# Antioxidant Activity Comparison between Young and Old Malaka Fruit (*Phyllantus emblica L.*) Extracts from Bandung, Indonesia

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***Abstract***
*Phyllantus emblica L. is a native plant from Indonesia which has been shown to have biological activty as antioxidant. The maturity of a plant could affect the different types and quantities of secondary metabolites and cause differences of biological activities. The objective of this research was to compare the antioxidant activity of young and old P. emblica fruit extracts from Bandung-Indonesia. Extraction was performed using macerator with different polarity of solvents as follow: n-hexane, ethyl acetate and ethanol. The extracts were vaporated using rotavapor. The antioxidant activity was tested using DPPH assay and monitored by TLC in order to find out the existing compounds which were responsible for antioxidant activity.*

*The results showed that the extracts from P. emblica performed antioxidant activity with $IC_{50}$ values of ≤50 μg/mL. Therefore, they were classified as very strong antioxidant. $IC_{50}$ value of young P. emblica fruit extract showed the smaller value than $IC_{50}$ value of old P. emblica fruit extract. The strongest antioxidant activty of all P.emblica extracts was showed by ethyl acetate extract from young P. emblica and it was shown by fenol substances. As conclusion, the young P. emblica fruit extract had stronger antioxidant acivity than the old P. emblica fruit extract.*

**Keywords:** Young and old fruit, *Phyllantus emblica* L., antioxidant, DPPH assay.

## Introduction

In the world of pharmacy, medicinal plants are used as raw material for medicine. Many of the researches provide biological activities information from various plants. Biological activity of a plant could be due to the presence of secondary metabolites in that medicinal plant[1]. Secondary metabolites in medicinal plant such as phenols and flavonoids are often referred to as bioactive components for various biological activities[2].

Flavonoids were widely used for some biological activities such as antioxidant[3]. Different types of phenol compounds could cause differences of antioxidant potency and antibacterial strength[4].

Type and quantity of secondary metabolite in medicinal plant could cause differences in the strenght and variety of biology activity potency[1]. Physiological processes in a plant, the maturity part of plant and environmental conditions[5] such as sunlight condition, air pressure and temperature could be factors to different types and quantity secondary metabolites[6,7].

*Phyllantus emblica* L. is one of the main species of plants that grow in various countries including Indonesia (West Java). *Phyllantus emblica* L. has a variety of biological activities such as antibacterial against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*[8]; antifungal against *Aspergillus Niger*, *Candida albicans*, *Penicillium nasturtium*[9]; inflammatory[10]; antidiarrheal[11-13]; antioxidant caused by phenolic groups[12] and antioxidants for anticancer[14]. However, the biology activity for antioxidants from maturity difference of *Phyllantus emblica* L. fruit had not been reported. According to Hasan et al[15], tannin and phenolic group were seconday metabolites in *P. emblica* fruit and could be responsible for antioxidant activity.

## Material and Methods

**Materials:** DPPH (2,2-diphenyl-1-picrylhydrazyl) and ascorbic acid were purchased from Sigma-Aldrich (MO, USA), young and old fruit of *P. emblica*, methanol P.a, ethanol, ethyl acetate, n-hexane and others analytical materials were used in this study.

### Methods

**Sample preparation**: *P. emblica* of young and old fruit were freshly collected from Bale Endah-Bandung, West Java-Indonesia on December 2016. *P.emblica* fruits were sorted, washed, dried at 40°C - 45°C and grinded into powder form.

**Extraction**: *P. emblica* of young and old fruit were extracted by using maserator with different polarity of solvent which were n-hexane, ethyl acetate and ethanol. Each sample was extracted with n-hexane in three repetitions, then the residue was extracted with ethyl acetat in three times repetition and the end residue was extracted with ethanol in three times repetition. Each extract was concentrated using rotary vaporator and resulted into thick n-hexane extract of young fruit *P.emblica* (YN), ethyl acetate extract (YE) and ethanol extract (YL), thick n-hexane extract of old fruit P.emblica (ON), ethyl acetate extracts (OE) and ethanol extract (OL).

**Monitoring of secondary metabolites**: Monitoring of secondary metabolites was performed against simplicia and fruit extract of *P.emblica* (YH, YE, YL, OH, OE and OL). A phenolic compound was identified by using $FeCl_3$ 10% reagent, a flavonoid using the amyl alcohol reagent, a tannin using gelatin, an alkaloid using Dragendorf and Mayer reagents, quinones using KOH 5%, saponins showing with a constant foam ± 10 minutes in water extracts, monoterpen and seskuiterpen using 10% solution of vanillin in $H_2SO_4$, steroid and triterpenoid using Lieberman-Burchard reagent[16].

**Antioxidant activity using the Blois method**: Antioxidant activity was adopted from the Blois methode[17]. DPPH solution of 50 ppm was used as control and ascorbic acid was used as antioxidant standard. Sample was prepared in various concentrations, then added with 50 ppm of DPPH (volume 1:1) and then incubated for 30 minutes. After incubation for 30 minutes, the absorbance was measured at λ 517 nm using a UV-visible spectrophotometry. Methanol was used as blank. Antioxidant activity was measured as a percentage of the sample against DPPH decrease absorbance. $IC_{50}$ of scavenge to DPPH was determined using the calibration curve of the antioxidant activity of samples in various concentrations.

**Monitoring of antioxidant compound in extract using TLC**: Monitoring of antioxidant compound was performed against extract which had the smallest value of $IC_{50}$. Admixture of ethyl acetate and n-hexane were used as eluents. $FeCl_3$ 1% was used for detecting the existence of phenolic compounds and citroboric 1% was used for detecting the existence of flavonoid compounds.

## Results and Discussion

**Extraction**: Each sample had differences of extract content per gram of simplicia and organoleptic by colors. Extract content per gram of *P.emblica* fruit simplisia was shown in fig. 1. Extract content of YE was the highest. It meant that ethyl acetat was the best solvent between n-hexane and ethanol for getting extract content of young *P.emblica* fruit. The organoleptic result showed green color for YN and ON, brown to dark for YE and OE and dark red for YL and OL.

**Monitoring of secondary metabolite**: A secondary metabolite of YL and OL is differences by tannin. Chemical compound of *P. Emblica* extract had been widely reported including tannin and phenol compounds. The production of secondary metabolites could be affected by the maturity of the plant[18]. The results showed that young and old fruits of *P. emblica* had some chemical compounds of flavonoid, phenolic, quinone and saponin. The differences in chemical compounds were indicated by the presence of tannin in young fruit of *P.emblica* but not in old fruit of *P.emblica* like that shown by YL.

**Antioxidant activity and $IC_{50}$ to scavenging of DPPH**: Antioxidant activity of a sample is shown by percentage of sample in 50 ppm to scavenging of DPPH. The antioxidant activity test was performed using Blois[17] method with DPPH. DPPH is a relatively stable free radical[19]. The DPPH solution is purple and shows absorption at 515-520 nm wavelength[20]. In case of scavenging of DPPH from the sample it will show a color change[21]. Antioxidant activity of fruit extract *P.emblica* from Bandung had differences which showed in range 54-94% for young fruit extract of *P.emblica* and 52-93% for old fruit extract of *P.emblica*.

Antioxidant activity of young fruit extract of *P.emblica* from Bandung was higher than old fruit extract of *P.emblica*. YE was the highest antioxidant activity when compared to YN, YL, ON, OE and OL. This result suggested that antioxidant compound in YE had the higher potency to scavenge the free radical DPPH. The previous reserach by Sumalatha[22] showed that antioxidant activity with scavenging of DPPH on ethanol extract fruit of *P.emblica* was 71,75%, water extract fruit of *P.emblica* showed $IC_{50}$ value to scavengging of DPPH as 51,3 μg/mL[23] and percentage of scavengging free radical DPPH at 100 μg concentration of extract leaves *P.emblica* was 82.053%[24]. The maturity part of plant could influence the type and quantity of secondary metabolite[1]. The type and quantity of secondary metabolite could influence the biology activity of medicinal plant.

$IC_{50}$ to scavenging of DPPH from each extract could be seen in fig. 3. $IC_{50}$ to scavenging of DPPH from young fruit extract of *P.emblica* had the smallest value than the old one. It means that a young fruit extract of *P.emblica* had the highest antioxidant activity than the old fruit extract. As we know that $IC_{50}$ is the concentration of sample which can reduce DPPH by 50%. $IC_{50}$ of all sample was compared with the standard of ascorbic acid. The smallest of $IC_{50}$ showed the highest antioxidant activity.

Antioxidant activity can be classified when showed by $IC_{50}$ value. According to Blois[17], sample which had $IC_{50}$ lower than 50 μg/mL was a very strong antioxidant. $IC_{50}$ value is the concentration of samples that can scavenge 50% of free radical DPPH activity. The highest antioxidant activity was indicated by the lowest value of $IC_{50}$. $IC_{50}$ of DPPH scavenging capacities of YN, YE, YL, ON, OE and OL were compared to $IC_{50}$ of ascorbic acid as a standard. As a result, YE had the lowest value of $IC_{50}$ compared to YN, YL, ON, OE and OL. ON had the highest value of $IC_{50}$ compared to YN, YE, YO, OE and OL. All extracts had a value of $IC_{50}$ lower than 50 μg/mL, while $IC_{50}$ DPPH of ascorbic acid was 2.468 μg/mL. This result suggested that all fruit extracts of *P.emblica* from Bale Endah-Bandung could be classified as a very strong potential antioxidant.

**Monitoring of antioxidant compounds**: Monitoring of antioxidant compound was performed on selected extract i.e. YE. It is based on the lowest $IC_{50}$ value which was

compared to other extract. Spot of YE which was positive by spraying of 0.2% DPPH was the same spot which was positive on phenolic compound. It was shown in fig. 4.

Antioxidant activity of extract can be present in suspected compounds that are capable in donating hydrogen on free radicals. Hydrogen donors could be groups of phenolic, flavonoid and carotenoid.[3,25] Phenolic and flavonoid compounds had the biggest group as compounds which have antioxidant activity in the medicinal plants[26]. Phenolic and flavonoid compounds were very soluble in polar solvents. Acidic compound of cinamic acid and benzoic acid contributed to the antioxidant activiy. Cinnamic acid had the higher antioxidan activity than benzoic acid[27,28]. Flavonoid compound in extract had a role to antioxidant activity. Position of hydroxyl group at C-3 and ortho position at C-3' and C-4' would increase the antioxidant activity in extract[28,29] and also with double bonds between C-2 and C-3[28,29].

Beside phenolic and flavonoid compound, carrotenoid compound in extract was also determined in improving antioxidant activity. Conjugated double bond in carrotenoid compounds affected the antioxidant activity[30,31]. Astaxanthin, β-carrotene dan α-tocoferol were determined in improving antioxidant activity. B-carrotene was effective as antioxidant acivity by interfering the chain reaction of free radical[30,32]. Carrotenoid compound was very soluble in nonpolar solvent as n-hexane.

TLC was used in monitoring antioxidant compound in extract. YE was monitored with TLC using ethyl acetate-n-hexane as eluent. The spotts were identified by $FeCl_3$ 1% for analyzing the presence of phenolic compounds, Citroborat 1% for analyzing the flavonoid compounds and DPPH 0,2% for analyzing the antioxidant compounds. The positive result from $FeCl_3$ 1% would give a brown to black colors in visible on the spotted, citroborat 1% would give light blue color in spotted by UV lamp at 366 nm and DPPH 0,2% would give a yellow color in spotted with purple background by visible. The results showed that YE performed positive to $FeCl_3$ 1% and citroborat 1%. The positive spotted by DPPH 0,2% showed the same positive spotted by $FeCl_3$ 1%.

This results suggested that the antioxidant compound were playing role in YE shown by phenolic group. Flavonoid in YE could contribute as antioxidant compund, but not as dominant compound. It was because that flavonoid in YE does not have hydroxyl ortho in C-3'and C-4' and does not have double bound between C-2 and C-3 and does not have hydroxyl position in C-4.

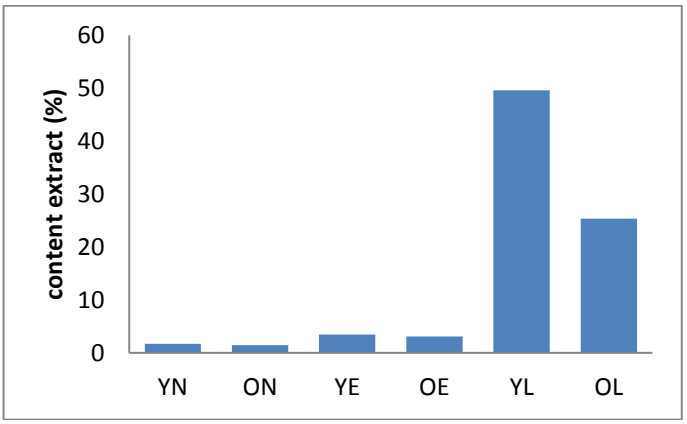


**Fig. 1: Content extract per gram of simplicia**

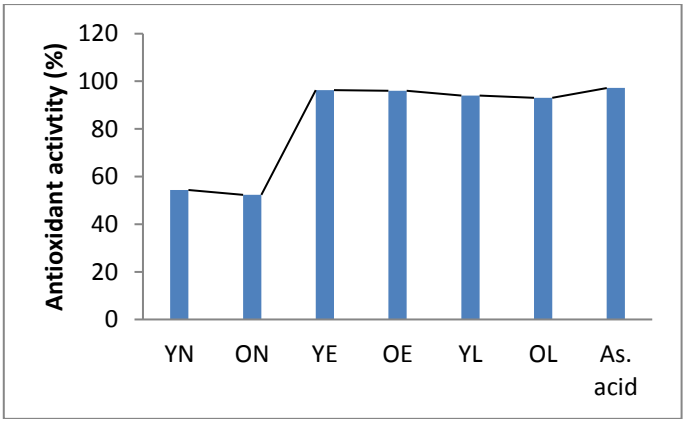


**Fig. 2: Antioxidant activity at 50 ppm extracts**

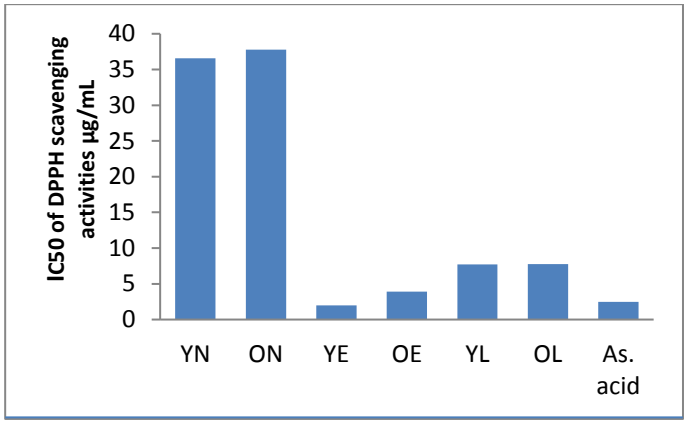


**Fig. 3: $IC_{50}$ of DPPH scavengging activities of extracts**

Note : YN (n-hexane extract of young fruit *P.emblica*; YE (ethyl acetate extract); YL (ethanol extract of young fruit *P.emblica*; ON (n-hexane extract of old fruit *P.emblica*; OE (ethyl acetate extracts; OL (ethanol extract of old fruit extract fruit *P.emblica*)

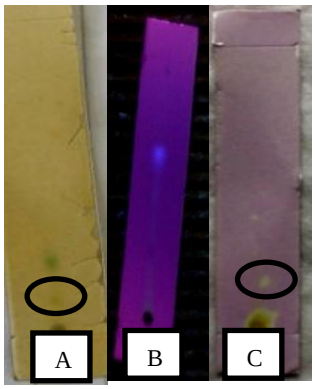


**Fig. 4: Antioxidant compound in YE with TLC**

Note : A (TLC with $FeCl_3$ 1%); B (TLC with Citroborat 1%); C (TLC with DPPH 0,2%)

## Conclusion

Antioxidant acitivity of young and old fruit extract of *P.emblica* had differences. Antioxidant activity from ethyl acetate young fruit extract of *P.emblica* had the highest activity than the other extracts. Phenolic compounds caused antioxidant activity of ethyl acetate young fruit extract of *P.emblica*.

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