# Effect of Extraction Technique on Antioxidant Capacity, Vitamin C, Total Phenol, and Total Flavonoid of *Bouea macrophylla* Griff Leaf

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# Effect of Extraction Technique on Antioxidant Capacity, Vitamin C, Total Phenol, and Total Flavonoid of *Bouea macrophylla Griff* Leaf

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**Abstract**. *Bouea macrophylla Griff* also known as gandaria is a local heritage plant of West Java. Aside from being a salad, gandaria leaves are also used as herbal remedies. Some parts of the gandaria tree contain bioactive compound that has health benefits. However, the research related to leaves is rare, especially about extraction techniques to obtain optimal bioactive content. The common preservation technique is drying process to make herbs storage time longer. Conventional drying techniques often reduce the ability and bioactive compound. This study compared antioxidant capacity, vitamin C, total phenolic, and total flavonoid on fresh leaves (FL), sundried leaf (SDL), roomdried leaf (RDL), and vacuum evaporator leaf extract (VELE) of gandaria. This study used spectrophotometry to analyze DPPH inhibition (antioxidant activity), total phenolic, total flavonoids, and titration methods to analyze vitamin C with 3 replicates. One-way ANOVA statistical test showed significant differences between 4 groups for antioxidant capacity (p=0.004), vitamin C (p=0.000), total phenol (p=0.000), and total flavonoid (p=0.031). The best $IC_{50}$ DPPH start from VELE 0.026±0.002 mg/mL, RDL 0.037±0.009 mg/mL, SDL 0.089±0.022 mg/mL, and FL 0.169±0.067 mg/mL. The highest vitamin C content per g extract start from FL 148.257±0.000 mg, RDL 148.121±0.235 mg, SDL 147.715±0.2346 mg, and VELE 145.819±0.813 mg. The highest total phenolic content per g extract start from VELE 117.836±1.831 mg GAE, RDL 45.772±0.618 mg GAE, SDL 31.711±1.707 mg GAE, and FL 18.289±2.360 mg GAE. The highest total flavonoid per g extract start from VELE 251.111±78.339 mg QE, RDL 131.111±89.106 mg QE, SDL 109.444±46.017 mg QE, and FL 53.889±20.839 mg QE. Further research about VELE of gandaria product development is very needed to make product that support health improvement.

**Keywords:** gandaria, leaf extract, antioxidant, phenolic, vitamin C

## 1. Introduction

Gandaria plants have different name in each region, including gandaria (Indonesia), jatake (Sunda), pao gandari (Madura), ma prang, somprang (Thailand), kundang, setar (Malaysia), and mango plum (United Kingdom) [1]. Gandaria is a species of Anacardiaceae. This plant grows in tropics climate, and is widely cultivated in West Java, Sumatra, Ambon, and Thailand. Figure 1 shows the gandaria trees in the Biotechnology area of LIPI (Indonesian Institute of Sciences) Cibinong. Gandaria plant

height ranges from 12-27 m, stem diameter 1.65 - 2 m, brownish stem color, round stem shape, grooved stem surface, direction of perpendicular stem growth, dichotomous branching, true woody stem type, and tap root [2].

**Figure 1**. Gandaria tree in the Biotechnology area of LIPI Cibinong

The length of gandaria leaves 9.5 – 11.5 cm, leaf width 3.5 cm, leaf stalk length 2.1 mm, leaf stalk width 1.6 mm, single leaf type, light green for young leaf, dark green for mature leaf, inverted oval leaf shape, tapered leaf tip , the base of the leaf is blunt, the leaf edge is a bit bumpy. The surface of the leaf is slippery, the structure of the leaf bone pinnates, the layout of the leaves alternates alternately, the location of the armpit flower or the end of the branch [2]. Figure 2 shows the appearance of gandaria leaf.

**Figure 2.** Gandaria Leaf

Table 1 shows the previous research related to the antioxidant potential possessed by parts of the gandaria plant. Based on table 1 the parts of the gandaria plant have antioxidant potential.

**Table 1.** previous studies related to potential antioxidant of gandaria plant

| Sample | Result | Literature |
|---|---|---|
| Ethanol extract of gandaria leaf | $IC_{50}$ antioxidant potential was 55.83 µg / mL. | [3] |
| Gandaria fruit juice | Gandaria fruit juice contains phenolic isolates and has antioxidant activity. | [4] |
| Cortex and stem of gandaria plant | Cortex and stem of gandaria plant contain phenolic isolates. | [5] |

The drying process purposed for preservation of food and agricultural products. The sun drying has been used since long times. The sundried method is appropriate for warm and dry areas. The ideal temperature for drying the sun is around 100$^{o}$F and the humidity must be 60% or less so that the dried herbs are perfect. In hot weather it can use glass which will help trap heat, absorbers that are too late to transmit heat and ventilation to help circulate air [6].

Room temperature about 25$^{o}$C and hummidity less than 60%. Leaf was dryed in room that has enough air circulation and protected from direct sunlight [6]. This study analyzed antioxidant potential, total phenol, total flavonoids, and vitamin C in leaves with conventional drying processes (roomdried and sundried) and extraction by vacuum evaporator compared with fresh leaf (FL). VELE (vacuum evaporator leaf extract) is an extract obtained from gandaria leaf juice evaporated in a vacuum evaporator.

## 2. Method

### *2.1 Sample preparation*

Sample of this study is gandaria leaf which are processed in several ways. Leaf are obtained from LIPI Bogor. The leaves are cleaned of dirt and washed with running water. Fresh leaf are stored in airtight containers and placed in refrigerator [7]. FL is blended to form small pieces. SDL (sundried leaf) is dried under the sunlight for 4 days so that the water content is <5%. RDL (roomdried leaf) is obtained from gandaria leaves dried at room temperature for 6 days so that the water content is <5%. VELE is obtained from gandaria leaf juice evaporated with a vacuum evaporator. Each extract was filtered by size 1000 mesh.

### *2.2 Total phenolic analysis*

Total phenolic were analyzed based on [8]. The standard gallic acid was made series 0-0.05 mg/mL in distilled water. 0,5 mL of each concentration was added to the Folin reagent 0.5 mL. Added 0.5 mL of Na2CO3 solution. Shaken until homogeneous and left for 2 hours at room temperature. Measured absorbance at wavelength 765 nm. A linear curve is made and produced the equation y = 21,574x-0.0207.

The sample extract was made with a concentration of 1 mg/mL in distilled water. 0.5 ml of each sample added Folin-Ciocalteau reagent 0.5 mL. Added 0.5 mL of Na2CO3 solution. Solution was shaken until homogeneous and left for 2 hours at room temperature. Absorbance was measured at 765 nm. The analysis was carried out 3 times replication duplo. Total phenolic was obtained as mg equivalent gallic acid / g extract by entering the absrobance of the sample into the equation in the standard curve.

### *2.3 Total flavonoid analysis*

The total level of sample flavonoids refers to the procedure of [8]. Quercetin was made in the concentration of 0-100 ppm in distilled water. 1 mL of each concentration plus 5% NaNO2 as much as 60 µL was mixed and incubated 5 minutes. Then added 10% $AlCl_3$ 60 µL mixed and incubated 6 minutes. Then added 10% NaOH as much as 400 µl, seen on the absorbance of 510 nm. The obtained standard curve equation is y = 0.0003x.

The next step is the determination of the total flavonoid content of the gandaria leaf extract. The sample extract was made with a concentration of 1mg / ml in distilled water, plus 5% NaNO2 60 µL mixed and incubated 5 minutes. Then added 10% $AlCl_3$ 60 µL mixed and incubated 6 minutes. Then added 10% NaOH 400 µL, seen on the absorbance 510 nm. The sample solution was made in 3 times replication duplo. Flavonoid levels obtained as equivalent quercetin by entering the absorbance of the sample into equations in the standard curve.

### *2.4 Vitamin C analysis*

Analysis of sample vitamin C levels was adapted from the AOAC 967.22 (45.1.15) method [9]. 10 g sample and 10 g of crystal oxalic acid were crushed together. Next, enter the ingredients into a 250 mL volumetric flask, tera using distilled water, than filtered. 1 mL of filtrate dilute 10 times. Titration is carried out with dye solution until it is pink for 15 seconds. Concentration of standart was made 0-10 mg/mL. The amount of mL of this dye solution is used to determine the equivalent of vitamin C.

*2.5 DPPH inhibiton analysis*

DPPH powder was made into concentration 0.2 mM. Vitamin C powder was made in concentrations of 0-10 µg/mL. Gandaria leaf were concentrated from 0.25 to 8 mg/mL. The 500 µl DPPH and 500 µl extracts of the samples were homogenized and stored in a dark room at room temperature for 30 minutes. Absorbance was seen using a spectrophotometer with wavelength 517 nm [10,11]

The concentration values of extract and vitamin C samples and percent inhibition were plotted on the x and y axes in the log regression equation, respectively. The $IC_{50}$ value was expressed as the value of x while the y value was expressed 50. $IC_{50}$ is the concentration of the sample needed to reduce DPPH free radicals 50% [11].

## 3. Result and Discussion

**Table 2**. Mean±standard deviation of total phenol, total flavonoid, vitamin C, and $IC_{50}$ DPPH

| Sampel | Phenol (mg GAE/g) | Flavonoid (mg QE/g) | Vitamin C (mg/g) | $IC_{50}$ DPPH (mg/ml) |
|---|---|---|---|---|
| FL | 18.289±2.360 (4) | 53.889±20.839 (4) | 148.257±0.000 (1) | 0.169±0.067 (4) |
| SDL | 31.711±1.707 (3) | 109.444±46.017 (3) | 147.715±0.235 (3) | 0.089±0.022 (3) |
| RDL | 45.772±0.618 (2) | 131.111±89.106 (2) | 148.121±0.235 (2) | 0.037±0.009 (2) |
| VELE | 117.836±1.831 (1) | 251.111±78.339 (1) | 145.819±0.813 (4) | 0.026±0.002 (1) |

Note: number inside () showed rank from best treatment.

**Table 3.** Result of anova one factor and post hoc test (t-test two tailed)

| Phenol | Flavonoid | Vitamin C | $IC_{50}$ DPPH |
|---|---|---|---|
| p=0.000 | p=0.031 | p=0.000 | p=0.004 |

Table 3 showed the significance value of the ANOVA one factor test. Based on one factor ANOVA test showed all variables, total phenol, flavonoids, vitamin C, and $IC_{50}$ DPPH had p value <0.05. That showed at least 1 treatment that had a significant difference. Further testing (post hoc) of each variable can be seen in table 4-7.

**Table 4.** Significance value of post hoc test (t-test two tailed) total phenol

| | SDL | RDL | VELE |
|---|---|---|---|
| FL | 0.002* | 0.000* | 0.000* |
| SDL | | 0.000* | 0.000* |
| RDL | | | 0.000* |

Table 4 showed that all treatments have a sign * which means that all treatments have a significant difference in the total phenolic content. Based on mean and standard deviation values in table 2, VELE was the best treatment with a significant difference.

**Table 5.** Significance value of post hoc test (t-test two tailed) total flavonoid

| | SDL | RDL | VELE |
|---|---|---|---|
| FL | 0.129 | 0.216 | 0.014* |
| SDL | | 0.727 | 0.054 |
| RDL | | | 0.155 |

Table 5 showed the flavonoid content was not significantly different except for FL and VELE. The content of flavonoids was significantly different in FL and VELE. Based on table 2, it concluded that the highest content of flavonoids is in VELE.

**Table 6.** Significance value of post hoc test (t-test two tailed) vitamin C

| | SDL | RDL | VELE |
|---|---|---|---|
| FL | 0.016* | 0.373 | 0.006* |
| SDL | | 0.101 | 0.017* |
| RDL | | | 0.009* |

Table 6 showed that the vitamin C content was significantly different between FL, SDL, RDL compared to VELE. It could be caused by exposure to heat to VELE for a long period of time which is 3 liters for 1.5 hours with a temperature of 60 minutes at a pressure of 0.6 atm. FL and RDL did not have a significant difference because it is possible that the temperature of 25$^{o}$C does not have a significant effect on the content of vitamin C. RDL and SDL were not significantly different because the difference in room temperature and sunlight did not significantly affect vitamin C. While SDL and FL were significantly different the influence of sunlight for 4 days has an effect on the vitamin C content compared to fresh leaves.

**Table 7.** Significance value of post hoc test (t-test two tailed) $IC_{50}$ DPPH

| | SDL | RDL | VELE |
|---|---|---|---|
| FL | 0.120 | 0.028* | 0.021* |
| SDL | | 0.020* | 0.008* |
| RDL | | | 0.112 |

The previous study revealed that sun-drying of green leafy vegetables cause a significant ($P < 0.05$) decrease in the vitamin C content (16.67-64.68% loss). Conversely it leads to a significant increase in the total phenolic content (6.45-223.08% gain), reducing property (16.00-362.50% gain) and free radical scavenging ability (126.00-5757.00% gain) of the green leafy vegetables. It could therefore be concluded that a significant decrease ($P < 0.05$) in Vitamin C content caused by sun- drying will not reduce the antioxidant activity of the green leafy vegetable, moreover, the phenolic constituent of the green leafy vegetables contributes more to the antioxidant properties of vegetables than ascorbic acid, as its increase on sun-drying cause a significant ($P < 0.05$) increases in the antioxidant properties of the green leafy vegetables, irrespective of the decrease in the ascorbic acid content [12].

The previous study [13] conducted a study of the content of vitamin C in some vegetables dried in a cabinet dryer with a temperature of 55 $^{o}$C, shade drying, and sun drying. In the study the highest vitamin C content was in the dryer cabinet, followed by shade drying, and sun drying. The maximum retention of vitamin C in the tray is dry and has high temperature and water for short time. Vitamin C that is sensitive to heat allows lower vitamin C content in VELE.

Anion radikal 2.2 *diphenyl* 1 *picryhydrazyl* (DPPH) is a stable free radical. DPPH barrier analysis by ingredients containing antioxidants measures the ability of the material to bind DPPH. The presence of unpaired electrons on DPPH gives a deep purple color which can be seen at the absorbance of 517 nm and the color will fade if DPPH binds to antioxidants [14]. The study [3] reported that the ethanol extract of gandaria leaves had antioxidant potential, namely $IC_{50}$ 55.83 mg/mL. The best $IC_{50}$ was obtained from the VELE sample 0.026 ± 0.002 mg/mL. When compared with ethanol extract [3] the VELE and RDL extraction techniques are better in DPPH inhibition ability. It may be caused DPPH binds to antioxidants such as phenols and flavonoids contained in the sample. Flavonoid and phenolic content is directly proportional to DPPH inhibition ability.

When compared with the standard vitamin C, extracts VELE there was no better DPPH inhibition ability. The inhibition of $IC_{50}$ DPPH by the standard vitamin C 0.01 mg/mL.

## 4. Conclusion

Best phenol, flavonoid content, and $IC_{50}$ DPPH in VELE treatment, while best vitamin C content in FL.

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