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Standardization of extract of *Leucaena leucocephala* (lmk) De Wit seeds by α-glucosidase inhibitor

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Abstract

Extracts of *Leucaena leucocephala* (lmk) De Wit seeds were collected from different areas in Java; they include Jakarta, Cengkareng, Bogor and Bandung. Each extract was examined for water content, ash content, acid-insoluble ash content, and α- glycosidase inhibition assay. The results showed that ethanolic extracts of *Leucaena leucocephala* (lmk) De Wit seeds collected from Bandung were found to be most active in inhibiting the formation of α-glycosidase compounds with an IC_{50} value of 7,46 ± 0,21 µg/ml, which was confirmed with ash content of 5,58 ± 0,04, acid-insoluble ash content of 0,55 ± 1,33, and water content of 26,22 ± 0,90.

Keywords: extracts of (*Leucaena leucocephala* (lmk) De Wit), α-glucosidase inhibition activity, different areas in Java.

Introduction

Diabetes is a complex and multifarious group of disorders characterized by hyperglycaemia, that has reached epidemic proportions in the present century. Several drugs such as biguanides and sulfonylureas are presently available to reduce hyperglycaemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems [1]. Management of diabetes without any side effects is still a challenge to the medical community. There is continuous search for alternative drugs. Therefore, it is prudent to look for options in herbal medicine for diabetes as well.

One therapeutic approach for treating diabetes is to decrease the postprandial hyperglycemia. This is done by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes α-glucosidase in the digestive tract. Inhibitors of these enzymes delay carbohydrate digestion, causing a reduction in the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [2]. Inhibitors of intestinal α-glucosidase have been used in the treatment of noninsulin-dependent diabetes mellitus (NIDDM) and represented at the huge proportion of antidiabetic drug market [3].

Indonesia is situated at equator area with an appropriate climate for the development of various vegetations, many of which have properties as herbal medicine. To improve the level of public health, plants that were previously used in traditional medicine need to be more optimally explored [4]. Unlike any other medical products, herbal medicine preparations pose some unique issues, particularly those related to product quality. This was due to the fact that the natural constituents contained in simplicia constitute the mixture of some secondary

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metabolites that may quantitatively (to some extent qualitatively) be changing due to a variety of genetic or environmental factors [5].

Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. But the most important challenges faced by these formulations arise because of their lack of complete standardization. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition [6]. One of Indonesian plants that have been used as traditional antidiabetic drugs is *Leucaena leucocephala* (lmk) De Wit seeds [7].

To develop *Leucaena leucocephala* (lmk) De Wit seeds as a herbal medicine preparation, some issues related to the quality of the extracts need to be taken into account, This was due to the fact that the natural constituents contained in simplicia constitute the mixture of some secondary metabolites that may quantitatively be changing due to a variety of genetic or environmental factors [4]. Therefore, the recent study aimed at determining the quality of ethanolic extracts of *Leucaena leucocephala* (lmk) De Wit seeds collected from four different areas; and the age of the seeds used in the study was based on the specific and non-specific parameters. The specific parameters were used to determine the level of chemical content; meanwhile, the non-specific parameters were used: to determine water content, to determine total ash content, and to determine acid-insoluble ash content. In addition, antidiabetic assay was conducted in vitro with the method of α-glucosidase inhibition.

Materials and Methods

Samples

Leucaena leucocephala (lmk) De Wit seeds were collected from four different areas: Bandung, Bogor, Cengkareng and Jakarta.. *Leucaena leucocephala* (lmk) De Wit seeds were collected from two groups of age; namely unripe seeds with an age of $\pm$ 1 month and ripe seeds with an age of $\pm$ 3 months. The materials were then washed with water, evaporated and subsequently grilled to powder before being extracted.

Extraction

Leucaena leucocephala (lmk) De Wit seeds were washed with water, evaporated to dryness, and then grilled to yield simplicia powder. The simplicia was immersed for 3 x 24 hours using 70% ethanol. It was then filtered with a piece of flannel fabric and subsequently concentrated using rotary evaporator to yield concentrated extracts.

Standardization of extracts

Determining water content

Carefully measure the substance in a premeasured container. Desiccate under a temperature of 105^{o} C for 5 hours, and then measure it. Continue the desiccation and measure within an interval of 1 hour to get a difference of no more than 0.25% between the consecutive measurements.

Determining Total Ash Content

The simplicia was slowly glowed to get the charcoal depleted; then, cool and measure. If in this way the charcoal cannot be depleted, add hot water, and then filter through an ash-free filter paper. The residue of filter paper was then rotated in the same crush. The filtrate was put into the crush, let to evaporate, rotated to its fixed weight, and then measured. Materials that had been aired were measured in terms of their contents.

Determining Acid-Insoluble Ash Content

The ash was collected following the determination of ash content, boiled in 25 ml of liquid sulfuric acid for 5 minutes; portions that were not soluble in the acid were collected, filtered through ash-free filter paper, washed with hot water, and glowed to reach their fixed weight. The aired materials were then assayed for their acid-insoluble ash content.

α-glucosidase Assay

The Samples and control were dissolved with 200 µl of DMSO. The dissolved samples became the main solution (15000 bpj), which would subsequently be made assay solution with a concentration of 1 bpj, 5 bpj, 10 bpj, 100 bpj, and 500 bpj. The solution was incubated under 30 ^{o}C for 5 minutes. Reaction began with the addition of 250 µL of α-glucosidase (0.075 unit); then incubate it once more for 15 minutes under 30 ^{o}C. Chemical reaction was ceased by adding 2 ml of Na_2CO_3 0,1 M, measured at λ 405 nm with acarbose as a positive control.

Percentage of inhibition was measured with the following formula: --

$$\% \text{ Inhibition} = \frac{\text{Abs}_{\text{negative control}} - \text{Abs}_{\text{treatment}}}{\text{Absorbance}_{\text{negative control}}} \times 100\%$$

Results and Discusion

The study was conducted with the aim of determining the quality of ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds as an antidiabetes collected from different areas and within the different harvesting times based on specific and non-specific parameters; the study also aimed at finding out the inhibition activity of α-glucosidase enzyme in vitro. Developing natural products to become a phytopharmaca preparation requires standardization that constitutes a sequence of parameters, procedures, and measurement techniques; the results were some elements, which were related to the quality paradigm in pharmacology. Quality here means the fulfillment of standard conditions (chemical, biological, and pharmacological standards) [5,6].

Table 1. The Result of Extract of *Leucaena leucocephala* (Lmk) De Wit seeds

Sample	Sample weight (g)	Extraction results fixed weight (g)	% rendemen
Jakarta $_{ripe}$	560	46,20	8,25
Jakarta $_{unripe}$	552,50	51,80	10,51
Cengkareng $_{ripe}$	541,70	43,98	8,12
Cengkareng $_{unripe}$	526	34,50	6,56
Bogor $_{ripe}$	555,90	42,70	7,68
Bogor $_{unripe}$	527,40	35,50	6,73
Bandung $_{ripe}$	549,94	45,70	8,31
Bandung $_{unripe}$	549,30	28,80	5,24

* Description : Fixed weights of the extracts were divided by simplicia weights multiplied by one hundred percents

Table 1 presents percent (%) of rendement from the extracts of *Leucaena leucocephala* (Lmk) De Wit seeds that had been assayed. The table further presents the value of extracts collected from $Jakarta_{ripe,}$ $Jakarta_{unripe}$, $Cengkareng_{ripe}$, $Cengkareng_{unripe}$, $Bogor_{ripe}$, $Bogor_{unripe,}$ $Bandung_{ripe}$, $Bandung_{unripe}$, respectively; i.e. 8,25 %, 10,51 %, 8,12 %, 6,56 %, 7,68 %, 6,73 %, 8,31 %, 5,24 %. Extracts from $Jakarta_{unripe}$ had a higher rendement (10,51 %); while extracts from $Bandung_{unripe}$ had a relatively lower rendement (5,24 %). Table 1 further shows that extracts from $Jakarta_{ripe}$ had a higher rendement (10,51 %) compared to those from $Bandung_{unripe}$ (5,24 %). The higher rendement value indicates more content of compounds in the extracts.

According to the ash content of *Leucaena leucocephala* (Lmk) De Wit seeds was not more than 4% and acid insoluble ash content of *Leucaena leucocephala* (Lmk) De Wit seeds was not more than 1% [4]. The table further presents acid insoluble ash content from Jakarta $_{unripe}$ (1,20 ± 0,51), Bandung $_{ripe}$ (1,14 ± 0,91) dan Bandung $_{unripe}$ (1,15 ± 1,33) that exceeded the required level as stated in the extract. This might be due to contamination of mineral elements both in intentional and in unintentional ways. Based on the determination of water content, it was found that extracts collected from Jakarta $_{unripe}$ had a higher value of content (31,27 ±. 36,47). The higher the water content of an extract, the easier fungi or bacteria develops on it, since water is a primary media for the development of fungi.

The study aims at determining ash contents, acid insoluble ash contents, and water contents. The extracts would be desiccated using a furnace with a temperature of 800 °C (for determining ash contents and acid insoluble ash contents) and an oven (for determining water contents). The results of each extract of *Leucaena leucocephala* (Lmk) De Wit seeds were presented through Table 2.

Table 2. The Result of Parameter of standardization

Parameter of standardization origin of simplicia	Ash content (%)	Acid insoluble ash content (%)	Water content (%)
Jakarta $_{ripe}$	5,55 ± 0,45	0,20 ± 0,2	27.49 ± 128.91
Jakarta $_{unripe}$	4,06 ± 0,09	1.20 ± 0,5	31.27 ± 36.47
Cengkareng $_{ripe}$	6,09 ± 0,77	0,42 ± 0,11	30,48 ± 0,36
Cengkareng $_{unripe}$	6,04 ± 0,03	0,76 ± 0,73	16,10 ± 1,36
Bogor $_{ripe}$	4,70 ± 0,06	0,40 ± 0,25	26,22 ± 0,90
Bogor $_{unripe}$	5,58 ± 0,04	0,55 ± 0,01	19,67 ± 0,42
Bandung $_{ripe}$	5,44 ± 0,01	1,14 ± 0,91	26,68 ± 0,76
Bandung $_{unripe}$	6,07 ± 0,13	1,15 ± 1,33	15,24 ± 0,48
$F_{calculation}$	7,9242	0,8282	5,5950

It is observable from Table 2 that statistical analysis on the parameter assay of ash content yielded $F_{calculation} > F_{table}$; showing that there is a significant difference between the values of ash contents; in which ash content of the extracts from $Cengkareng_{ripe}$ was higher (6,09 ± 0,77) than that of the extracts from $Jakarta_{unripe}$ (4,06 ± 0,09). Statistical analysis on the acid insoluble ash content yielded $F_{calculation} < F_{table}$; showing that there is no significant difference between the values of acid insoluble ash contents, in which the acid insoluble ash contents of the extracts from $Jakarta_{unripe}$ was higher (1,20 ± 0,55) than that of the extracts from $Jakarta_{ripe}$ (0,20 ± 0,02). Statistical analysis on the water contents results in $F_{calculation} > F_{table}$; indicating that there is a significant difference between the water contents, in which water contents of the extracts from $Jakarta_{unripe}$ was higher (31,27 ± 36,4708) than that of extracts from $Bandung_{unripe}$ (15,24 ± 0,4784).

The recent study was conducted to determine the concentration of inhibiting capacity of ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds collected from different areas during the different harvesting times. Eight ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds was assayed for their activity in inhibiting α-glucosidase by cleaving carbohydrates into glucose in vitro using acarbose as positive controls.

Table 3. The Result of Assay in Inhibiting α-glucosidase in vitro

Extracts	IC_{50}
Jakarta $_{ripe}$	12,26 ± 1,41
Jakarta $_{unripe}$	9,63 ± 0,29
Cengkareng $_{ripe}$	8,81 ± 0,82
Cengkareng $_{unripe}$	9,05 ± 0,10
Bogor $_{ripe}$	7,46 ± 0,21
Bogor $_{unripe}$	15,45 ± 4,83
Bandung $_{ripe}$	16,50 ± 1,15
Bandung $_{unripe}$	8,88 ± 0,14
Acarbose	1,76 ± 0,89

Table 3 presents IC_{50} values of some ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds in inhibiting α-glucosidase. The presence of inhibition to alpha-glucosidase activity of all extract of ripe and unripe could be caused by the presence of carbohydrate, which is suspected to be the competitive inhibitor for alpha-glucosidase enzyme. This is appropriate with the substrate of alpha-glucosidase which is food carbohydrate, such as starch and glycogen.

The tables indicates that ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds collected from $Bogor_{ripe}$ has an IC_{50} value of 7,46 ± 0,21 µg/ml; it is higher than the ethanolic extracts from $Jakarta_{ripe}$ (12,26 ± 1,41 µg/ml). Table 3 presents IC_{50} values of inhibition essay of α-glucosidase; extracts from $Bogor_{ripe}$ has a lower value (7,46 ± 0,21 µg/ml), showing that ethanolic extracts from $Bogor_{ripe}$ is stronger in terms of its effectiveness than extracts from any other areas and from acarbose in inhibiting the activity of α-glucosidase enzyme when cleaving the carbohydrates into glucose. The greater effectiveness of ethanolic extracts of *Leucaena leucocephala* (Lmk) De Wit seeds collected from $Bogor_{ripe}$ was confirmed by the data on standardization parameters including ash content, acid insoluble ash content, water content, and geographical area of the area from which the plants originate. $Bogor_{ripe}$ has an ash content of (4,70 ± 0,06), a value approximating the required standard; the acid insoluble ash content meets the required criteria. Similarly, geographical area of Bogor is contributing to the quality of the extracts of *Leucaena leucocephala* (Lmk) De Wit seeds, since the geographical area of Bogor is most appropriate for the optimal development of *Leucaena leucocephala* (Lmk) De Wit seeds. Bogor has a minimum height of 190 m and maximum height of 330 meters with mean annual rain fall 3.500 – 4000 mm; and the most appropriate place for *Leucaena*

leucocephala (Lmk) De Wit seeds is an area with a height of over 490 meters and a rainfall of 3900 mm.

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