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The effect of extraction methods on total phenolic, flavonoid and antioxidant capacity of Loloh Sembung *(Blumea balsamifera)*

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Abstract

A Loloh is commonly consumed by Balinese people and often used as a therapeutic herbal drink. Various extraction methods can be employed to produce loloh. The research aims to determine the effect of extraction methods on total phenolic content, flavonoid and antioxidant capacity of loloh sembung *(Blumea balsamifera)*. The concentrations of dried sembung leaves were 1, 3, 5 and 7 grams and 100 mL of water was used as solvent. The extraction methods used were infused and decoction. The analysis was done for determining the total phenolic, total flavonoids, and total antioxidant capacity (DPPH and FRAP). The results showed no significant difference between infuse and decoction methods. Higher total phenolic, flavonoid, and antioxidant capacity were produced by loloh sembung at a concentration of 1%.

Introduction

Balinese people have been consuming herbal drinks since ancient times because they assumed that consumption of herbal drink is safer than synthetic medicine (Kusumawati and Yogeswara, 2016). The Balinese traditional herbal drink commonly known as loloh (Leurs, 2009) can be made through various methods, such as boiled, brewed, kneaded by hand or with a blender. Consumption of loloh as medicinal preparations has a long tradition in every region in Bali. The loloh is commonly used to treat various diseases and this ancient believes referring to a book known as Usada Taru Pramana. In Usada Taru Pramana, mentions all various types of plants that can be used as herbal medicines and also the extraction methods are written specifically (Suryadarma, 2006). In general, Balinese people utilized extract from part of a plant as a loloh. The part of plants that can be used as a loloh are roots, stems, seeds, fruit and leaves (Kusumawati and Yogeswara, 2016).

Sembung is one of herbal plants that can be used as a loloh. The Usada taru pramana describes that sembung leaves can be used as herbal medicine to treat fever (Kusumawati and Yogeswara, 2016). Some research suggests that sembung has efficacy as an anti-inflammatory, improves blood circulation, inhibits bacteria growth, and maintaned body temperature (Mursito, 2002; Ali *et al.*, 2005; Norikura *et al.*, 2008; Sakee *et al*., 2011). Several research reported that these properties partly due to flavonoid and phenolic content in herbs. Flavonoids are the most abundant phenolic content in herbs and have a beneficial effect on human health. Flavonoids have the capacity as free radical scavenger in biological systems and provide antioxidant protection. Certain flavonoid compounds provide a protection against free radicals which are associated with pathological damage such as hypertension and cardiovascular disease (Galleano *et al.*, 2010; Mlandenka *et al.,* 2010).

Balinese people traditionally prepared loloh sembung using boiling method or kneaded by hand. Previous studied by Kusumawati and Yogeswara (2016) have shown that boiling and brewing methods exhibit different antioxidant activities. The boiling method gives a higher antioxidant capacity was 5.55±0.01 mg GAE/g dried leaves. Moreover, boiling 7% of dried sembung leaves was able to produce free radicals scavenger up to 99.25% (Kusumawati *et al*., 2016). However, the boiling process may lead to loss of compounds that act as antioxidants. Efforts should be made to minimize the loss of bioactive components caused by direct heating.

Another extraction method which involves indirect heating should be applied to produce

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high antioxidant activity in loloh sembung such as infusion or decoction. Fotakis *et al*. (2016) reported the infusion of herbal plants method positively affected the extractability of the phenolic compounds compared to decoctions. In contrast, Rodrigues *et al.* (2016) reported that the infusion and decoction of Limonium algarvense flowers have no significant differences were observed for both extracts ($p>0.05$). The applications of infusion and decoction as an extraction method to prepared loloh sembung and its effect on bioactive properties have not yet been studied. Thus, the aim of the present work was to study the impact of different extraction method on antioxidant activity, flavonoid and phenolic content of loloh sembung. Proper extraction methods could offer functional properties of loloh sembung.

Materials and Methods

Materials

Blumea balsamifera fresh leaves were collected from Bajera Village, Tabanan, Bali, Indonesia and were harvested in March 2016. 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, 2,2-diphenyl-1-picrylhydrazyl (DPPH), alumunium chloride, 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ), and $Fe_2SO_4.7H_2O$ were purchased from Sigma-Aldrich chemical.

Preparation of dried sembung leaves

Fresh sembung leaves were washed, drained, and dried in a shaded place for 14 days (Kusumawati *et al*., 2014) at a temperature of 31.93±1.07°C. The dried sembung leaves were pounded using a blender (Philips) to powder. Then, the sembung leave powder was sieved using an 80 mesh sieve. The powder of sembung leave was kept at a temperature of 4°C prior to the analysis.

Loloh sembung extraction methods

Loloh sembung was extracted using infusion and decoction methods. Extraction was conducted by dissolving dried sembung leaves in 100 ml water. Infusion was carried out at a temperature of 90°C for 15 minutes while the decoction was carried out at a temperature of 100°C for 30 minutes. Infusion and decoction used the duplex pan, and the sample was placed on the top of the pan. The extract obtained was then filtered. The filtrate was analyzed for total phenolic content, total flavonoid content, and antioxidant capacity.

Analysis of extract

Total phenolic content (TPC) assay

The total phenolic content of the extract of loloh sembung was determined according to Ammar *et al.* (2015). Extract (100 µL) was dissolved in 6 µL distilled water and 500 µL Folin-Ciocalteau reagent. The solution was mixed for 1 mins. 1.5 mL of 20% Na_2CO_3 was added; then, distilled water was added up to the mark of a flask (10 mL). The solution was mixed, which was then incubated at a room temperature for 30 mins. Absorbance was measured at 765 nm using Shimadzu 1650 UV-vis spectrophotometer. TPC was expressed as gallic acid equivalents (GAE) in mg per g of extract.

Total flavonoid content (TFC) assay

The total flavonoid content of the loloh sembung extract was determined according to Chang *et al.* (2002). Extract (0.5 mL) was added to 0.1 mL of 10% alumunium chloride (in ethanol), 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water. The solution was a vortex for 20 mins and then incubated at a room temperature for 30 mins. Absorbance was measured at 415 nm using Shimadzu 1650 UV-vis spectrophotometer. TFC was expressed as quercetin equivalents (QE) in mg per g of extract.

DPPH assay

The total antioxidant capacity of loloh sembung was determined using DPPH method according to Hanani *et al.* (2005). Extract (1 mL) was added to 2 mL of 0.004% 2,2-diphenyl-1-picrylhydrazyl (DPPH) (in methanol). The solution was incubated in a dark room for 60 mins. Absorbance was measured at 517 nm using Shimadzu 1650 UV-vis spectrophotometer. TAC with DPPH method was expressed as quercetin equivalents (QE) in mg per g of extract.

FRAP assay

The total antioxidant capacity of loloh sembung was determined using FRAP method according to Konczak *et al.* (2010). Extract (10 µL) was dissolved in 30 µL distilled water and added to 200 µL FRAP (consisting of a mixture of ferric chloride and 2, 4, 6 - tripyridyl - s- triazine). The solution was incubated for 4 mins. Absorbance was measured at 600nm using Shimadzu 1650 UV-vis spectrophotometer. FRAP was expressed as Fe^{2+} equivalents in mmol Fe^{2+} per g of extract.

Statistical analysis

All the analyses were performed in triplicate

and the results were expressed as mean±standard deviation. The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey methods range tests using SPSS statistics 20 software. ANOVA data with a $p<0.05$ were considered statistically significant.

Results and Discussion

The dried sembung leaves have glaucous color and aroma of tea. The yield obtained from drying fresh sembung amounted to 6.68%. The loloh was extracted by infusion (at a temperature of 90°C for 15 mins) and decoction (at a temperature of 100°C for 30 mins). The loloh sembung was formulated with a various concentrations i.e 1%, 3%, 5% and 7%. After the extraction processed, the filtrate was collected and filtered using Whatman filter paper no 1. The liquids are called loloh. Then, the loloh was analyzed for total phenolic, flavonoids, and total antioxidant capacity using DPPH and FRAP methods.

Total phenolic and flavonoid contents of loloh sembung

The total phenolic contents of dried sembung leaves in water extracts were varied between extraction processes. The total phenolic contents of loloh sembung were in range between 5.88±0.01 and 27.42±0.05 mg GAE/g sample (Table 1). The standard curve equation was y=7.142x-0.0341, r^2= 0.9863. The statistic result showed that both infusion and decoction methods showed no significant difference in TPC. Similar result was reported by Rodrigues *et al.* (2016) that the infusion and decoction from *Limonium algarvense* flowers have no significant differences were observed for both extracts ($p>0.05$). The concentration of 1% of dried leaves showed higher total phenolic content. Kusumawati and Yogeswara (2016) reported that total phenolic content of loloh sembung extracted using brewing method was 13.15±0.11mg GAE/g sample than those to boiling method. Furthermore, it also suggest that brewing method gives the best result compare to boiling method in terms of total phenolic content. Higher TPC content in the extraction of loloh sembung can be associated with the loss of soluble components of nonphenolic compounds such as monosaccharides, disaccharides, and oligosaccharides soluble fiber and protein (Nithiyanantham *et al*., 2013). High temperature and long extraction time can affect the release of the tightly bound compounds and improve the efficiency of extraction (Marete *et al*., 2009; Wita and Yogeswara, 2016). The optimum temperature of extraction method to increase the total phenolic content was above 25-65°C (Tsai *et al*., 2012).The treatment process using high temperature will increase the potential of antioxidant activity due to the formation of new compounds that serve as antioxidant activities (Marete *et al.*, 2009; Nithiyanantham *et al*., 2013). The polyphenolic components of higher plants may act as antioxidants or agents of other mechanisms contributing to anticarcinogenic actions (Catherine e*t al*., 1996).

The total flavonoid contents of dried sembung leaves in water extracts were varied between extraction methods. The total flavonoid contents of loloh sembung equivalent to quercetin were between 113.64±0.19 and 266.66±0.65 mg QE/g sample (Table 1). The standard curve equation obtained was y=0.2716x-0.0092, r^2= 0.9976. The statistic results indicate that both infusion and decoction methods showed no difference in TFC. The concentration of 1% of the loloh sembung exhibited higher TFC. Several studies on the flavonoid constituents of sembung have been reported such as flavones, monoterpenes and triterpenes (Nesa *et al.*, 2004). The TFC of the extract exhibited biological activity of the extract. Flavonoids contained in extracts showed the presence of antioxidant and anticancer activities (Catherine *et al*., 1996; Bae *et al.*, 2012).

Total antioxidant capacity (TAC) of loloh sembung

The antioxidant activities of the extract have the capacity as a free radical scavenger (Shimada *et al*., 1992; Catherine *et al*., 1996). The total antioxidant capacity of loloh sembung was analyzed to determine the ability as a free radical scavenger in water extract. The TAC of loloh sembung was determined using DPPH method was between 2998.19±5.13 and 19096.45±53.84 mg QE/g sample (Table 1). Further, the TAC of loloh sembung was determined using FRAP method equivalent to ferosulfate, which was between 50.73±0.45 and 334.96±1.81 mmol Fe^{2+}/g sample (Table 1). The high ability of free radicals scavenger produced by infusion and decoction methods indicated that the components act as antioxidants consist of heat resistant compounds. Kusumawati and Yogeswara (2016) reported that loloh sembung made using boiling method exhibit higher antioxidant capacity compare to brewing method. In addition, Kusumawati *et al.* (2015) reported that loloh tempuyung has many heat resistant compounds and hence, heating process is required to extract the loloh tempuyung. Tsai and She (2006) reported that there was a bond between the phenolic compounds with matrix proteins in beans during the heating process. Interactions between proteins and phenolic compounds may lead the protein to be

Table 1. Total phenolic, flavonoid and antioxidant capacity (DPPH and FRAP methods) of loloh sembung

Extraction method	Concentration	TPC (mgGAE/g sample)	TFC (mgQE/g sample)	TAC DPPH (mg QE/g sample)	FRAP (mmol FeE/g sample)
Infuse	1%	24.75±0.05^{c}	243.65±0.65^{d}	18423.86±35.89^{d}	322.46±1.36^{d}
	3%	13.37±0.03^{b}	196.58±0.65^{c}	6767.34±11.96^{c}	133.55±0.45^{c}
	5%	8.19±0.01^{a}	139.03±0.26^{b}	4167.01±0.00^{b}	77.63±0.18^{b}
	7%	6.54±0.71^{a}	113.64±0.19^{a}	2998.19±5.13^{a}	50.73±0.45^{a}
Decoction	1%	27.42±0.05^{c}	266.66±0.65^{d}	19096.45±53.84^{d}	334.96±1.81^{d}
	3%	12.27±0.05^{b}	181.39±0.87^{c}	6644±5.98^{c}	125.54±0.30^{c}
	5%	7.63±0.02^{a}	144.27±0.39^{b}	4116.24^{b}	77.25±0.36^{b}
	7%	5.88±0.01^{a}	125.14±0.46^{a}	3030.82±5.13^{a}	51.38±0.19^{a}

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

stable and the antioxidant capacity increase during the heating process. Nesa *et al*. (2004) reported that extract of sembung leaves contained quercetin, rhamnetin, luteolin and luteolin-7-methyl ether reacted rapidly with DPPH radical at concentration ranges from 100–150l g/ml. Previous studied by Jimoh *et al.,* (2011) revealed that, water extract of Sonchus asper and Sonchus oleraceus leaves at 1% exhibit high antioxidant activity and thus could serve as free radical scavenging activity. In addition, water extract at 1% have 92.9% of DPPH radical scavenging abilities and this result were slightly less than those of ascorbic acid and BHT. Similar results were also reported by Takao *et al.*, (2015) found that, 1% of Myrtaceae leaves extract prepared with infusion extraction method exhibit high radical scavenging activity, high phenolic content and strong antioxidant activity index. Furthermore, TPC are strongly correlated with antioxidant activity index.

There was a highly significant ($p<0.01$) positive correlation between the DPPH/FRAP and TPC value for loloh sembung (Pearson R for DPPH = 0.992 and for FRAP = 0.996) and between the DPPH/FRAP and TFC value for loloh sembung (Pearson R for DPPH = 0.951 and for FRAP = 0.965). The results indicate that high antioxidant capacity is associated with high phenolics and flavonoids contents.

Conclusion

All of the loloh sembung concentrations exhibited different extents of antioxidant capacity. The results of the present study showed that the concentration of 1% in the loloh sembung contains the highest amount of phenolic and flavonoid compounds, thus exhibiting the highest antioxidant capacity. The statistical results indicate that both infusion and decoction methods produced no differences in terms of TPC, TFC, and TAC significantly.

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