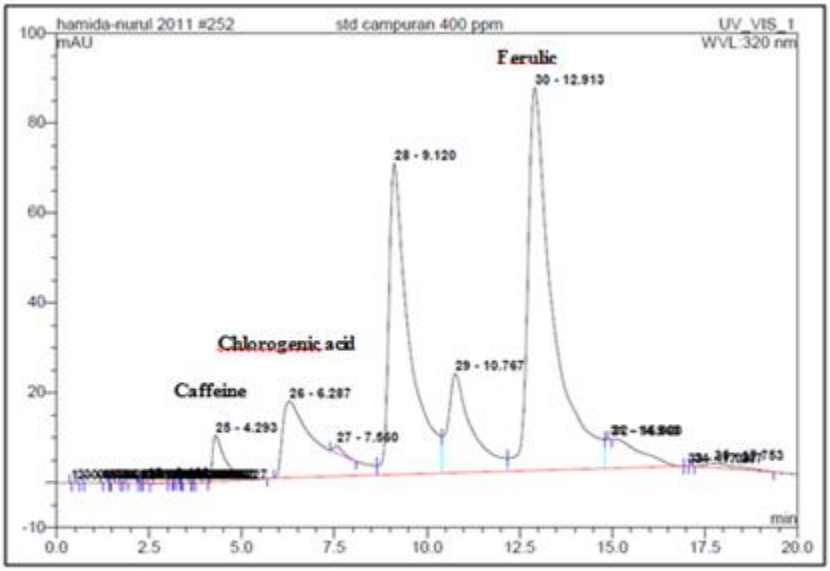

Figure 3. Chromatogram of HPLC of Stadard Caffeine, Chlorogenic acid, and ferulic acid 400 ppm

Tabel 8. Retention time and AUC Extract Coffee bean From Pangalengan by HPLC

Name	Retention time (min)	AUC	Consentration (ppm)
Caffeine	4.33	5.400	482.25
Chlorogenic acid	6.06	104.727	2158.5
Ferulic Acid	12.83	0.000	0.000

Tabel 9. Retention time and AUC Extract Coffee bean From Garut by HPLC

Name	Retention time (min)	AUC	Concentration (ppm)
Caffeine	4.34	9.353	834.025
Chlorogenic acid	6.08	83.824	1727.5
Ferulic Acid	12.91	0.189	1.136

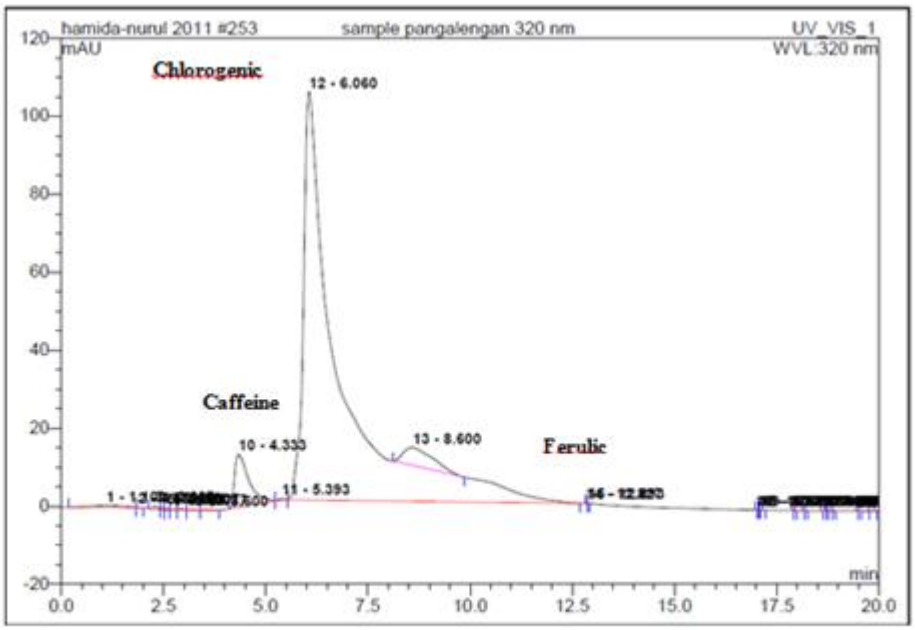

Figure 4. Chromatogram of HPLC in Determination biomarker of extract coffee bean from Pangalengan

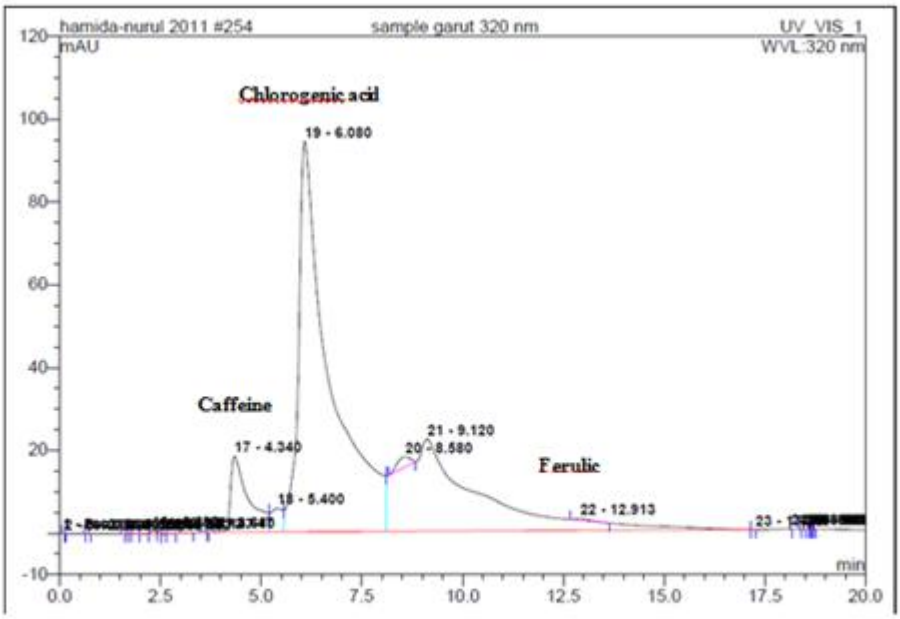

Figure 5. Chromatogram of HPLC in Determination biomarker of extract coffee bean from Garut

Tabel 10. Retention time and AUC Extract Coffee bean From Tasikmalaya by HPLC

Name	Retention time (min)	AUC	Concentration (ppm)
Caffeine	4.33	10.858	967.0
Chlorogenic acid	6.08	76.130	1569.12
Ferulic Acid	12.93	1.776	10.68

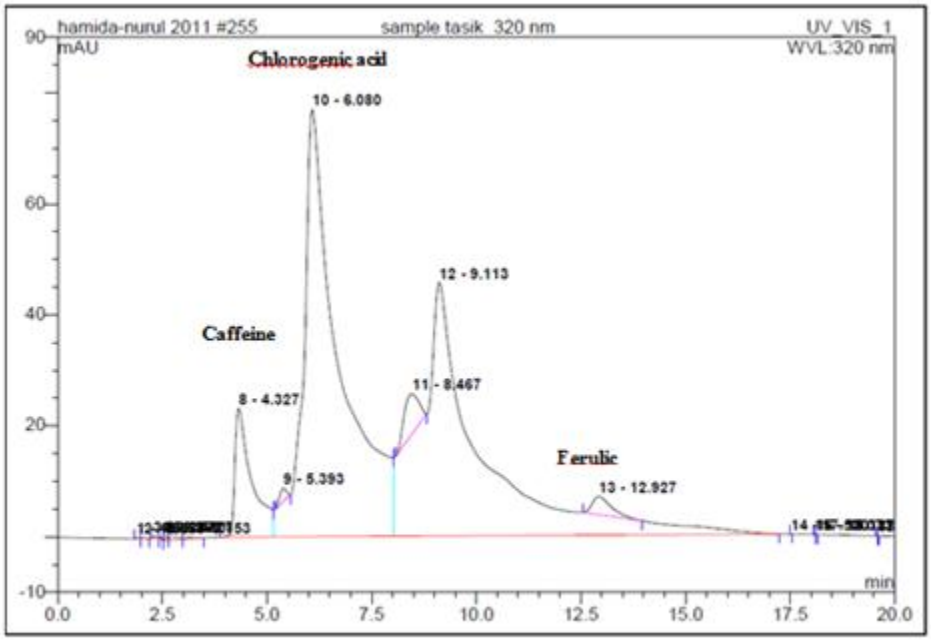

Figure 6. Chromatogram of HPLC in Determination biomarker of extract coffee bean from Tasikmalaya

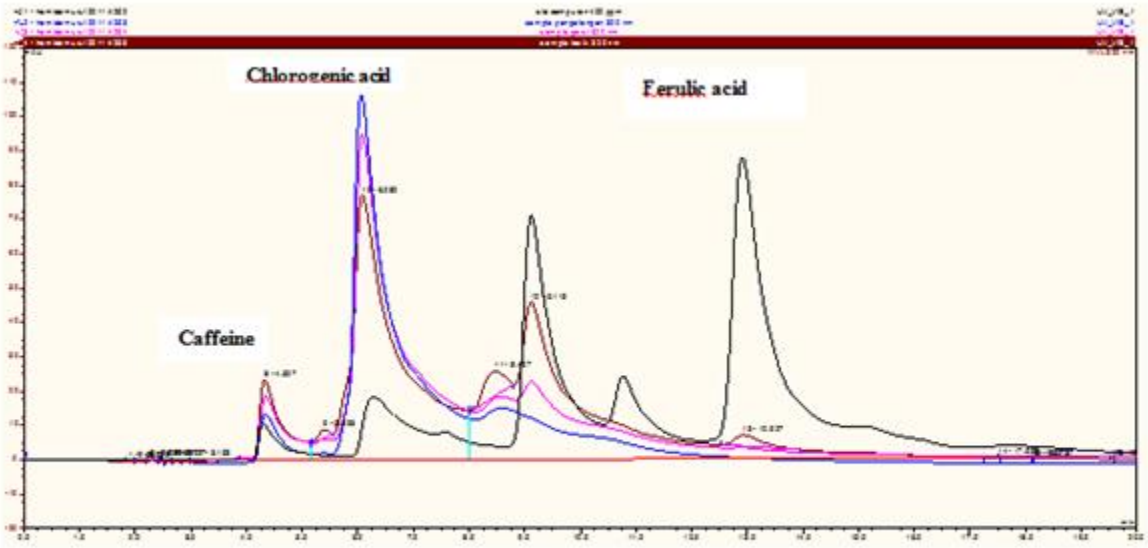

Figure 7. Chromatogram of HPLC in Determination biomarker of extract coffee bean

Discussion

Non-Specific parameters

In the Table 1 and 2 were known that the non-specific parameters of both crude drug and extracts obtained from each region has a different value. This is due to differences in terms of both climate and soil conditions. Therefore, the value set on the standardization of value ranges of all the values calculated. Determination of water content was used distillation by toluene. The water content was set to maintain the quality of the extract. In addition to the determination of water content, could also determine the amount of other substances volatile at crude drug or extract. The less water content in crude drug and extracts the slight possibility of contamination by mold growth. The water content can be affected by habitat or environment. The water content in the crude drugs and extracts determine the acceptability, freshness and durability extract. According to the literature in the moisture content should not be more than 10%. The results showed the water content of crude drug determination of Arabica coffee beans between 7.20% -7.73%, and the determination of water content of Arabica coffee beans extract between 5.833% -6.667%. Extracts and botanicals to meet the requirements which the water content both have a value below 10%. Determination loss of dry is the percentage of compounds that disappeared during the heating process.

Loss of dry is a reduction in material weight after drying in a way that has been assigned to the drying temperature 105° C[4]. In the loss of dry assay calculated loss of dry are substances that are volatile at temperatures 105° C, while other substances which are difficult to evaporate will remain after reaching a constant weight[3]. The results of the determination crude drug shows the loss of dry between 7.833% -10.667% and extract between 18.167% -21.830%. In this study persenstase lower water content loss of dry and qualified. Total ash aims to provide an overview of internal and external mineral content from the beginning to obtain the extract[3]. After calculation, the total ash content in the crude drug containing inorganic compounds with values range between 7.28% -8.24%. Acid insoluble ash content is a continuation of the determination of total ash content which aims to determine the magnitude of the internal and external mineral content in crude drugs and extracts which are not soluble in acidic solvents. This provides a profile of possible metal or contamination, and other inorganic compounds are not soluble in acid. To test materials obtained crude drug acid insoluble ash content ranging from 0.144% -0.176%. The results of the determination, the extract containing inorganic compounds with values range between 1.77% -3.28% and other inorganic compounds that do not dissolve in acid ranged between 0.0098% - 0.0210%. Gravity of Arabica coffee bean extract determined by using a pycnometer. The weight of the type intended to establish the characteristics of an important ingredient that is used for testing the identity and purity[11] results showed determination of specific gravity between the arabica coffee bean extract from 0.891 to 0.928.

Specific parameters

Determination of the specific parameters of the assay consists of level soluble content. The results can be seen in the following Table 3. Parameters soluble content used to determine the amount of chemical compounds in the crude drug and extracts. Soluble content specified parameters as raw material test parameters of traditional medicine because of the amount of chemical compounds in the crude drug and extracts will closely associated with reproducibility in the pharmacodynamic activity of the crude drug[5]. Determination of these parameters aims to provide an overview initial amount of compounds that can be extracted[3]. Water solvent used for dissolving polar compounds and ethanol to dissolve the less polar compounds. Levels water-soluble content in crude drug between 7.83% -10.67% and the levels ethanol-soluble contents 6.67% -8.00%. As for the test sample extract, Levels water-soluble content 29.5% -32.5% and levels ethanol-soluble content 32.000% -38.166%. Based on the results of the determination, for the test material crude drug water-soluble content value is higher than the levels ethanol-soluble content. As for the ethanol-soluble extract of the test material is higher than the levels of water soluble content. This indicates that the test substance derived from Pangalengan, Tasikmalaya and Garut more contain substances which are polar making it easier to dissolve in water than ethanol. While the test material which is already extract, more substances containing nonpolar making it easier to dissolve in ethanol than water. The difference in the number of levels of water soluble extract and ethanol to dissolve in crude drug and extract were investigated due to differences in solubility of chemical compounds each test material in water and ethanol solvent.

Chemical Test Ingredients

Using the method presented here, the caffeine, chlorogenic acid, and ferulic acid content in different arabica coffee samples, which were collected in several areas in West Java, was measured. Three samples of 10 gram of arabica coffee bean powder were weighted for each source area (Pangalengan, Garut, and Tasikmalaya). Then, each samples were extracted by soxhlet using ethanol 70% and the liquid extract were evaporated to obtain the condensed extract. Condensed extract were weighted 0.1 gram then diluted to aquabidest and the sample was submitted to the SPE process. Standard solutions were also prepared and measurements were then made for standards and all the samples. The measured results show that all the Arabica coffee bean, collected from three different source areas in West Java, contain caffeine, chlorogenic acid and ferulic acid. Depending on the source area, the consentration of caffeine is the highest corresponding to Tasikmalaya 0.967 mg/g(967 ppm), Garut 0.834 mg/g(834.025 ppm), and the lowest to Pangalengan 0.482 mg/g(482.25 ppm). Also the consentration of chlorogenic acid with the highest corresponding to Pangalengan 2.158 mg/g(2158.5 ppm), Garut 1.727 mg/g (1727.5 ppm), and the lowest to Tasikmalaya 1.569 mg/g (1569.12 ppm). Meanwhile the ferulic acid from Pangalengan was unmeasured, while the highest consentration of ferulic acid was from Tasikmalaya 0.01068 mg/g (10.68 ppm) and Garut 0.00136 mg/g (1.136 ppm).

Conclusion

Determination of standard parameters of non-specific and specific preset could be used for considerations on the processing of crude drug Arabica coffee beans in the manufacture of the drug so that the quality of the extract to be used is ensured. It can also be used as a quality control for standard products. The difference in the results obtained for the determination of standardization is influenced by the environment (soil and atmosphere), climate, temperature, and degree of acidity from Arabica coffee beans..

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