

Physical Characteristics, Total Phenolic, and Total Flavonoid Contents of *Coccinia grandis* (L.) Voigt Leaves Extract

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Abstract: *Coccinia grandis* (L.) Voigt is a plant that has been widely used for the treatment of various types of diseases. The phenolic and flavonoid content of plant extracts largely determine their pharmacological activity. The aim of this study was to determine the total phenolic and total flavonoid contents of the ethanol extract of *C. grandis* leaves. The Leaves of *C. grandis* were obtained in the area of Dalung, North Kuta, Badung, Bali. The powdered *C. grandis* leaves were extracted by maceration method using 70% ethanol followed by evaporation using an oven at 45°C to obtain a thick extract. The non-specific parameters of the extract were then determined, such as moisture content, ash content, and acid insoluble ash. Total phenolic content was determined using the follin-ciocalteu method and total flavonoid content was determined using the aluminum chloride method. Based on the research results, the yield of *C. grandis* leaf extract was 19.36%. *C. grandis* leaves extract has a description of a thick and sticky extract, blackish brown color, distinctive aroma, and a bitter sour taste. The water content of the ethanolic extract of *C. grandis* leaves was 9.93±0.03% (g/g). The total ash value of the simplicia and ethanolic extract of *C. grandis* leaves were 20.76±0.15% (g/g) and 27.38±0.19% (g/g), respectively. The acid insoluble ash content of the extract was 4.05±0.30% (g/g). The total phenolic and total flavonoid contents of the extract were 111.92±0.49 µg GAE/mg dry extract and 73.60±3.53 µg QE/mg dry extract, respectively.

Keywords: *Coccinia grandis*, extract, standardization, phenolic, flavonoid

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INTRODUCTION

Many plants contain bioactive compounds that are responsible for treating various diseases. These plants have been well known as herbal plants or medicinal plants. Herbal remedies can come from any part of the plant from leaves, roots, seeds, bark, to flowers [1]. About 75-80% of the world's population rely on herbal medicine as a major part of their traditional therapy [2]. Toxic side effects of conventional medicines and lack of medication for many chronic diseases are the two main things that cause the high use of these herbal

medicines. In fact, the World Health Organization (WHO) encourages the use of herbal medicines, especially in developing countries, to reduce the financial burden on the state.

The pharmacological activity of a medicinal plant is caused by the presence of phytochemical compounds such as alkaloids, phenolics, flavonoids, tannins, steroids, saponins, and terpenoids that are produced from the plant secondary metabolism [3]. Phenolics are compounds that have one or two aromatic rings with one or more hydroxyl groups. Phenolics are

mostly found in polyphenols, especially from fruits, vegetables, cereal, nuts, and derivative products such as fruit juices, tea, coffee, chocolate and red wine. Phenolic is considered to have the highest antioxidant content compared to other phytochemicals [4]. Flavonoids are the largest group of phenolic compounds found in plants that have biological activities such as antimicrobial, anticancer and can provide protection against cardiovascular disease [5]. Flavonoids are known as pigment dyes in plants that function as antioxidants and are reported to have strong free radical scavenging activity based on their ability to transfer hydrogen or electrons and chelate transition metals [6]. In addition, flavonoids are bioactive phenols with low molecular weight that have antidiabetic activity and have been widely studied as promising substances that are significantly attractive to use for safe diabetes therapy [7]. The total flavonoid content in the extract, as measured by the aluminum chloride colorimetric method, was 54.72 ± 1.81 mg catechin/g extract and several studies have reported that flavonoids have antioxidant properties and hydroxyl groups that have free radical scavenging activity [8].

Coccinia grandis (L.) Voigt is a plant that grows wild in many areas in Africa and Asia including India, Sri Lanka, Indonesia, China, Malaysia, the Philippines, East Papua, Guinea, Vietnam and northern regions [9]. All parts of this plant are traditionally used for various medicinal purposes. *C. grandis* leaves are used in traditional Indian medicine for the treatment of a number of ailments including diabetes, wounds, ulcers, inflammation, skin eruptions, fever, asthma and coughs. Several studies have shown that *C. grandis* leaves extract has anti-inflammatory, analgesic and antipyretic activity [10], hepatoprotective [11–13], antibacteria [14,15], and antidiabetes [16,17]. In previous studies, we reported the determination of total phenolic and flavonoid contents from *C. grandis* leaves water extract and their combination with *Averrhoa blimbi* L. fruits [18] and *Blumea balsamifera* L. leaves [19]. In this study, we extracted the simplicia of *C. grandis* leaves using 70% ethanol solvent. The thick extract obtained was then determined for its physical parameters, total phenolic content, and total flavonoids.

MATERIALS AND METHODS

Materials. *C. grandis* leaves were harvested in Dalung, North Kuta District, Badung Regency, Bali. Whole plant was determined at the Pharmacognosy Laboratory, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta with number: 20.03.08/UN1/FFA/BF/PT/2020. The chemicals used in this study were ethanol, n-hexane, ethyl acetate, sulfuric acid, distilled water, folin ciocalteu reagent, sodium carbonate, gallic acid, aluminum chloride, potassium

acetate, quercetin, hydrochloric acid, and toluene. All chemicals are of pro analysis quality.

Preparation and Extraction of *C. grandis* Leaves.

Fresh *C. grandis* leaves were collected and washed under running water and then drained for 24 hours at room temperature. *C. grandis* leaves were dried in the oven at 50°C for 3 days. The dried leaves of *C. grandis* were powdered and stored in an airtight plastic container. One part of *C. grandis* leaves simplicia powder was put into a glass bottle and 10 parts of 70% ethanol were added until all the powders were completely immersed. The maceration process was carried out for 24 hours, with stirring every 3 hours. The mixture was filtered and the maserate was poured into a large container bottle. The macerated waste was remacerated 2 times and the macerate was collected in a container bottle. The ethanol solvent was then evaporated using an oven at 45°C. Furthermore, the thick extract was obtained, put in a dark bottle and stored in the refrigerator.

Determination of Extraction Yield. The extraction yield was expressed as a percent. The yield was calculated by dividing the mass of the thick ethanol extract produced by the mass of dry leaf simplicia multiplied by one hundred [20].

Determination of Physical Characteristics of the Extract. Organoleptic observation was carried out by recognizing physically using the five senses in describing shape, smell, color and taste [21]. Other parameters such as loss of drying, moisture content, ash content, and acid insoluble ash content were determined following the procedures of the World Health Organization for herbal extracts [22].

Determination of Total Phenolic Content.

Determination of total phenolic content was conducted using the Folin-Ciocalteu colorimetric method from Attanayake et al. [23]. A total of 1.0 ml of 0.05 g/ml extract solution was mixed with 1.0 ml of 95% ethanol, 5.0 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. The mixture was allowed to react for 5 minutes and 1.0 ml of 5% sodium carbonate was added. After that, it was thoroughly mixed and placed in a dark container at room temperature (27°C) for one hour and the absorbance was measured spectrophotometrically at 725 nm. The quantification was carried out with a standard curve of gallic acid (0–50 µg/ml). The results were expressed in terms of gallic acid equivalent GAE/g dry extract.

Determination of Total Flavonoid Content. The total flavonoid content of the plant extract was determined using the aluminum chloride colorimetric method. A total of 0.50 ml of extract solution was mixed with 95% ethanol (1.5 ml) followed by the addition of 10% aluminum chloride (0.10 ml), 1 M potassium acetate

(0.10 ml) and distilled water (2.8 ml). The resulting mixture was incubated at 27°C for 30 minutes. The absorbance of the mixture was measured spectrophotometrically at 415 nm. The flavonoid content was calculated using a standard calibration of quercetin solution in the range of 0-50 µg. The results were expressed as micrograms of quercetin equivalent (QE)/g dry extract [23].

Data Analysis. The research data were expressed as mean±standard error of mean (SEM) and the experiment was carried out in at least three replications.

RESULTS AND DISCUSSION

Physical Characteristic of *C. grandis* Extract. In this study, several tests were applied to the *C. grandis* simplicia and extract. The result of the determination of physical characteristic of *C. grandis* simplicia and extract is depicted in Table 1. The extraction yield of 193.57 g was obtained from 1 kg of dry simplicia. If calculated based on the percentage, the extraction yield was 19.36%. The organoleptic test results showed that the ethanol extract of *C. grandis* leaves was characterized by a thick and sticky extract, blackish brown color, distinctive aroma, and bitter sour taste. The organoleptic test aims to provide an initial description of the extract in a simple and objective manner. The results of this test can explain the shape, color, smell and taste of the extracts made using the five senses [24].

Table 1. Selected physical characteristic of *C. grandis* simplicia and extract.

Indicators ^[a]	Simplicia	Extract
Species	<i>Coccinia grandis</i> (L.) Voigt	-
Extraction yield (%)	-	19.36
Organoleptic	-	Thick and sticky extract, blackish brown color, distinctive aroma, bitter sour taste
Loss of drying (% (g/g))	8.29±0.09	-
Moisture content (% (g/g))	-	9.93±0.03
Ash content (% (g/g))	20.76±0.15	27.38±0.19
Acid insoluble ash (% (g/g))	-	4.05±0.30

Results are expressed as mean ± standard deviation. The experiment was carried out in at least 3 replications

Loss of drying is an important parameter in standardizing both simplicia and extracts. The determination is conducted by drying the simplicia at a temperature of 105°C for 30 minutes or until a constant weight is obtained. This aims to provide a maximum limit on the number of compounds lost in the drying process. In this study, the loss of drying of *C. grandis* leaves simplicia was 8.29±0.09% (g/g). At a drying temperature of 105°C, the components of the simplicia lost are water and compounds with lower boiling points. An extract must have a low water content so that it is not easily overgrown by fungi. When the extract is moldy, its pharmacological activity is reduced. The water content in the extract should not exceed 10% [25]. The extract obtained from this study had a moisture content of 9.93±0.03% (g/g). These results indicate that the ethanolic extract of *C. grandis* leaves has a moisture content that matches the required moisture content. If the water content is low, the fungus growth in the extract will be inhibited. The determination of the total ash content of the extract aims to determine the internal mineral content in the extract, while the determination of the acid insoluble ash content aims to determine the external mineral content [26]. In this study, the total content of ash and acid insoluble ash were 27.38±0.19% (g/g) and 4.05±0.30% (g/g), respectively. These results indicate that the inorganic mineral content of the extract is very high. Many factors can affect these results, such as: the natural conditions in which the plant grows, the process of washing unclean leaves, to the process of evaporating the extract using an oven.

Total Phenolic and Flavonoid Contents. Phenolic and flavonoid are part of secondary metabolites produced by plants that have pharmacological activities such as anti-inflammatory, antiviral, antimicrobial, antidiabetic, anticancer, and antioxidant. Flavonoids are linked with several diseases in human since they have a broad spectrum of health-promoting effects. Flavonoid-rich plant extracts showed antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties. They also inhibited the activity of enzymes, such as xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase and phosphoinositide 3-kinase [27]. The concentration of phenolic and flavonoid compounds in plants varies, which is influenced by many factors including: soil condition, irrigation and climate [28]. In this study, the determination of total phenolic content was carried out by the Folin-Ciocalteu method with gallic acid used as the standard. The linear regression equation $y = 0.0073x + 0.0013$ with $R^2 = 0.9994$ is obtained from the plot of gallic acid concentration vs absorbance. Determination of total flavonoid was carried out using the aluminium chloride colorimetric method with

quercetin as a standard. The linear regression equation obtained is $y = 0.0075x + 0.0008$ with $r =$

0.9991. The standard curves of gallic acid and quercetin are shown in Figure 1.

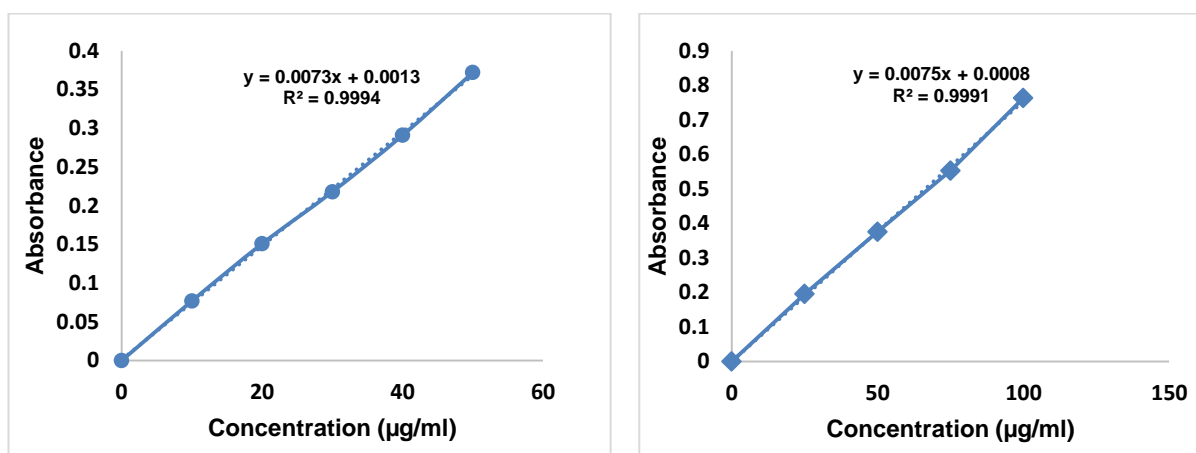


Figure 1. Standard curves of gallic acid (left) and quercetin (right)

Based on calculations using the linear regression equation, the total phenolic and flavonoid extract of *C. grandis* leaves were 111.92 ± 0.49 µg GAE/mg d.e. and 73.60 ± 3.53 µg QE/mg d.e., respectively (Table 2). Several researchers have also reported the results of total phenolic and flavonoid determinations of plant extracts. Total phenolics and flavonoids from methanol extract of *M. spinosa* leaves were reported to be 90.08 ± 0.44 mg GAE/g d.m. and 58.50 ± 0.09 QE/g d.m. [29]. The methanol extract of the root of *Arisaema jacquemontii* Blume was reported to contain a total phenolic of 45.17 ± 1.70 mg GAE/g d.m. and a total flavonoid of 35 ± 2.20 QE/g d.m. [30].

parameters of the extract and the simplicia of *C. grandis* provide an overview of the quality of the extract used. The total phenolic content of *C. grandis* leaves extract was found to be 111.92 ± 0.49 µg GAE/mg d.e. While the total flavonoid content of *C. grandis* leaves extract was 73.60 ± 3.53 µg QE/mg d.e.

Table 2. Total phenolic and flavonoid content *C. grandis* simplicia and extract.

Linear regression equation	Total phenolic (µg GAE/mg d.e.)	Total flavonoid (µg QE/mg d.e.)
$y = 0.0073x + 0.0013$	111.92 ± 0.49	-
$y = 0.0075x + 0.0008$	-	73.60 ± 3.53

Results are expressed as mean \pm standard deviation. The experiment was carried out in at least 3 replications; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; d.e.: dry extract.

CONCLUSION

This study provides information regarding the physical and chemical characteristics of the ethanolic extract of *C. grandis* leaves. The extraction yield of *C. grandis* leaves using ethanol solvent was 19.36%. The extract of *C. grandis* leaves is characterized by a thick and sticky extract, blackish brown color, distinctive aroma, and a bitter sour taste. The test results of the physical

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