Introduction

*Allium odorum* is thought to originate from China, spread to Japan, Korea, India, Nepal, Thailand, and the Philippines. It is an annual plant that grows in clumps, leaves the width of 5–10 mm, solids and slightly sprouts, leaves reach 20 cm long, dark green, distinctive aroma, 40 cm tall flower stalks, white color, grow upright, solid flower stalks, and square.[1,2] This plant is known in Indonesia with various regional names such as singando (Palembang), chives or doubles (Sundanese), kecai, kucai, pucai (Javanese), bucay (Madura), ganda (Minahasa), bring iosina (Gorontalo), kocai (Bugis), kusai (Roti), and ganda (Halmahera and Ternate).[3] Through this paper, it will be called as ganda leaf. The usefulness of kucai leaves is not yet widely known. However, there are several uses that have been reported based on several libraries as follows: In Japan, China, Korea, India, Nepal, Thailand, and the Philippines are traditionally known to be effective in restoring fatigue.[1] The leaves are commonly used as spices that can be consumed in both fresh and dry forms. Clinically, this plant is used as an anti-tumor, digestive disorder in the intestine. In Thailand, the seeds are used in the treatment of a toothache.[4] In another literature, it is stated that ganda leaves have antibacterial and antifungal activity both for Gram-positive and Gram-negative bacteria.[5,6] It also reported as antioxidant, anti-inflammation, antiplatelet, anti-allergic, and cytotoxicity, reduce the risk for heart disease or cancer.[7]

Ganda leaves contain sodium, potassium, calcium, phosphorus, magnesium, manganese, Vitamins A, B1, B2, C, sulfur compounds, quercetin-3-glycosides, glucose, galactose, furalic acid, p-kumarat acid, malic acid, citric acid, palmitic acid, linoleic acid, and linolenic acid.[1,8] So far, the information about the parameters used to determine the quality of double leaf extract has not
been widely disclosed. On this basis, the idea arose to conduct research on the parameters used for quality testing of ganda leaf extract. The parameters for determining the quality of double extracts need to be known to complete information about these plants.

MATERIALS AND METHODS

Materials

Ganda (A. odorum) leaves were collected from Monaco plantations, Lembang, West Java and determined at the taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran. Chemical used was Dragendorff reagent (mixture of solution Bi (NO$_3$)$_3$ in HNO$_3$), Lieberman-Burchard reagent (mixture of anhydrous acetic acid and concentrated sulfuric acid), Mayer reagent (mixture of HgCl$_2$ solution in water and KI in water), magnesium (Mg) powder (CV Agung Menara Abdi), vanillin sulfate, chloroform, ethyl acetate, and toluene (PT Brataco). Unless stated otherwise, all chemicals were analytical grades.

Equipment

The equipment used in this study was macerator, mortar, stomper, drip plate, water bath, 254 nm ultraviolet (UV) lamp and 366 nm lamps, gas chromatography-mass spectrometry (GC-MS) (Shimadzu GC-17 A; GC/MS. QP5050A), microscope (Nixon), digital cameras (Nikon), and ovens (Memmert).

Methods

A collection, determination, and processing of materials: Ganda leaves were collected and then cleaned from the soil, washed using running water and then dried. Then, sliced or chopped with ± 3 cm long and dried with indirect sunlight for about 2 weeks. After drying, the sample was ground until smooth, then stored in a clean and closed container.

Extraction

The extraction method used in this study was maceration. The selection of this method was done to prevent the occurrence of damage to the thermolabile chemical compounds contained in the ganda leaves leaf. The maceration was carried out by soaking the sample in the macerator then leaving it for 24 h at room temperature with stirring occasionally. The solvent replacement was carried out during 3 × 24 h.

Examination of Quality Control Parameters from the Extract of A. odorum

These examinations included organoleptic, macroscopic, microscopic, phytochemical screening, non-specific parameter, examination of specific parameters, thin-layer chromatography (TLC), and GC-MS referring to official literature.

Organoleptic Examination

Macroscopic and organoleptic examinations performed on sample included physical characteristics such as size, color, surface characteristics, texture, friability, and the surface of the fault or the plane.

Microscopic Examination

The examination was carried out on simplicia in the form of powder with chloral hydrate media to see parenchymal fragments containing oil cells, starch grains, tracheal fragments, and other marker characteristics possessed by simplicia.

Phytochemical Screening

Tests were carried out on ganda leaf extract which includes: Alkaloid, flavonoid, saponin, triterpenoid, steroid, quinone, and monoterpenoids/Sesquiterpenes. The test was based on the Farnsworth method.

Check for Non-specific Parameters

Here extracts of specific gravity and drying losses were carried out.

Determination of Moisture Content

Determination of moisture content is carried out by distillation using toluene.

Determination of Ash Total Contents

Approximately 2–3 g which had been carefully weighed, was inserted into the silicate crucible which had been spawned and ground, evenly distributed. Then, it was spawned slowly until the charcoal was used up, cooled, then weighed. Ash content was calculated against the weight of the initial extract.

Determination of Non-soluble Acid Levels

The ash obtained from the above ash content was boiled with 25 mL dilute hydrochloric acid for 5 min, then the insoluble part of the acid, filtered through crucible glass or ash-free filter paper, washed with hot water, spilled until the weight remained, then weighed. The levels of insoluble ash in acids were calculated against the weight of the initial extract.

Specific Parameter Checking

Organoleptic extract: The organoleptic examination of double leaf thick extract uses the senses to describe shape, color, smell, and taste.

Determination of Water Soluble Compounds

A total of 5 g of extract was macerated for 24 h with 100 mL of chloroform LP water using clogged flask while repeatedly shaking it for the first 6 h and then left for 18 h. After filtering, 20 mL of filtrate is then evaporated to dryness in a shallow flat base cup that has been anchored; the residue is heated at a temperature of 105ºC to a fixed weight. The level
Determination of Soluble Ethanol Compounds
A total of 5 g of extract was macerated for 24 h with 100 mL ethanol (95%) using a clogged flask while shaking repeatedly for the first 6 h and then left for 18 h, then filtered quickly by avoiding ethanol evaporation, then 20 mL of filtrate evaporated to dryness in a shallow evaporator dish with a flat base, the residue was heated at a temperature of 105ºC to a fixed weight, then weighed. The level of the compound dissolved in ethanol was calculated against the initial extract.

Chemical Content of Extracts
This test included the determination of essential oil levels. The number of extracts was estimated to produce 1 mL–3 mL of essential oil add a number of extracts that had been carefully weighed into the pumpkin connect with the scale and cooling section. Boil the contents of the pumpkin with the appropriate heating until the essential oil was completely distilled and not added again in the scale container. If the volume of essential oil was accommodated in a sealed container, the recording could be done with readings up to 0.1 mL, and the volume of essential oils for every 100 g extract could be calculated from the weight of the extract weighed.

Specific Parameters
This test was carried out with TLC and GC-MS.

TLC
Ganda leaf extract was applied to the TLC silica gel GF 254 plate using a microcapillary right at the 1 cm line from the bottom of the plate. The TLC plate was then put into a chromatographic vessel which had been saturated first with the developer of chloroform-methanol (9:1), then developed to a certain extent. After that, the deadline of the developer was marked, then the spots that occur were observed under 254 nm and 366 nm of UV light and sprayed with the appearance of 10% sulfuric acid spots in methanol, then heated at 110ºC until color appears, then calculated the value of Rf.

GC-MS
This test the content of ganda leaf ethanol extracts using GC-GC (Shimadzu GC-17 A; GC/MS. QP5050A).

RESULTS
Organoleptic Examination Results
Ganda leaves had a width of 5–10 mm, were dense and slightly sprouted, leaf length reached 20 cm, dark green, distinctive aroma, the height of flower stalk reached 40 cm, white, grew with erect flower stems, solid, and square.

Microscopic Examination Results
The results of the microscopic examination of ganda leaf powder showed that the herb contained cover hair, stomata, oxalate crystals, parenchyma, oil cells, epidermis, and vessels.

Phytochemical Screening Results
Phytochemical screening was an initial description of the compound content in one simplicia. The results of phytochemical screening are shown in Table 1.

Huzaifa et al. reported that the phytochemical tests of Allium sativum (garlic) in its aqueous of bulb showed the presence of flavonoids, alkaloids, saponins, tannins, and cardiac glycosides. Abdul Kadir et al. mentioned in the phytochemical analysis of Allium cepa L. Ethanolic extract revealed the presence of saponins, tannins, flavonoids, and alkaloids while steroids were not detected. Tiwari et al. showed that phytochemical analysis of A. sativum n-butanol fraction showed the presence of steroids, triterpenoid saponins, and carbohydrates. Usharani et al. showed the presence of alkaloids, tannins, saponins, phenols, flavonoids, and volatile oil that were common to all the A. odorum ethanol extract.

Extraction
After maceration with ethanol, evaporating with rotary evaporator followed with drying at water bath, it was found that the viscous ethanol extract yield of ganda leaves was 19.10 %. w/w.

Specific Weight Determination
The results of the determination of the specific density of extract at the three determinations were 1.98% w/v.

Table 1: Phytochemical screening of ganda leaf extract

<table>
<thead>
<tr>
<th>No</th>
<th>Chemical compounds</th>
<th>Odorum leaves ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Polyphenols</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Tannin</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>Quinone</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Triterpenoids</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>Monoterpenoid/sesquiterpenoid</td>
<td>+</td>
</tr>
</tbody>
</table>

(+): Detected, (−): Not detected

Table 2: Extract parameter of ganda leaves extract

<table>
<thead>
<tr>
<th>No</th>
<th>Determination</th>
<th>Results (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drying shrinkage</td>
<td>7.44</td>
</tr>
<tr>
<td>2</td>
<td>Water content</td>
<td>12.90</td>
</tr>
<tr>
<td>3</td>
<td>Ash total content</td>
<td>5.20</td>
</tr>
<tr>
<td>4</td>
<td>Insoluble acid ash</td>
<td>0.95</td>
</tr>
<tr>
<td>5</td>
<td>Water-soluble compounds</td>
<td>10.97</td>
</tr>
<tr>
<td>6</td>
<td>Soluble ethanol compounds</td>
<td>13.70</td>
</tr>
<tr>
<td>7</td>
<td>Essential oil content</td>
<td>0.60</td>
</tr>
</tbody>
</table>
The results of determining the extract parameters are shown in Table 2. All data at this table were an average value after three determinations.

**TLC Results**

The developer of TLC was chloroform-methanol (9:1), the appearance of 254 nm UV light spots, 366 nm, and 10% sulfuric acid reagent in methanol as shown in Table 3.

**DISCUSSION**

In this study, the simplicia used was ganda leaves \((A.\ odorum\ L.)\). The selection and washing of ganda leaves were intended to remove impurities that cause contamination and interfere with the process of determining extract parameters.

Making extracts were done by macerating \(3 \times 24\) h by replacing solvents every day and occasionally stirring so that the solvent interacts with simplicia. The solvent replacement was intended to attract the compound content in simplicia as much as possible. The thick extract produced was blackish brown with a distinctive odor. In making the extract, it was used 95% ethanol solvent, because 95% ethanol had a small water content, thus minimizing the possibility of an enzymatic reaction in simplicia.

The extract quality requirements consist of various general (nonspecific) parameters, specific parameters, and test for the chemical content of ganda leaf extract. The non-specific parameters determined were specific gravity, drying losses, water content, total ash content, and acid insoluble ash content. Specific parameters determined were the levels of water-soluble compounds and levels of soluble ethanol compounds. The content test carried out was the determination of essential oil content, TLC, and GC-MS.

The results of phytochemical screening showed ganda leaf extract containing alkaloid compounds, flavonoids, steroids, polyphenols, quinones, and monoterpenes/sesquiterpenes.

From the results of non-specific parameters, it was known that ganda leaf extract had a specific gravity value of 1.84, drying losses of 7.44%, water content of 12.9%, total ash content of 5.2%, and acidic insoluble ash content of 0.95%, whereas the results of specific parameters known as leaf extract double have a water-soluble compound content of 10.97% and a level of 13.7% soluble ethanol compound. From the test of the chemical content of the extract, it could be seen as follows.

**Table 3: TLC results of ganda leaves extract**

<table>
<thead>
<tr>
<th>Spots</th>
<th>(R_f)</th>
<th>UV 254 nm</th>
<th>UV 366 nm</th>
<th>10% (H_2SO_4) in MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04</td>
<td>Blue</td>
<td>-</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>Purple</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>0.25</td>
<td>Purple</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>0.33</td>
<td>Purple</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>0.51</td>
<td>-</td>
<td>Red</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>0.68</td>
<td>Purple</td>
<td>Red</td>
<td>Light green</td>
</tr>
<tr>
<td>7</td>
<td>0.73</td>
<td>-</td>
<td>Red</td>
<td>Light green</td>
</tr>
<tr>
<td>8</td>
<td>0.81</td>
<td>-</td>
<td>Red</td>
<td>Light green</td>
</tr>
<tr>
<td>9</td>
<td>0.91</td>
<td>-</td>
<td>Purple</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.95</td>
<td>-</td>
<td>Red</td>
<td>Dark green</td>
</tr>
</tbody>
</table>

**Table 4: GC-MS of ganda leaves extract**

<table>
<thead>
<tr>
<th>No</th>
<th>(R_f)</th>
<th>Compounds</th>
<th>Peak area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.430</td>
<td>2 diisooctyl esters, phthalic acid, 2- (hexadecyloxy) - 3 - (octadesiloxy)</td>
<td>3.3</td>
</tr>
<tr>
<td>2</td>
<td>28.095</td>
<td>propyl ester, lauric acid, 2- (1-oxododesil) oxy -1,3 – propanediol ester, hexadecanoic acid</td>
<td>3.48</td>
</tr>
<tr>
<td>3</td>
<td>29.038</td>
<td>Phosphonic acid, 2- (1-oxododesil) oxy -1,3 – propanediol ester, hexadecanoic acid</td>
<td>43.31</td>
</tr>
<tr>
<td>4</td>
<td>29.583</td>
<td>Phosphonic acid</td>
<td>4.31</td>
</tr>
</tbody>
</table>

GC-MS: Gas chromatography-mass spectrometry
that ganda leaf extract had an essential oil content of 0.06%, in TLC patches were produced with different Rf values on UV 254 nm, UV 366 nm, and 10% sulfuric acid. From the results of GC-MS, it was known that ganda leaf extract contains 2 diisooctyl esters, phthalic acid; 2- (hexadecyloxy) - 3 - (octadesiloxy) propyl ester, lauric acid; 2- (1-oxododesil) oxy-1,3-propanedyl ester, hexadecanoic acid; phosphonic acid.

CONCLUSION

The results of this determination could be used as a preliminary description of the quality of ganda leaf extract quality, but the results of this determination cannot yet be used as an A. odorum leaf extract quality standard, because the determination of parameters only came from one region. Further investigation of a different area of ganda leaves is suggested in order to obtain general parameters of ganda leaves.

ACKNOWLEDGMENT

The authors wish to thank Vivi Salfika for technical support.

REFERENCES