Antioxidant activity of loloh Malaka fruit (*Phyllanthus emblica L.*) in Ayurveda Medication: How it supports environmental conservation

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Abstract. Traditional medication method in Bali use the Ayurveda principle known as Usadha. Usadha used herb or fruit as main component of medicine. One of them is Malaka fruit (*Phyllanthus emblica L.*) and commonly in Bali known as kalimaka, kalimoko, kamlika. Malaka can be processed through boiling using water and consumed as traditional drink (loloh). Malaka fruit has been known to contain vitamin C as antioxidant. Therefore, this research aims to determinent the antioxidant activity of malaka fruit loloh using DPPH (2,2-difenil-1-pikrilhidrazil). The result showed that malaka fruit loloh obtained was antioxidant activity with antioxidant capacity 164 mg/ 100 mL and IC50 691,1 µg/Ml. Further contains vitamin C obtained was 0,184 mg/100mL, Flavonoid was 3,15 mg/100mL, phenol was 375 mg/100mL and tannin was 546 mg/mL. Based on these result malaka fruit loloh can be use as on alternative for traditional medication.

1. Introduction

In everyday life, we cannot be free from free radical compounds. Cigarette smoke, fried foods, grilled foods, excessive sun exposure, motor vehicle emission, synthetic drugs, toxins and air pollution are some of the sources of free radical compounds in the body. The increasing number of free radicals in the body can trigger the emergence of degenerative diseases such as cancer, diabetes, inflammation and cardiovascular disease [1].

To protect ourselves from free radicals, the body produces anti-free radical compounds or also be called as antioxidants. Antioxidants are compounds in small amounts compared to substrates that can delay or prevent the occurrence of free radical reactions. Antioxidants are naturally produced in the body, but the amount is very limited to compete with free radicals produced by the body itself. Therefore, it is necessary to have antioxidants from outside the body. The use of synthetic antioxidants has high effectiveness but is not safe for health. Currently the use of synthetic antioxidants has been limited because it turns out that the results of research that has been done that antioxidants such as BHT (Butylated Hydroxy Toluent) can poison toward experimental animals and are carcinogenic. Therefore, the food and medicine industry has switched to developing antioxidants derived from nature [2].

Ayurveda is a knowledge of eastern medicine that is better known from India. Ayurvedic comes from the word Ayu (ayur, ayuh, ayus) and vida. Ayu, Ayus means living a healthy and longevity. Vida, vid means knowing, knowledge. In Balinese society, the principle of traditional Balinese medicine uses the Ayurveda principle which in Bali is known as usadha. Usadha is a treatment using natural ingredients.
such as animals, minerals and plants. Treatment with natural ingredients such as plants can be processed and mixed up so that they can be drunk as loloh. Loloh is one form of traditional Balinese drinks which in Indonesian is interpreted as herbal medicine. One of the plants that can be used is Malaka (Phyllanthus emblica L). In India, Malaka fruit is one of the most important elements in Ayurvedic medicine. Malaka fruit contains flavonoids, phenols and vitamin C which have the potential as antioxidants [3]. Based on research, phenolic group compounds show patent antioxidant activity. The correlation between levels of phenolic compounds or flavonoids with antioxidant activity using DPPH method is very high. According to Ayurveda, Malaka fruit can balance the three elements of tridosha because it contains 6 flavors (sad taste) which are sweet, bitter, spicy, salty, sour and sour. Malaka as a rasayana fruit (rejuvenation) is one of the fruits for care so that it is youthful and longevity. Some types of health problems can be overcome by using Malaka fruits such as to improve digestion, treat constipation, reduce fever, cleanse the blood, reduce cough, relieve asthma, treat myopic eyes, increase stamina and increase intelligence [4].

The use of Malaka as a traditional medicine has not been widely used by the society because until now there has been no scientific information or research that clearly states that Malaka fruit has antioxidant power to reduce free radicals. In order to provide more evidence of the benefits, in this study toward antioxidant activity of loloh Malaka fruit (Phyllanthus emblica L) was carried out using DPPH method (1.1 Diphenyl-2-pikrilhidrazil). DPPH method provides sample reactivity information tested with a stable radical. DPPH provides strong absorption at a wavelength of 517 nm in dark purple. Free radicals cause colors that are proportional to the number of electrons taken. While the selection of the method of extraction with the form of loloh because of the tendency of Balinese people now consume traditional drinks like loloh.

2. Material and method

2.1. Research design
This research uses two research designs, namely: descriptive explorative and experimental. Descriptive explorative research included extraction and identification of compound contents while the experimental research used laboratories in vitro which aimed to test the free antiradical activity of DPPH as the antioxidant capacity of the loloh Malaka fruit (Phyllanthus emblica L.) from Datah Village, Abang District, Karangasem Regency, Bali.

2.2. Research material
The chemicals used in this study were aquadest, 10% NaOH, concentrated sulfuric acid, 2 N hydrochloric acid, benzene, Liebermann-Burchard reagent, Dragendorf reagent, Meyer reagent, sodium chloride, 95% ethanol, DPPH, Iodine solution 0, 01 N, 1% starch, quaresetin, AlCl2 solution, 50% ethanol, Follin-Ciocalteu reagent, 5% Na2CO3 solution, Follin-Denish Reagent and Gallic acid.

2.3. Research instrument
The instruments used in this research were knives, blenders, glass beaker, analytical balance, Erlenmeyer, sample bottles, infusion pans, funnels, filter paper, flannel, jars, 10 mL and 100 mL flask, drop pipette, volume pipette, vortex, centrifuge, micro pipette, test tube, rotary vacuum evaporator, aluminum foil, UV-Vis spectrophotometer and a set of titration devices.

2.4. Working procedure

2.4.1. Material preparation. Fresh fruit of Malaka (Phyllanthus emblica L.) is collected and cleaned of sticky dirt, washed with running water after it has been extracted to become loloh.

2.4.2. Manufacturing of loloh Malaka fruit. Loloh malaka fruit was made of 40% content by weighing 40 grams of fresh Malaka then giving 50 mL of warm water, then blending. Malaka fruit extract obtained
is then filtered. The filtrate obtained is then measured in volume until it has a concentration of 40%. The filtrate obtained is called the loloh Malaka fruit. Furthermore, loloh Malaka fruit was tested quantitatively by vitamin C test, flavonoid test, phenol test and tannin test while the antioxidant activity test was carried out by testing the antioxidant capacity and IC50% test.

2.4.3. Quantitative test of antioxidant activity

a) Measurement of Vitamin C [5]. As much as 1 mL (1 gr) Malaka and put into Erlenmeyer. Then 1 mL of 1% starch solution is added. The sample is then shaken until it is homogeneously mixed. Then titrated with 0.01N iodine solution, from clear to constant blue.

b) Measurement of total Flavonoids [6]. Quasarin standard solution was made as much as 0.5 mL with a concentration of 0.00 ppm; 2.00 ppm; 4.00 ppm; 6.00 ppm, 8.00 ppm; and 10 ppm taken from 100 ppm quasarin stock solution (0.01 g quasarin dissolved in 100 mL of distilled water). The sample of loloh Malaka fruit (Phyllanthus emblica L.) was prepared by weighing 0.1 g of the sample of loloh Malaka which was distorted and filtered. Analysis of total flavonoid content was carried out by preparing a sample of standard solution of quasarin with various variations and samples of loloh fruit of Malaka (Phyllanthus emblica L.). Then 2 mL of each quasarin standard dilution series and 2 mL of each filtrate were added 2 mL of AlCl2 solution. The sample was cortexed and incubated for 30 minutes then absorbance was measured at a wavelength of 415 nm.

c) Measurement of total Phenol [5]. A standard solution of gallic acid 0.5 mL was made with a concentration of 0.00 ppm; 10.00 ppm; 20.00 ppm; 40.00 ppm; and 80.00 ppm taken from 100 ppm gallic acid stock solution (0.01 g of gallic acid dissolved in 100 mL of distilled water). The sample loloh Malaka fruit (Phyllanthus emblica L.) was prepared by being treated the same as weighing 0.1 g of the sample, then divorxerted and filtered, the filtrate was collected. Analysis of total phenol content was carried out by preparing a sample of standard gallic acid solution with various variations of 0.5 each series of standard dilution of gallic acid put into a test tube. For the sample filtrate loloh Malaka fruit (Phyllanthus emblica L.) pipetted as much as 0.05 mL added 0.45 mL of 85% methanol so that all samples and standard solutions of total gallic acid were 0.5 mL each. All samples were added with 0.5 mL of Folin-Ciocalteu reagent, vortexed and allowed to stand for five minutes. Then 4 mL of 5% Na2CO3. Divorxeted again and allowed to stand for 30 minutes then the absorbance is measured at a wavelength of 760 nm.

d) Determination of Tannin Degree [5]. A standard solution of 0.5 mL of tannic acid was made with a concentration of 0.00 ppm; 2.00 ppm; 4.00 ppm; 6.00 ppm, 8.00 ppm; and 10 ppm taken from a 100ppm quercetin stock solution (0.01 g of tannic acid dissolved in 100 mL of distilled water). The sample loloh Malaka fruit (Phyllanthus emblica L.) was prepared by treating the same as weighing 0.1 g of sample. Analysis of total tannin content was carried out by preparing a sample of a standard solution of tannic acid with various variations, samples of loloh Malaka fruit (Phyllanthus emblica L.). Each series of standard dilutions of gallic acid is put into a test tube. For filtrate samples of loloh Malaka fruit (Phyllanthus emblica L.) pipetted as much as 0.01 mL added 0.49 mL of distilled water so that all samples and the total standard solution of tannic acid were 0.5 mL each. All samples were added with 0.5 mL of Folin-Denis reagent, distorted and allowed to stand for five minutes. Then add 4 mL of 5% Na2CO3. Divorxed again and allowed to stand for 30 minutes then the absorbance is measured at a wavelength of 725 nm.

e) Determination of antioxidant capacity [6]. A standard solution of gallic acid 0.5 mL was made with a concentration of 0.00 ppm; 2.5 ppm; 5.0 ppm; 7.5 ppm; and 10.0 ppm taken from a 100
ppm gallic acid stock solution (0.01 g of gallic acid dissolved in 100 mL of distilled water). The sample loloh of Malaka fruit (Phyllanthus emblica L.) was prepared by being treated equally by weighing 0.1 g of the sample, then homogenized and centrifuged at 3000 rpm for 15 minutes. (Phyllantus emblica L.). Each series of standard dilutions of gallic acid is put into a test tube. For filtrate samples of loloh Malaka fruit (Phyllanthus emblica L.) pipetted as much as 0.1 mL added 0.4 mL of distilled water so that all samples and the total standard solution of tannic acid were 0.5 mL each. All samples added 3.5 mL 0.1 mM DPPH, homogenized, and incubated 30 minutes then absorbed at a wavelength of 517 nm.

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\text{Antioxidant Capacity} = \frac{ppm(x) \times \text{Total volume} \times Fp \times 100\%}{\text{massa sampel (mg)}} \tag{1}
\]

Regression: \(y = ax + b\)

Explanation:
\(X = \text{sampel concentration}\)
\(FP = \text{dilution factor}\)

\(f)\) Determination of IC\(_{50}\) grade [6]. A sample of 0.5 mL loloh Malaka fruit (Phyllanthus emblica L.) sample solution was made with a concentration of 0.00 ppm; ppm; 0.25 ppm; 0.50 ppm; 0.75 ppm; and 1.00 ppm. Each sample of pipette as much as 0.1 mL added 0.4 mL of distilled water, then added 3.5 mL of 0.1 mM DPPH, homogenized, and incubated 30 minutes then absorbed at a wavelength of 517 nm.

\[
\% IC = \frac{\text{abs. kontrol} - \text{abs. sampel}}{\text{abs kontrol}} \times 100\% \tag{2}
\]

3. Results and discussion
Based on the results of the research, it was found that Loloh Malaka fruit was white and cloudy with homogeneity. The cloudy white color produced is extraction from fresh Malaka juice mixed with warm water solvent. The use of warm water serves to reduce the number of contaminants contained in water. Where the water used is boiling water and left until the temperature is 400C. The use of warm water to boiling can accelerate the extraction process and attract more active compounds contained in Malaka fruit (Phyllanthus emblica L). The simple screening process using gauze caused the loloh of Malaka (Phyllanthus emblica L.) fruit to be homogeneous with a small amount of sediment at the bottom of the sample loloh of Malaka fruit. Picture of loloh Malaka fruit as shown below.

![Figure 1. Loloh malaka fruit (Phyllanthus emblica L.)](image)

Quantitative test of loloh of Malaka fruit (Phyllanthus emblica L.) includes checking parameters of vitamin C levels, flavonoids, phenols, tannins, antioxidant capacity, and IC\(_{50}\)%. The results of the analysis are presented in the table 1.
Table 1. Test results of vitamin C levels, flavonoids, phenols, tannins, antioxidant capacity, and IC50% loloh of malaka fruits (*Phyllanthus emblica* L.).

| No | Parameter Test | Amount     | Sampel loloh Malaka Fruit (*Phyllanthus emblica* L.) |
|----|----------------|------------|------------------------------------------------------|
| 1  | Vitamin C      | mg/100 mL  | 0.184                                                |
| 2  | Flavonoid      | mg/100 mL  | 3.15                                                 |
| 3  | Phenol         | mg/100 mL  | 375                                                  |
| 4  | Tannin         | mg/100 mL  | 546                                                  |
| 5  | Antioxidant Cap.| mg/100 mL  | 164                                                  |
| 6  | IC50%          | µg/mL      | 691.1                                                |

3.1. Vitamin C degree of loloh Malaka fruit (*Phyllanthus emblica* L.)

In determining vitamin C levels of loloh Malaka fruit (*Phyllanthus emblica* L.) was obtained at 0.1848 mg / 100 mL. This shows low levels of vitamin C obtained. Vitamin C is ascorbic acid, a chemical compound that dissolves in water. Vitamin C has many benefits in the body, so vitamin C supplements are found on the market. However, without supplementation we can also meet the need for vitamin C by consuming fruits. The content of vitamin C in fruits or vegetables is not as high as vitamin C in tablets. But when viewed from the need for vitamin C 100 mg / day, consumption of fruit and vegetables is sufficient to meet the needs of vitamin C. Plus in many fruits there are also other substances that are also useful for the body. So that it can also meet the needs of substances other than vitamin C.

Vitamin C or ascorbic acid can be found in plant tissues. This vitamin has an important role for cellular metabolism. As one of the antioxidant compounds, vitamin C is able to eliminate ROS (radical oxygen species) like O$_2^-$, H$_2$O$_2$, HO, ONOO$^-$. The effectiveness of flavonoids in counteracting radical DPPH largely depends on structure, hydrophobicity, biological activity, and also oxidative activity. The ability to terminate the radical chain reaction by flavonoids depends on the presence of the o-hydroxyl group on the B ring. This allows the formation of intramolecular hydrogen bonds between the hydroxyl groups that increase the stability of the phenoxy radical.

3.2. Flavonoid degree of Loloh Malaka fruit (*Phyllanthus emblica* L.)

Based on the table above the levels of flavonoids in loloh Malaka fruit (*Phyllanthus emblica* L.) are 3.15 mg /100 mL. Components of flavonoids and phenol polyphenols in plants are known to have multifunctional properties as reducing agents, donating hydrogen atoms as antioxidants and reducing the formation of oxygen singlets [7].

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3.3. Phenol degree of Loloh Malaka fruit (*Phyllanthus emblica* L.)

Based on analysis of phenol levels of loloh Malaka fruit (*Phyllanthus emblica* L.) was obtained at 375 mg / 100 mL. Polyphenol components in plants are known to have multifunctional properties as reducing agents, donating hydrogen atoms as antioxidants and reducing the formation of oxygen singlets. Determination of total phenol levels in this study using Follin Chiocalteu reagent. This method is based on the strength of reducing the phenolic hydroxy group. All phenolic compounds including simple phenols can react with Follin Chiocalteu reagents. The existence of an aromatic nucleus on phenol compounds can reduce phospholibidad phosphotungstate to blue molybdenum. According to Bettuzi, et al., this compound is a group of polyphenols that have strong antioxidant activity [8].

3.4. Tannin degree of Loloh Malaka fruit (*Phyllanthus emblica* L.)

In determining the tannin content of loloh Malaka fruit (*Phyllanthus emblica* L.) fruit was obtained at 546 mg / 100 mL High and low phenolic concentrations in the sample one of them is caused by the selection of methods in extracting the sample. The tannin content of the Malaka fruit causes this fruit to have a bitter and bitter taste. Bitter and bitter taste is produced by tannin content found in the sample loloh. The tannin content of loloh malaka fruit causes astringent taste. In Ayurveda astringent taste can relieve the element kapha (water) in the body.
In terms of nutrition, tannins have good and bad nutritional effects. Excessive consumption of tannin is toxic to health. This is supported by research conducted by Irene Mueller in 2007 that high levels of tannin have a negative effect on animal nutrition and health because it is toxic. In addition, an increase in tannin levels will also cause a decrease in protease enzymes in converting proteins to amino acids and can bind cellulose, pectin and vitamin B12. Therefore, further tests need to be carried out to determine the effectiveness of tannin accordingly so as to obtain a good tannin effect if consumed properly.

3.5. Antioxidant Capacity of Loloh Malaka fruit (Philanthus emblica L.)

In this research, antioxidant measurements were carried out using DPPH method which was marked by changes in color from purple to yellow after incubation for 30 minutes. The purpose of incubation is to accelerate the reaction between DPPH radicals and samples that act as antioxidants. Based on the results of the analysis, the value of antioxidant capacity was 164 mg / 100mL. Factors that influence the increase in antioxidant capacity are concentration, temperature and heating time. This is supported by Yuliarti's research that the antioxidant activity of Tempuyung leaves extracted with ethanol is increasing. In this study, water was used as a solvent in the making of loloh fruit Malaka. This shows that the antioxidant capacity of Malaka fruit has not reached the optimal point.

The antioxidant capacity of an ingredient is affected by the components in the material that are able to move to inhibit oxidation. Components of antioxidants include phenol compounds and vitamin C. The selection of the extraction process and the solvents used are determinants of the high and low antioxidant capacity in the sample. The type and polarity of extraction solvents can affect single electron transfer and hydrogen atom transfer which is a key aspect in testing antioxidant activity. The results showed a comparable correlation between the level of secondary metabolite compounds and the form of bioactive components to an antioxidant activity from the samples of plants analyzed.

3.6. IC 50 Grade Loloh Malaka fruit (Philanthus emblica L.)

Based on the results of the research obtained, the IC50 value for loloh malaka fruit was 691.1 µg / mL. Specifically, an ingredient is said to have antioxidant properties if it has an IC value of 50% ranging from 200-1000 µg / mL so that with a value of 691.1 ppm, loloh fruit malaka has the potential as an antioxidant. The presence of phenol and vitamin C states that in loloh the fruit of Malaka (Philanthus emblica L.) in the ingredients or samples is able to move to inhibit oxidation. Because the antioxidant capacity of an ingredient depends on the antioxidant component, namely phenol and vitamin C compounds. Curcuma loloh malaka fruit (Philanthus emblica L.) can be seen in Figure 2.

![Figure 2. Curve of IC50 Loloh Malaka fruit.](image-url)
counteract free radicals [14]. This proves that the natural ingredients of some plants that are around us have antioxidant potential which is good for health. The potential of these antioxidants if processed in the right way and concentration is more profitable than consuming food with the addition of dangerous synthetic antioxidants.

4. Conclusion
Based on the results of the research that has been done, the following conclusions are obtained:

- Malaka (*Phyllanthus emblica* L.) fruit has antioxidant activity with an antioxidant capacity of 164 mg / 100 mL with IC50 691.1 µg / mL so that it has the potential as a natural antioxidant.

- Parameters test to support the antioxidant activity of *lohol* Malaka (*Phyllanthus emblica* L.) gave a specific value that is vitamin C content of 0.1848 mg / 100 mL, flavonoids 3.15 mg / 100 mL, phenol 375mg / 100 mL, and 546 mg / 100 mL tannin.

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