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Characterization of Ethanolic Extract of Saluang belum (*Luvunga sarmentosa* Kurz) Root

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A B S T R A C T

Saluang belum (*Luvunga sarmentosa* Kurz) is a member of the endemic plants of Central Kalimantan, which the local community has traditionally used as a traditional medicine to cure several kinds of diseases. Characterization of the ethanolic extract of Saluang belum root is needed to facilitate the consistency of safety and quality. Therefore, this research needs to be carried out to complete the data on the characterization of the ethanolic extract of Saluang belum root. The material used is Saluang belum root collected from Palangkaraya, Central Kalimantan, Indonesia. There is no standard for Saluang belum extract. The results showed that name of the extract was Sarmentosa radix extractum; Indonesian name was Saluang belum; Latin name was *Luvunga sarmentosa* Kurz; part that used was root; reddish dark brown colour; typical smell; bitter flavour; contains carbohydrate, allylic alcohol and terpenoid compounds; density 0,82 g/ml; water content 4,865%; total ash content 1,74%; acid insoluble ash content 0,31%, negative residual solvent; Pb contamination 3,206 mg/kg; Cd contamination <0,001 mg/kg; total plate count 1,0 x 10^2 colonies/g; mold contamination 1,0 x 10^2 colonies/g; yeast contamination < 1,0 x 10^0 colonies/g.

1. Introduction

In the evolution of human society, medicinal plants have played an important role. Medicinal plants are the primary component of traditional medicines, and numerous modern medicines are partially derived from medicinal plants (Hosseinzadeh *et al.*, 2015). Plants produce phytochemicals, which are bioactive substances derived from plants, to serve as a form of defense. More than a thousand phytochemicals have been identified and can be obtained from various sources (Kumar *et al.*, 2023). Medicinal plants are generally regarded as safe and effective. Characterization of extract is necessary to determine the quality of extract (Pahriyani and Arista, 2021).

Saluang belum (*Luvunga sarmentosa* Kurz) is a member of the endemic plants of Central Kalimantan, which has been traditionally used by the local community as a traditional medicine to cure several kinds of diseases such as lumbago and kidney pain as well as to increase vitality (Anggriani, 2018). Ethanolic extract of Saluang belum root contains flavonoids, steroids, and tannins (Wati *et al.*, 2018). The ethanolic extract of Saluang belum may affect the development of spermatocyte and spermatid cells in mice, which are indicated to increase male fertility (Musfirah, Bachri and Nurani, 2016) and be able to improve sperm quality and sperm viability (Musfirah *et al.*, 2016). In addition, Ethanolic extract of Saluang belum has aphrodisiac activity in Wistar albino rats (Wati *et al.*, 2018).

Based on the great potential of the Saluang belum as a medicinal plant, it needs to be done characterize the root of ethanolic extract of Saluang belum. Characterization root of the ethanolic extract of Saluang belum is needed to facilitate the consistency of the safety and quality of the root extract of Saluang belum. Therefore, this research needs to be carried out to complete the data on the characterization of the ethanolic extract of Saluang belum root.

The advantages in terms of the pharmacological effects of the curcumin and paracetamol combination make these two active pharmaceutical ingredients have a great potential to be mixed in solid pharmaceutical dosage forms. However, until now there has been no study on the physical interactions that occur between the mixture of curcumin and paracetamol. Therefore, this study aims to characterize the physical interactions that occur in the binary mixture between curcumin and paracetamol and determine its impact on the solubility of curcumin.

2. Research Methods

Materials

The material used are Saluang belum, ethanol 96%, aquadest, xylol, Pb, Cd, HNO_3, H_2O_2, Plate Count Agar (PCA), Buffered Peptone Water (BPW), analytical balance (Ohaus®), sieve number 40 (Pharmalab®), oven (Thermo scientific®), round bottom flask (Duran®), rotary evaporator (IKA®), evaporator cup (Anumbra®), pycnometer (Iwaki ®), distillation apparatus (Iwaki®), waterbath (Memmert®), erlenmeyer (Pyrex®), hot plate (Maspion®), furnace (Nabertherm®), Atomic Absorption Spectrometry (SSA) (Shimadzu®), Gas Chromatography-Mass Spectrometry (GCMS) (Shimadzu®), volumetric flask (Iwaki®), petri dish (Pyrex®), measuring cup (Iwaki®), funnel (Pyrex ®), micropipette (OneMed®), incubator (Memmert®).

Experiments

1. Sample preparation

Saluang belum was collected from Palangkaraya, Central Kalimantan, Indonesia and the plant was verified by an expert botanist from Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia with number UGM/FA/ 4535b /M/03/02.

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The time of this research was from December 2018 to July 2019. The location of the research was carried out at the Borneo Lestari Banjarbaru Natural Product and Pharmaceutical Technology Laboratory, Banjarbaru Industrial Research and Standardization Center and Drug, Food and Cosmetic Testing Laboratory at the Islamic University of Indonesia, Yogyakarta.

The root of Saluang belum is washed, then air-dried, cut into small pieces and then dried using an oven at a temperature of 50°C. Then, ground into powder and sieved through sieve number 40. Dry powder of Saluang belum root was macerated using ethanol 96% 3 x 24 hours while stirring. The filtrate was evaporated using rotary evaporator.

2. Characterization of ethanolic extract of saluang belum

Extract identity

The extract identity consists of the given name of the extract, the Indonesian name of the plant, the Latin name of the plant and the part of the plant used.

Organoleptic

The organoleptic test uses the senses to demonstrate the characteristics quickly, and objectively. The odour, colour, and flavour of the Saluang belum extract were described as organoleptic parameters.

Chromatogram profile

Porcelain cup was prepared, add 0.05 g of extract, then dilute with 10 ml of ethanol. The extract was taken using a capillary tube and then dropped onto GF 254 silica gel plate which had been activated for 15 minutes at 105°C. Then it was eluted using n-hexane: ethyl acetate (7:3). The spots were viewed under a 254 nm 366 nm UV lamp then visualized with H_2SO_4 10% and the spots were viewed under visible light.

Density

Weight the pycnometer and then calibrated by setting the weight of the pycnometer and water at 25°C and then weighing (W1). Prepare the pycnometer the temperature of the filled pycnometer was adjusted to 25°C, add extract at ± 20°C into the pycnometer and weighed (W2) and the density is determined. The formula can be seen in Formula 1.

$$d = \frac{W2-W0}{W1-20} \quad (1)$$

Note: d = density (g), W0 = pycnometer weight (g), W1 = pycnometer weight + water (g), and W2 = pycnometer + extract (g)

Water content

Water content is determined through distillation. Beaker glass was prepared and add Xylol which is saturated with water in advance. After standing, the two layers of water and xylol will separate, and the water layer will be discarded. 5 g of the extract (W) was added into 200 ml of xylol saturated water. Carefully the flask was heated at 110°C for 1 hour and wait until separated, the water volume was measured (V) and water percentage is determined. The formula can be seen in Formula 2.

$$\% \text{ Water content} = \frac{V}{W} \times 100\% \quad (2)$$

Note: V = volume of water (ml), W = weight of extract (g)

Total ash content

Silica crucible was prepared and weight it when lit (W0), then 2 g of extract (W1) was added to silica crudible. After that, the extract was heated by a furnace at 600 ± 25°C until the charcoal wears out (W2). The total ash content is expressed in % W/W. The formula can be seen in Formula 3.

$$\% \text{ Total Ash Content} = \frac{(W2 - W0)}{W1} \times 100\% \quad (3)$$

Note: W0 = mass of silica crucible (g), W1 = mass of extract (g), W2 = mass of silica crucible + ash (g)

Acid insoluble ash content

25 ml of dilute sulfuric acid was added to beaker glass, ash obtained from the total ash content (W1) was added into silica crucible and determine for 5 minutes, filter and rinse with hot water. Then, silica crucible was heated in a furnace at 600 ± 25°C until the charcoal runs out (W2). The acid-insoluble ash content of the air-dried material was calculated. The formula can be seen in Formula 4.

$$\% \text{ Acid Insoluble Ash Content} = \frac{(W2 - W0)}{W1} \times 100\% \quad (4)$$

Note: W0 = mass of silica crucible from total ash content (g), W1 = mass obtained from total ash content (g), W2 = mass of crucible + ash (g).

Residual solvent

The tool used to determine the residual solvent is Gas Chromatography-Mass Spectrometry (GCMS). GC-MS instrument was prepared at a temperature of 70°C to 200°C, 0.1% of the sample was prepared by dissolving the extract in methanol and the standard is ethanol. Quantitative measurement of ethanol content is determined by calculating the sample peak area (Peak1) compared to the standard peak area (Peak0) multiplied by the standard concentration. The formula can be seen in Formula 5.

$$\% \text{ Residual Solvent} = \frac{\text{Peak1}}{\text{Peak0}} \times 100\% \quad (5)$$

Note: Peak1 = peak area of the sample, Peak0 = peak area of standard

Heavy metal contamination

Determination of Pb and Cd contamination using Atomic Absorption Spectrometry (SSA). A beaker glass was added 1 g of extract (W) and dissolve it in 20 ml of HNO_3, stand it for 24 hours. After that, the mixture was heated at 100°C for 10 minutes, and cooled. 2 ml H_2O_2 30% was added and filtered. Then, add the distilled water (V). SSA was measured the samples, then calculates the heavy metal content. The formula can be seen in Formula 6.

$$\text{Heavy metal contamination} = \frac{\bar{x} \times V}{W} \quad (6)$$

Note: $\bar{x}$ = average concentration (ppm), V = final volume (ml), W = weight of sample (g)

Total plate count

Measuring flask was prepared and added 1 g of extract (W). Then Buffered Peptone Water (BPW) was added to flask to obtain a 10^{-1} dilution and shake until dissolved, followed by a 10^{-2} dilution (DF). 1 ml of the dilution was added into a petri dish, then 5 ml of Plate Count Agar (PCA) media which has been melted at a temperature of approximately 45°C was poured. The petri dish is shaken gently until the sample is completely mixed. Then let it sit until the mixture in the petri dish freezes. The petri dish was turned upside down in an incubator at 35°C for 24 hours. Colony growth was counted on each plate containing 30-300 colonies after 24 hours ($\sum$ colonies). TPC in colonies/g of sample was calculated by multiplying the average number of colonies on the plate by the appropriate dilution factor. The formula can be seen in Formula 7.

$$\text{Total Plate Count} = \frac{\sum \text{colony}}{\text{DF} \times W} \quad (7)$$

Note: $\sum$ colony = total colony (colony), DF = dilution factor, W = weight of sample (g)

Mold and yeast contamination

PCA medium and 1 ml of each dilution (DF) were poured into the petri dish. The petri dish is shaken gently and then allowed to

freeze. Then incubated at 25°C for 7 days. Record the results as the number of molds and yeasts/g sample (∑ colony). The formula can be seen in Formula 8.

$$\text{Total Mold \& Yeast Contamination} = \frac{\sum \text{colony}}{\text{DF} \times \text{W}} \quad (8)$$

Note: ∑ colony = total colony (colony), DF = dilution factor, W = weight of sample (g)

3. Result and Discussion

Characterization of extract is necessary to determine the quality of extract (Pahriyani and Arista, 2021). The results of characterization of ethanolic extract of Saluang belum shown in **Table 1**. Extract identity parameter to determine the authenticity of the sample used. The proccess of determination is crucial because ingredients possess similar physical characteristics with other plants in the same genus (Muthia *et al.*, 2021). Name of the extract is *Sarmentosa radix extractum* with Indonesian name is Saluang belum, Latin name is *Luvunga sarmentosa* Kurz, and the part used is root. Organoleptic test based on sensory from the sample obtained a reddish dark brown color, typical smell and bitter. Thin Layer Chromatography (TLC) used to determine the chromatogram profile of ethanolic extract of Saluang belum four spots were identifiend and predicted using H_2SO_4 10% spray reagents. The use of sulfuric acid commonly used to detect carbohydrates and terpenes or known as universal reagent (Agatonovic-Kustrin *et al.*, 2019). After being sprayed using H_2SO_4 10%, a blue color is observed at Rf (1) 0,63 predicted to be a carbohydrate and green color on Rf (3) 0,46 predicted to be an allylic alcohols (Agatonovic-Kustrin *et al.*, 2019) and on Rf (5) 0,25 shows a yellow green color predicted to be a terpenoid compound (Guntarti *et al.*, 2017). The results of chromatogram profile show in **Figure 1**.

Density is ratio of the solid material density to the density of water at 20°C (Dhir *et al.*, 2018). There are several tools for determining density, namely pycnometer, hydrostatic balance, Reimann balance, Mohr Westphal balance. Pycnometer was used because the sample used was small and simple. The average yield for determining the density of ethanolic extract of Saluang belum root was 0,82 ± 0,00 g/ml. Density describes the amount of mass per unit volume to provide a boundary between liquid extract and thick extract, apart from that, density is also related to how to determine the purity of a substance whose density is determined (Depkes RI, 2000).

Determination of the water content is to establish a minimum range the amount for water content in the material. In determining the water content, the distillation method is used because it is more efficient in time and cost than the titration method and the results are more valid than the gravimetric method, where the water content is affected by other volatile substances.

The average result for determining the water content of ethanolic extract of Saluang belum root is 4,865% ± 1,40. Low water content may prevent degradation in quality or damage caused by the growth of microorganisms, so that it can be for long-term storage (Pradana, 2017).

The total ash content provides a perspective of the internal and external mineral content (Setyawati, Wijayanti and Wiratmini, 2019). To create organic compounds, extracts were heated to high temperatures, at which point their derivatives decomposed and evaporated, leaving behind only minerals and inorganic elements (Marissa Angelina *et al.*, 2015). The average results for determining the total ash content of ethanolic extract of Saluang belum root were 1,74% ± 0,93 and the acid insoluble ash content was 0,31% ± 0,14. The acid insoluble ash content is lower than the total ash content because only certain metals are still present (Alegantina, 2015). The large total ash content in Saluang belum shows that the extract obtained contains a lot of minerals. Meanwhile, the presence of ash levels that are insoluble in acid indicates the presence of sand or other impurities that are still present (M. Angelina *et al.*, 2015).

Determination of residual solvent aims to ensure that during the process does not leave residual solvent. It can give unwanted side effects if the remaining solvent enters the body (Saifudin, Rahayu and Teruna, 2011). The result for determining the residual content ethanolic extract of Saluang belum root were negative.

The purpose of heavy metal contamination screening is to verify the extract does not exceed permissible level of certain heavy metals that are harmful to human health. Lead (Pb) and cadmium (Cd) are the heavy metals contamination tested using AAS. Pb can cause damage to the formation of red blood cells, and Cd can cause organ damage because it can precipitate in the body (Indiriwati, 2017). Unless the soil is already polluted, the concentration of heavy metals in the soil is naturally quite low (Nur, 2013). Sources of Pb and Cd pollutants mostly come from various industries (Ahyar, Bengen and Wardiatno, 2017). The average results for determining Pb and Cd contaminations in ethanolic extract of Saluang belum were heavy metal Pb 3,206 mg/kg ± 0,00 and Cd < 0,001 mg/kg ± 0,00, both of which did not exceed the standard heavy metal Pb 10 mg/kg and heavy metal Cd not exceeding 0.3 mg/kg (BPOM RI, 2006). Microbial, mold and yeast contamination test attempts to ensure that the extract is not contaminated exceeding the set limit because it can reduce the stability of the extract and harmful. The average yield for determining the microbial contamination of ethanolic extract of Saluang belum was 1,0 x 10^2 colonies/g.

Table 1. Characterization of ethanolic extract of Saluang belum.

No.	Parameter	Unit	Result
1	Extract identity	-	Name of the extract: Sarmentosa radix extractum Indonesian name: Saluang belum Scientific name: *Luvunga sarmentosa* Kurz Part: Root
2	Organoleptic	-	Colour: Reddish dark brown Odour: Typical smell Flavour: Bitter
3	Chromatogram profile	-	Spot 1 Carbohydrate; spot 2 not identified; spot 3 carbohydrate; spot 4 allylic alcohol; spot 5 steroid
4	Density	g/ml	0,82 ± 0,00
5	Water content	%	4,865 ± 1,40
6	Total ash content	%	1,74 ± 0,93
7	Acid insoluble ash content	%	0,31% ± 0,14
8	Residual solvent	-	Negative
9	Pb contamination	mg/kg	3,206 ± 0,00
10	Cd contamination	mg/kg	<0,001 ± 0,00
11	Total plate count	colonies/g	1,0 x 10^2
12	Mold contamination	colonies/g	1,0 x 10^2
13	Yeast contamination	colonies/g	< 1,0 x 10^0

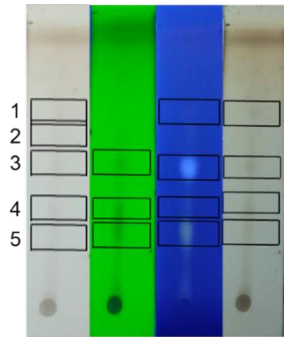

Figure 1. Chromatogram profile using silica gel 60 GF_{254} stationary phase, n-hexane : ethyl acetate (7:3) mobile phase; (a) light; (b) 254 nm; (c) 366 nm; (d) light + H_2SO_4 10%

The results of the determination of microbial contamination fall within the requirements for microbial contamination, namely ≤ 10^4 colonies/g (BPOM RI, 2006). The average yield for determining the mold and yeast contamination of ethanolic extract of Saluang belum root was 1,0 x 10^2 colonies/g. The results of the determination of mold and yeast contamination fall within the requirements ≤ 10^3 colonies/g (BPOM RI, 2006). Determination of residual solvent aims to ensure that during the process does not leave residual solvent. It can give unwanted side effects if the remaining solvent enters the body (Saifudin, Rahayu and Teruna, 2011). The result for determining the residual content ethanolic extract of Saluang belum root were negative.

The purpose of heavy metal contamination screening is to verify the extract does not exceed permissible level of certain heavy metals that are harmful to human health. Lead (Pb) and cadmium (Cd) are the heavy metals contamination tested using AAS. Pb can cause damage to the formation of red blood cells, and Cd can cause organ damage because it can precipitate in the body (Indiriwati, 2017). Unless the soil is already polluted, the concentration of heavy metals in the soil is naturally quite low (Nur, 2013). Sources of Pb and Cd pollutants mostly come from various industries (Ahyar, Bengen and Wardiatno, 2017). The average results for determining Pb and Cd contaminations in ethanolic extract of Saluang belum were heavy metal Pb 3,206 mg/kg ± 0,00 and Cd < 0,001 mg/kg ± 0,00, both of which did not exceed the standard heavy metal Pb 10 mg/kg and heavy metal Cd not exceeding 0.3 mg/kg (BPOM RI, 2006). Microbial, mold and yeast contamination test attempts to ensure that the extract is not contaminated exceeding the set limit because it can reduce the stability of the extract and harmful. The average yield for determining the microbial contamination of ethanolic extract of Saluang belum was 1,0 x 10^2 colonies/g. The results of the determination of microbial contamination fall within the requirements for microbial contamination, namely ≤ 10^4 colonies/g (BPOM RI, 2006). The average yield for determining the mold and yeast contamination of ethanolic extract of Saluang belum root was 1,0 x 10^2 colonies/g. The results of the determination of mold and yeast contamination fall within the requirements ≤ 10^3 colonies/g (BPOM RI, 2006).

4. Conclusion

The results showed that name of the extract was Sarmentosa radix extractum; Indonesian name was Saluang belum; Latin name was *Luvunga sarmentosa* Kurz; part that used was root; reddish dark brown colour; typical smell; bitter flavour; contains carbohydrate, allylic alcohol and terpenoid compounds; density 0,82 g/ml; water content 4,865%; total ash content 1,74%; acid insoluble ash content 0,31%, negative residual solvent; Pb contamination 3,206 mg/kg; Cd contamination <0,001 mg/kg; total plate count 1,0 x 10^2 colonies/g; mold contamination 1,0 x 10^2 colonies/g; yeast contamination < 1,0 x 100 colonies/g. There is no standard yet for Saluang belum extract. Hence, we cannot decide if it meets the requirements.

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