

Effect of *loloh sembung* (*Blumea balsamifera*) maturity stage on antioxidant activity

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ABSTRAK

Latar belakang: *Loloh sembung* (*Blumea balsamifera*) adalah minuman tradisional masyarakat Bali yang digunakan untuk mengobati penyakit. Perbedaan kematangan daun sembung akan mempengaruhi aktivitas antioksidan loloh sembung.

Tujuan: Penelitian ini bertujuan untuk menentukan aktivitas antioksidan loloh sembung yang dibuat dari berbagai jenis kematangan daun sembung yang diekstrak dengan menggunakan pelarut air.

Metode: Serbuk daun sembung pada tingkat kematangan yang berbeda (muda, dewasa dan tua) direbus untuk menghasilkan loloh sembung. Analisis kandungan antioksidan meliputi analisis total fenolik, total kandungan flavonoid dan aktivitas antioksidan ferric reducing antioxidant power (FRAP).

Hasil: Hasil analisis menunjukkan bahwa daun sembung dengan tingkat kematangan tua menunjukkan kandungan total fenolik dan aktivitas antioksidan yang tinggi dibandingkan dengan daun dewasa dan muda, yaitu masing-masing sebesar $0,85 \pm 0,005$ GAE/g dan $0,66 \pm 0,003$ mmol Fe^{2+} /g sampel. Sedangkan, daun dengan tingkat kematangan dewasa menunjukkan total flavonoid yang tinggi, yaitu sebesar $0,39 \pm 0,006$ QE/g. Berdasarkan korelasi Pearson, perbedaan tingkat kematangan daun menunjukkan korelasi positif dengan kandungan total fenolik, total flavonoid dan aktivitas antioksidan (FRAP).

Kesimpulan: Perbedaan tingkat kematangan daun sembung menunjukkan korelasi positif dengan kandungan total fenolik, total flavonoid dan aktivitas antioksidan (FRAP). Penelitian ini menunjukkan bahwa loloh sembung berpotensi sebagai minuman fungsional yang memanfaatkan kearifan lokal.

KATA KUNCI: tingkat kematangan daun, total fenolik, total flavonoid, FRAP

ABSTRACT

Background: A *Loloh sembung* (*Blumea balsamifera*) is a traditional herbal drink from Bali and widely used to treat several diseases by Balinese people. *Sembung* leaf at different maturity stages would affect antioxidant activity of loloh sembung.

Objectives: The objective of the research was determined antioxidant activity of loloh sembung prepare from different maturity stages of sembung leaf and extracted using water.

Methods: *Sembung* leaves powder at different maturity stages (young, mature and old) was boiled to produce loloh sembung. The analyses of antioxidant activity of loloh sembung included total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing antioxidant power (FRAP).

Results: The results showed that old leaves were significantly higher in TPC and FRAP values compare to mature and young leaves, i.e 0.8575 ± 0.005 GAE/g and 0.6625 ± 0.003 mmol Fe^{2+} /g sample respectively. However, the mature leaves revealed significantly high TFC, i.e 0.3972 ± 0.006 QE/g. Based on Pearson's correlation coefficient, the difference of maturity stage exhibited positive correlation with TPC, TFC and FRAP.

Conclusion: The different of maturity stage exhibite showed positive correlation with TPC, TFC and FRAP. This study suggested that loloh sembung had a promising prospect as functional drink based on local wisdom.

KEYWORDS: leaf maturity stage, loloh sembung, total flavonoids, total phenolic (*italic*)

INTRODUCTION

Sembung is a medicinal herbal plant that is widely used to treat several diseases in a traditional community of Southeast Asia (1). Recent study reported that sembung is commonly used to aid postpartum recovery, high antioxidant activity and ACE inhibitory activity (1, 2, 3). The beneficial effect is due to high flavonoid content in sembung. Various flavonoids have been isolated from the leaves and these flavonoids responsible for antioxidant activity, ACE inhibitory activity, anti-inflammatory activity and sudorific properties (4, 2, 3). Previous studied by Ali et al (2005) reported that new constituents of flavonoids 3,4,5-trihydroxy-3,7-dimethoxyflavanone, 3,4,5-trihydroxy-7-ethoxyflavanone and new biflavonoid, 3-O-7-biluteolin have been successfully isolated from sembung leaves using soxhlet extraction methods (4).

Consumption of herbal medicine in Balinese community has long been used as traditional remedies. This tradition has been passed down to generations. Sembung is commonly consumed as herbal drink (*loloh*) by Balinese people and several methods such as boiling and kneading by hand are employed to produce *loloh* sembung. Our previous studied Kusumawati and Yogeswara (2016) reported that different extraction methods exhibited various antioxidant capacity, phenolic content and flavonoid content in *loloh* sembung (3). Some old leaves are

commonly used to produce *loloh* sembung and the maturity stage of the leaves gives a significant impact on bioactive compounds of *loloh* sembung.

Different maturity stage of the leaves plays significant role on bioactive compounds in plant extract (5, 6). Norlham et al (2015) reported that young *Cosmos caudatus* leaves exhibited high antioxidant activity compare to old and mature leaves. This result was in agreement with Yun et al (2014), it reported that old blueberry leaves exhibit high phenolic content (5). In addition, Morales et al (2017) have revealed that phenolic compound of *Hovenia dulcis* pseudofruit have increase through the maturity process (7). A high phenolic compound in the leaves has strongly correlated with the antioxidant activity (8). However, there is a little information regarding the effect of maturity degree on antioxidant capacity of *loloh* sembung be. Therefore, the aim of this study was to determine the antioxidant capacity of sembung leaves at different maturity stages to produce *loloh* sembung.

MATERIALS AND METHODS

Sembung fresh leaves (young, mature and old) that seen at **Figure 1** were collected from Tabanan, Bali, Indonesia and were harvested in March 2017. The young leaf is leaf primordium nearby the stem tip that have most epidermal hairs; the mature leaf is bifacial leaf that have less epidermis hair; the old leaf is

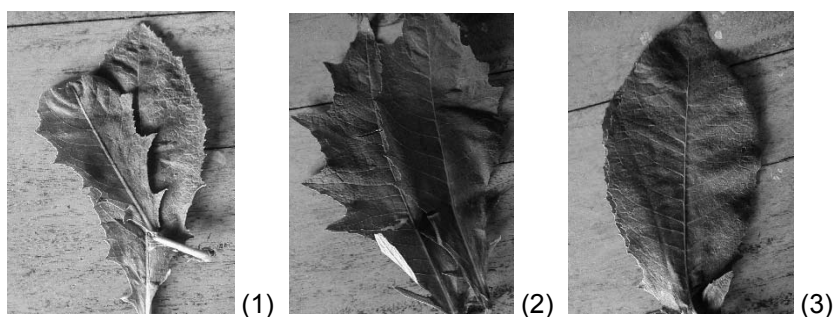


Figure 1. Sembung leaf (1) young; (2) mature; (3) old

have not epidermis hair (9). The leaves were collected and identified in Plant Taxonomy Laboratory, Faculty of Biology, Udayana University, Bali. Folin-Ciocalteu, methanol, ethanol and sodium carbonate (analytical grade, *Merck*), gallic acid, quercetin, aluminium chloride, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), $\text{Fe}_2\text{SO}_4 \cdot 7\text{H}_2\text{O}$ were purchased from *Sigma-Aldrich* chemical and aquades.

Preparation of loloh sembung

Sembung leaves at different maturity stages were washed, rinsed and dried under the shade. Subsequently, the leaves were pounded into a powder with a blender (Phillips) and sieved it into 80 mesh. Sembung leaves were collected and divided into three parts i.e young, mature and old leaves. The leaves were washed, rinsed and drye under the shade for fourteen days. Dry leaves were pounded with a blender and sieved into 80 mesh to obtain sembung powder. Dried sembung powder (7% w/v) was boiled for seven minute to produce loloh sembung.

Antioxidant capacity

A ten microliter of extract were dissolved in 30 μl of distilled water and thoroughly mixed with 200 μl FRAP (mixture of iron chloride and 2, 4, 6 - tripyridyl - s - triazine). The aliquots were incubated for 4 minutes in room temperature and the absorbance were measured with spectrophotometer UV-Vis and was read at 600 nm. FRAP value was expressed as Fe^{2+} equivalent in $\text{mmol Fe}^{2+}/\text{g}$ dried extract. (10)

Total phenolic content

Determination of phenolic content was measured using Follin ciocalteu methods with slight modification. Gallic acid was used as a standard. Fifty μl (extract sample) was mixed with 250 μl Follin

ciocalteu and incubated for 1 minute. Briefly mixed with 750 μl Na_2CO_3 20% and vortex for 1 minute and followed by incubation in room temperature for 2 hours. Extract sample were then added with 5 ml of aquades and the absorbance were read at 760 nm. Phenolic content value was expressed as gallic acid equivalent/g dried extract (11).

Total flavonoid content

A 50 μl extract were mixed with 4 ml aquades and 0.3 ml NaNO_2 10% and incubated in room temperature for 6 minutes. After 6 minutes, distilled water was added until reach final volume of 10 ml and followed by vortexing for 1 minute. Subsequently, the mixture was kept for 15 minutes. Absorbance was read at 510 nm and distilled water was used as a blank. Quercetin was used as a standard and flavonoid was expressed as Quercetin equivalent/ dried extract (12).

Statistical analysis

All experiments were conducted in triplicated and the results were expressed as mean \pm standard deviation. Two-way analysis of variance (ANOVA) of the data was carried out using SPSS 20.0 software. Mean differences were established by the Tukey's range tests. The significant differences ($p < 0.05$) between the means were performed to determine the effect of stage maturity on the TPC, TFC and FRAP of loloh sembung.

RESULTS

The results of this study exhibited that loloh sembung prepare from different maturity stage have different total phenolic, flavonoid and antioxidant capacity (FRAP methods), as presented in **Table 1**.

Table 1. Total phenolic, flavonoid and antioxidant capacity (FRAP methods) of loloh sembung prepare from different maturity stage

Maturity stage	Total Phenolic Content (TPC) (mgGAE/g sample)	Total Flavonoid Content (TFC) (mgQE/g sample)	Antioxidant Capacity FRAP (mmol FeE/g sample)
Young	0.38 \pm 0.004 ^a	0.13 \pm 0.003 ^a	0.65 \pm 0.002 ^b
Mature	0.81 \pm 0.005 ^b	0.40 \pm 0.006 ^c	0.63 \pm 0.003 ^a
Old	0.86 \pm 0.005 ^c	0.32 \pm 0.005 ^b	0.66 \pm 0.003 ^c

*Different letter in the same coloum indicates significant difference ($p < 0.05$)

DISCUSSION

Yield of *loloh sembung*

The yield extract from young, mature and old leaves obtained in this study was 9.21%, 16.78% and 12.95% respectively. Differences in yield extraction was due to young leaves content less water compare to mature and old leaves. Biochemical properties of the stomata in each mature stages effect the water content in plant leaves (13). Boiling method was employed to produce *loloh sembung* by using 100 ml of distilled water at 80°C for 7 minutes, since this method was commonly used by the Balinese people to produce *loloh sembung* (14). Total phenolic content, flavonoid content and FRAP analysis were carried out to determine the antioxidant activity of *loloh sembung*.

Total phenolic content

The total phenolic content in *loloh sembung* ranged from 0.38 ± 0.004 to 0.86 ± 0.005 GAE/g respectively. The results showed that old leaves contained high phenolic content 0.86 ± 0.005 GAE/g. The phenolic content of the leaves were increased through the maturity process while the young and mature leaves exhibit low total phenolic content. All samples showed significant TPC according to Tukey test as can be seen in **Table 1**.

This studied were in agreement with several researches reported that a number of old leaves exhibit high total phenolic content while young and mature leaves exhibit low total phenolic content. High total phenolic content in old leaves were due to optimum synthesis of phenolic compound through aging process. The maturity of the leaves was also effect the production of secondary metabolite and total phenolic content. Thus, the older leaves reflect high TPC compounds compare to young and mature stages present in the sample. Maieves et al (2015) reported that total antioxidant capacity has closely related with total phenolic content (15).

Total flavonoid content

The total flavonoid content in *loloh sembung* ranged from 0.13 ± 0.003 to 0.32 ± 0.005 QE/g respectively. These results showed that mature

leaves exhibit high flavonoid content present in the sample. Whereas, young leaves and old leaves showed lower total flavonoid content in the extract sample. Previous studied by Oleyade et al (2012) reported that mature leaves seem to exhibit high flavonoid content compare to young and old leaves (16). High flavonoid content is probably due to the alteration of bioactive compounds in the leaf tissue during maturity process. Mature leaves exhibit low phenolic content and yet, exhibit high flavonoid content. High flavonoid content is also due to high absorption of the mature leaves to the sunlight which may lead to over synthesis of flavonoid compound. Mature leaves have a wider surface area and hence have a high absorption ability to the sunlight compare to young and old leaves (17).

In contrast, different results were reported by other studied (6, 18, 19) that flavonoid content were increased in young leaves compare to mature and old leaves. This result suggests that different type of leaves exhibit various phenolic content at different maturity stages.

Antioxidant capacity

In biological tissues, mechanisms of their antioxidant are extremely complex (20). In this study, the antioxidant capacity of *loloh sembung* was evaluated using FRAP method. The antioxidant activity by FRAP in *loloh sembung* ranged from 0.65 ± 0.002 to 0.66 ± 0.003 mmol Fe²⁺/g sample respectively. This result showed that old leaves exhibit high antioxidant capacity 0.66 ± 0.003 mmol/g sample. Tukey test analysis showed that all samples have a significant antioxidant capacity

These results were in agreement with Kamal et al (2012) reported that old leaves exhibit high antioxidant capacity analyzed using FRAP method (17). The antioxidant capacities are relatively increased with plant maturity. During maturity process, the bioactive compound is increasing and depend on the biosynthetic pathways and mechanisms of metabolic control.

There was a highly significant ($p < 0.01$) positive correlation between the maturity stage and TPC value in *loloh sembung* (Pearson R= 0.91), TFC value (Pearson R= 0.71), as well as antioxidant

capacity (FRAP) value in *loloh* sembung (Pearson $R=0.26$). The results indicate that maturity stage affected to total phenolics contents, total flavonoid contents and antioxidant capacity.

Sembung leaf has high flavonoid content and widely distributed in plants. Flavonoid is a polyphenolic compounds and possess antioxidant activity in biological activity (21, 22). Flavonoids exhibit various biological effects including antibacterial activity, anti-inflammatory and anti-allergic. Flavonoids act as antioxidant by chelating reactive oxygen species and lead to inhibition of enzymes responsible for superoxide anion production. Resulting prevention of radical alkoxyl and peroxy radicals formation (23).

CONCLUSIONS AND RECOMMENDATION

As a conclusion, old sembung leaves exhibit high phenolic content and high antioxidant capacity while mature sembung leaves showed high flavonoid content. Hence, mature sembung leaves can be utilized to produce *loloh* sembung with a high flavonoid content. Therefore, *loloh* sembung with its high flavonoid content may be considered to have the ability such as antibacterial, anti-inflammatory, vasodilatory and anticancer.

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