# ANTIOXIDANT CAPACITY OF FRANGIPANI (*Plumeria alba*) POWDER EXTRACT

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## ABSTRACT

This research aimed to identify the antioxidant capacity, vitamin C (ascorbic acid), and total phenolic compounds of frangipani flower powder. The powder was extracted using ethanol, methanol, acetic acid, and water (aquadest). Antioxidant capacity of each extract were determined using *2,2-diphenyl-1-picrylhydrazyl radical scavenging* method, vitamin C were determined using *2,4 Dinitrophenylhydrazine*, and total phenolic compounds were determined using *Folin Ciocalteu reagent*. All of parameters were measured by spectrofotometer. The result shows that the highest value of antioxidant capacity was ethanolic extract (18.19%) and the lowest value was acetic acid extract (12.74%). The highest value of vitamin C was aqueous extract (3.49 mg/100g) and the lowest value was acetic acid extract (3.02 mg/100g). The highest value of total phenolic content was aqueous extract (25.49 mg GAE/g) and the lowest value was acetic acid extract (22.74 mg GAE/g). In conclusion, the higher antioxidant capacity was not always followed by the higher of vitamin C and total phenolic compounds.

Key word: *antioxidant capacity, vitamin C, phenolic compounds, extract of frangipani powder*

## INTRODUCTION

Radicals are chemical compounds or atoms with one or more unpaired electrons, and free radicals that have moved out the immediate molecular environment of their generation. There are several free radicals in the body such as superoxide ($O_2$*), hydroxyl radical (OH*), hydrogen peroxide ($H_2O_2$) and nitric oxide radical (NO*).

Free radical can trigger lipid peroxidation, as well as the oxidation of protein and DNA, causing extensive damage to body cells. Oxidative stress resulted from an imbalance of oxidizing species and natural antioxidant in the body has been thought to have contributed to aging, cell apoptosis, and severe diseases such as cancer, Parkinson's disease, Alzheimer's disease, and even cardiovascular

disorders (Halliwell dan Gutteridge, 2000). The antioxidant plays an important role in protection of cell, lipid, protein and DNA against oxidative stress and maintain a balance between the various toxic oxygen species. Antioxidant means 'against oxidation'. An antioxidant is any substance that retard or prevents deterioration, damage or destruction by oxidation. It is a classification of several organic substances including vitamin A, E, and C, and polyphenol such as tannin, flavonoid, ferulic acid, gallic acid, and catechin (Prakash, 2001 ; Kumalaningsih, 2007).

Many studies shows that a high dietary intake of fruits, vegetables, and some spices are supposed to reduce the risk of degenerative deseases. Many fresh fruits and vegetables have been found to contain natural antioxidants, mainly phenolic compounds (ferulic acid, gallic acid, catechins, antocyanin, tannin, and flavonoid), vitamin C, and tocopherol (Gill *et al*., 2002).

Frangipani is one of popular flower in Bali Island. In Bali, especially at spa and beauty salon, and for vegetarian club, frangipani is ussually consumed as Frangipani tea. Frangipani is believed can reduce the fever and diarhea, and also can be used for genital deseasses. Aqueous extract of dried frangipani ($90^oC$) have antioxidant capacity as 7.44% and total phenolic compounds as 18.7 mg GAE/g (Wrasiati *et al*. 2008). Therefore, the deep research on frangipani need to be carried out. This research investigated antioxidant capacity, vitamin C, and total phenolic compounds of Cendana Frangipani powder which was extracted using water (aquadest), ethanol, methanol, and acetic acid.

## MATERIALS AND METHODS

**Materials** : Frangpani flower (*Pumeria alba*), aquadest, acetic acid 50%, ethanol 96%, methanol 80%, cloroform, aseton, hexane, KCl , $H_2SO_4$, $K_2SO_4$, NaOH, folin-ciocalteu phenol, gallic acid monohydrat (standard), DDPH radical scavenging, 2,4-Dinitrophenylhydrazine, meta-phosphoric acid, triclhoroacetic acid, sullfuric acid, and $Na_2CO_3$.

**Methods**

**Extraction**: Frangipani flowers were dried in cabinet drier at $60^oC$ until the water content 8%. After that the dried frangipani was milled become powder. Each five grams of the samples (powder) were extracted in 200 ml of aquadest, 96% ethanol,

80% methanol, and 50% acetic acid, at 28$^{o}$C for 24 h. After filtration, the filtrate of ethanol (ethanol extract) and methanol (methanol extract) were evaporated under vacuum at 45$^{o}$C. The filtrate of aquadest (aqueous extract) and acetic acid (acetic acid extract) were dried in freeze drier at -52$^{o}$C. All extract were stored as powder. The research prosedure was showed in Figure 1.

**Determination of Total Phenolic Compounds** : The content of the total phenolic compounds was evaluated by using Folin-Cocalteu colorimetric method. A total of 0.2-0.4 g of each sample was weighed in a 10 mL flask and filled up with distilled water. The extract was centrifuged (5000 rpm) for 5 min. The 200 µL sample solution was mixedwith 800 µL of $Na_2CO_3$ solution and 1 mL of Folin reagent. The samples stood for 120 min at room temperature before the absorbance was measured at 750 nm. Gallic acid monohydrat was used as a standard, and the result was expressed as gallic acid equivalents (mg GAE/g extract) (Liu *et al*., 2002).

**Determination of Antioxidant Capacity *in vitro*** : It was determined by DPPH method and the results were expressed as percent (%)(Okawa *et al*., 2001). The *in vitro* antioxidant capacity of prepared flower extracts were investigated by DPPH free radical scavenging assay with alittle modifications. The stock solution was prepared by dissolving 24 mg of DPPH with 100mL of methanol and then stored at -20$^{o}$C until needed. The working solution was obtained by mixing 10 mL of the stock solution with 45 mL of methanol to obtain the absorbance of 1.1 ± 0.02 units at 515 nm using spectrofotometer. Frangipani powder extracts (150 µL) were allowed to react with 2.850 µL of the DPPH solution for 1 h in the dark. Then the absorbance was taken at 515 nm. The antoixidant capacity was calculated as decrease in absorbance value using the formula :

$$(\%) = (Ao - A1/Ao) \times 100\%$$

Where, Ao is absorbance of the control (without sample) and A1 is the absorbance of mixture containing the sample.

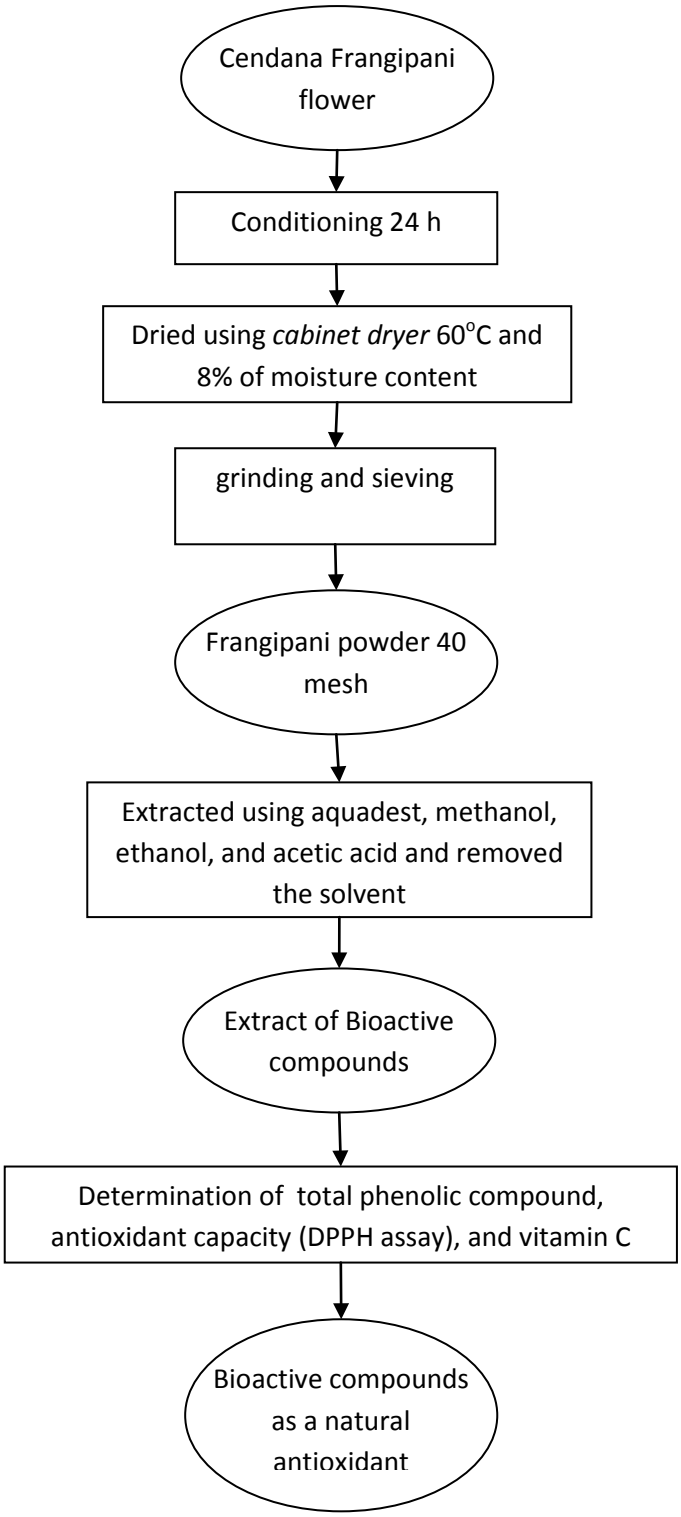



Figure 1

The procedure used in the study

**Determination of Vitamin C** : The vitamin C content was analyzed photometrically after oxidation (catalyzed by copper ions) of ascorbic acid to dehydroascorbic acid, which reacts with 2,4-dinitrophenylhydrazine to form a red complex. The absorbance was measured at 520 nm. The 0.3-0.6 g sample was mixed with 5 mL 0f meta-phosphoric acid and vortexede for 1 min. After centrifugation of the mixture, the liquid layer was transferred into a flask. This extraction was repeater 4 times for nonliquid samples. A total of 200 µL was mixed with 300 µL of trichloroacetic acid and centrifuged at 12000 rpm for 5 min. Afterward 100 µL of 2,4—dinitrophenylhydrazine reagent was added and then mixed. The solution was heated (60$^{o}$C) and shaken for 1 h. The sample was cooled for 5 min on ice and added 400 µL sulfuric acid. The samples were kept in the dark for 20 min and the absorbance was measured at 520 nm. Ascorbic acid solution was used for calibration.

## RESULTS

Fresh Frangipani, dried frangipani, frangipani powder and the extract of frangipani powder were presented in Figure 2. The dried extract of frangipani powder was presented in Figure 3. The total phenolic compounds, vitamin C, and antioxidant capacity of frangipani flower extract were presented in Table 1.

Figure 2
(I) Fresh Flower, (II) Dried Flower, (III) Frangipani Powder, (IV) The extract

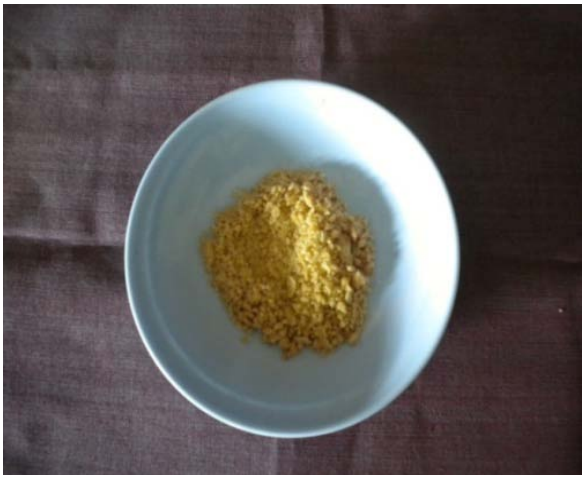


Figure 3
Dried extract of frangipani powder

Table1
Total phenolic compounds and antioxidant capacity of frangipani flower extract

| No | Parameters | Aqueous extract | Ethanol extract | Acetic acid extract | Methanol extract |
|---|---|---|---|---|---|
| 1 | Antioxidant capacity (%) | 17.05 | 18.19 | 13.84 | 17.91 |
| 2 | Vitamin C (%) | 3,49 | 3,08 | 3,13 | 3,02 |
| 3 | Total phenoic compounds (mg GAE/g) | 25.49 | 24.50 | 23.74 | 24.59 |

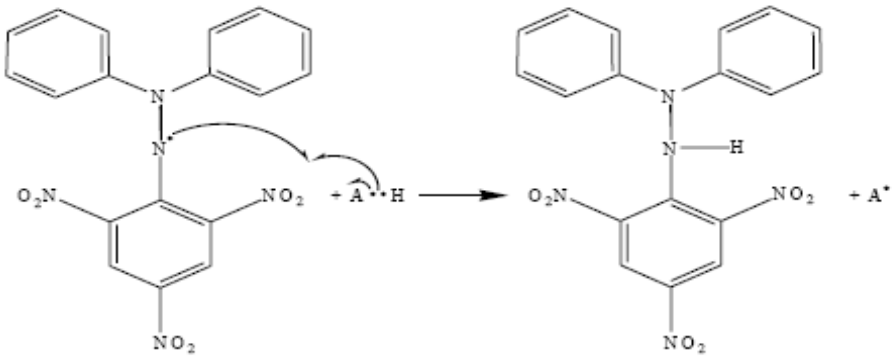
The solvent which was used to extract the frangipani powder were polar protic solvent, because they have O-H bound. In the present study, the extracts were prepared using 96% ethanol, 80% methanol, 50% acetic acid, and aquadest. Aquadest has the highest dielectric constant (80), followed by methanol (33), ethanol (24), and acetic acid (6.2). The dielectric constant shows polarity of the solvent. Aquadest has the highest polarity and acetic acid has the lowest polarity.

Ethanol extract has the highest antioxidant capacity and acetic acid extract has the lowest antioxidant capacity. Antioxidant capacity of ethanol extract as 18.19% and antioxidant capacity of acetic acid as 13.84%. Aqueous extract has the highest total phenolic compounds (25.49 mg GAE/g) and vitamin C (3.49%). Acetic acid extract has the lowest phenolic compounds (23.74 mg GAE/g) and vitamin C (2.09%)

## DISCUSSION

### Antioxidant capacity

DPPH is free radical compound and has been widely used to test the free radical-scavenging ability of various samples. It is a stable free radical with a characteristic absorption at 515-517 nm (Kubola and Siriamornpun, 2008). In the present study, it was used to study the radical-scavenging effects of frangipani powder extract. As antioxidants donate protons to this radical, the absorption decreases. The mechanism was presented in Figure 4.



radical DPPH + Antioxidant stable DPPH + Antioxidant

Figure 4
Mechanism of free radical-scavenging of DPPH (Huang *et al*., 2005)

Ethanol extract has the highest antioxidant capacity and acetic acid extract has the lowest antioxidant capacity. The antioxidant capacities of each extract may depend on structural factors such as the number of phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, and free carboxylic group (Bhuiyan *et al*., 2009). Ethanol and methanol have ability to degrade the cell wall and to solve bioactive compounds of the plant. Bhuiyan *et al*. (2009) reported the antioxidant capacity of ethanolic and methanolic extract of *Zizyphus mauritiana* were higher than the aqueous extract. Voigt (1995) stated that the ability of solvent to extract the cell content is influenced by their activity to loosing the cellulose cell wall and to solving the active components of cell content. Besides, the polarity similarity of solvent with

the component to be solved is also influencing the ability of solvent to extract the cell content.

**Total Phenolic Compound**

Phenolic compounds are secondary metabolites which synthesize in plants. They posses biological properties such as antioxidant, anti-aging, anti-carcinogen, anti-inflammation, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities. Many researchers reported that many medicinal plants contain a large amount of polyphenol, in which those polyphenol influencing the antioxidant effect (Prakash, 2001; Kumalaningsih, 2006)

The aaqueous extract has the highest total phenolic compounds (25.49 mg GAE/g) and acetic acid extract has the lowest phenolic compounds (23.74 mg GAE/g). Water has ability to solve phenolic compounds such as tannin, antocyanin and antoxantin. Cowan (1999) stated that tannin and antocyanin are solved in water. The results of this research agree with Hodzics *et al.* (2009) who is stated that the extraction of cereal such as oat, barley, corn, and wheat by water at 40$^{o}$C was resulting in a high total phenol, because those cereals had a high content of tannin. The highest total content of phenol of aqueous extract was not always followed by the highest antioxidant capacity, because not all of the phenol compound had antioxidant activity. According to Okawa *et al*. (2001) that generally the compound that has the antioxidant activity is the phenol compounds which have hydroxyl group on ortho and para position of -OH and -OR group.

**Vitamin C**

Vitamin C (ascorbic acid, ascorbate) is a water-soluble vitamin found widely in plant. Fruit and vegetable consist a lot of vitamin C. It is synthesized by plant from several precursors and is abundant in leaves and, in particular, the chloroplast. It may play a role in photosynthesis, stress resistance, and plant growth and development (Padayatty *et al.*, 2002 *in* Cadenas and Packer, 2002). Vitamin C is a six-carbon lactones that is synthesized from glucose in the liver of most mammalian species, but not by human, non-human primates and guinea pigs. These species do not have the enzyme gulonolactone oxides, which is essential for synthesis the

ascorbic acid from glucose (Padayatty *et al*., 2003). Therefore, human must supply vitamin C from fruit, vegetables, flower and others part of plants (Carr and Frei, 2002 *in* Cadenas and Packer, 2002).

The water extract of frangipani powder has the highest vitamin C. Because vitamin C is too polar, so it is tent to solve easier in the water compared with in others solvent such as acetic acid, methanol and ethanol. This is supported by Amrun and Umiyah (2005), who is reported that the vitamin C of Kenitu fruits (*Chrysophyllum cainito* L.) was tent to part more in water compared with in methanol. So, the antioxidant capacity in water extract was determined by the vitamin C content of the fruits.

## CONCLUSION

Ethanol extract has the highest antioxidant capacity and acetic acid extract has the lowest antioxidant capacity. Antioxidant capacity of ethanol extract as 18.19% and antioxidant capacity of acetic acid as 13.84%. Aqueous extract has the highest total phenolic compounds (25.49 mg GAE/g) and vitamin C (3.49%). Acetic acid extract has the lowest phenolic compounds (23.74 mg GAE/g) and vitamin C (2.09%). The highest total content of phenol of aqueous extract was not always followed by the highest antioxidant capacity, because not all of the phenol compound had antioxidant activity.

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