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Antioxidant Activity of Aqueous Extracts of Dehydrated Lunuwila

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Introduction

Lunuwila (*Bacopa monnieri*) was extensively reported for its health promoting antioxidant capacity (Biswas *et al*., 2012). It is widely used in traditional medicine for treating different diseases and illnesses and enhancing memory and cognitive functions of the brain (Stough *et al*., 2001; Prabhakar *et al*., 2008; Charles *et al*., 2011; Shinomol *et al*., 2011). The first written reference to traditional medicine in Sri Lanka, which was composed by the King Buddhadasa (Paul, 1954), provides substantial information on preparation of decoctions from medicinal plants, and the knowledge is handed down from generation to generation. Such preparations are different from other extraction techniques as a known quantity of medicinal plant/s in a specified volume of water is heated in clay pots for a specified time until the original volume is brought down to one-eight. Extraction method affects the antioxidant capacities (Shah and Bojja, 2014) and most of the studies on antioxidant capacity of *Lunuwila* have been undertaken using alcoholic extracts. However, alcoholic extractions are mostly not performed by traditional medical practitioners in the preparation of decoctions as most of the individuals opt for aqueous preparations of medicinal plants, probably because of cultural and religious norms. Even though this plant has been used from time immemorial as a green leafy vegetable, particularly as a salad given to children to acquire brain boosting effects, bitter flavour limits its consumption. Therefore, this study focused on investigating the effect of heating dehydrated *Lunuwila* in water, until the original volume reduced to one-eight and two-eight, on antioxidant capacity of the decoctions. The results of this study could be useful in the process of developing, beverages, functional foods and nutraceuticals.

Materials and methods

Fresh *Lunuwila* samples collected from the Galle area in Sri Lanka were washed thoroughly with running tap water, allowed to air dry at room temperature (30 ± 2 ^{o}C) for 2 h and partially dried to remove excess moisture using an oven (SIBTA –Thermotec oven, serial no-7y0099). The partially dried samples were dehydrated at 50 ±1 ^{o}C for 12 h) (Silpa *et al.*, 2019) in a cabinet air dryer (Zhejingsujing equipment, model 101A-6, China), allowed to cool (30 ± 2 ^{o}C) and packaged in metalized PET pouches (32 Gauge) for further use.

Moisture content of fresh and dehydrated *Lunuwila* samples were determined. Dehydrated *Lunuwila* samples were cut into approximately 1 cm long pieces. As instructed by a traditional medicine practitioner, a ratio of 6:100 (g/ml) between dehydrated sample and distilled water (pH 6.8) was used for aqueous extraction in clay pots. Two extraction ratios of 8:1 and 8:2 were used as treatments in triplicate. The samples were heated in a water bath maintained at 60 ± 5 °C. Heating was carried out until the final volume of the extract reached 12.5 and 25 % from its initial volume to obtain extraction ratios of 8:1 and 8:2 respectively. Time taken for reduction in the volume was monitored during heating. All the extracts were stored under refrigeration conditions (0 - 4 °C) in glass bottles (250 mL) for further use.

DPPH radical scavenging activity (DPPH-RSA) of the aqueous extracts was determined in triplicate according to the method described by Hatano *et al.*, (1988) with slight modifications. Ferric reducing power (FRP), lipid peroxidation inhibition activity (LPIA), total antioxidant capacity (TAC) and total saponins (TS) of the aqueous extracts were determined in triplicate according to the methods described by Oyaizu (1986), Ohkowa *et al*., (1979), Prieto *et al*., (1999) and Uematsu *et al*., (2000) respectively. Dry yield of each extract was determined in triplicate by vortexing each aqueous extract (5 mL) for 5 min and drying at 80 ± 1 °C in an oven (SIBTA –Thermotec) until the liquid was totally vaporized.

The results were expressed as mean ± standard deviations (SD). The one-way analysis of variance (ANOVA) followed by Tukey's Studentized range test was employed to find out whether individual means of treatments were significantly different ($P < 0.05$).

Results and Discussion

Lunuwila is a seasonal herb easily grown during rainy seasons in the wet, marshy and damp areas. Short shelf life of *Lunuwila* caused by high moisture content and mechanical damages due to succulent nature limits its use year around. Therefore, extending the shelf life of this

seasonal herb by reducing the moisture content ensures its availability. Drying of *Lunuwila* in cabinet driers at 50 °C was reported to preserve Bacosides, one of the most important groups of constituents responsible for health benefits (Silpa *et al.*, 2019). Dehydration at 50 ± 1 °C for 12 h in a cabinet air dryer reduced the moisture content of *Lunuwila* from 84.97 ± 1.75 to 11.41 ± 0.27%. Time required for a volume reduction of 8:1 and 8:2 in the preparation of decoctions by heating dehydrated *Lunuwila* in distilled water was 135 and 90 min respectively.

Due to the complex nature of phytochemicals possessing antioxidant activity of medicinal plants, need to employ at least two or more methods to evaluate total antioxidant activity of plant extracts is suggested (Gunathilake and Ranaweera, 2016). Therefore, DPPH-RSA, FRP, LPIA, TAC and TS were employed in this study to measure the antioxidant activity of the decoctions. Dose dependency of antioxidant activity of *Lunuwila* was suggested based on FRP, hydrogen peroxide radical assay, nitric oxide radical scavenging, superoxide radical scavenging and LPIA (Shah *et al*., 2012). Similarly, dose dependency of antioxidant activity of aqueous decoctions, regardless of the extraction ratios of 8:1 and 8:2, was evident as indicated by the results of DPPH-RSA, FRP, LPIA, TS and TAC (Fig. 1). DPPH-RSA increased with the increase in concentration, which shows concentration dependent scavenging

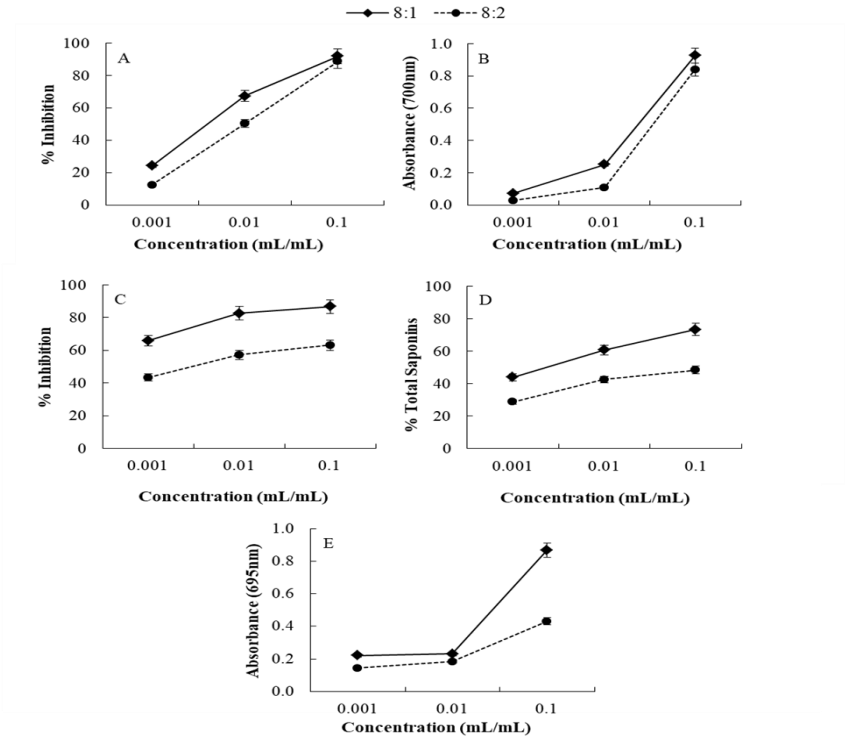

Fig 1. DPPH radical scavenging activity (A), ferric reducing power (B), lipid peroxidation inhibition activity (C), total saponins (D) and total antioxidant capacity (E) of aqueous extracts of dehydrated *Lunuwila.*

activity (Fig. 1A). This is due to increase in antioxidant activity in terms of hydrogen atom-donating capacity (Amarowicz *et al.*, 2004). Moreover, better extractability of phytoconstituents possessing hydrogen atom-donating capacity when extracted in 8: 1 than in 8:2 was evident (Fig. 1A). Concentration dependency of FRP of aqueous extract of *Lunuwila* was evident regardless of the extraction ratio (Fig. 1B). Extractability of constituents possessing FRP, which is higher in 8:1 extraction than that in 8:2 extraction, may probably be due to longer heating time of the former than the latter. As such phytoconstituents reduce the Fe^{3+}/ferricyanide complex to the ferrous form due to their ability to donate electrons and reduce the oxidized intermediates generated in the process of lipid peroxidation, they are

reported to act as primary and secondary antioxidants (Amarowicz *et al*., 2004). The ability of the extracts to inhibit peroxidation of lipids is evaluated using LPIA, where the presence of an antioxidant in the medium inhibits auto-oxidation of linoleic acid, resulting ultimately in low production of the coloured ferric thiocyanate complex (Ohkowa *et al*., 1979). Increase in LPIA was evident in a concentration dependent manner (Fig. 1C). As phytoconstituents such as flavonoids and other polyphenols have been reported to be effective in inhibiting lipid peroxidation (Shah *et al*., 2012), extraction in 8:1 probably resulted in significantly higher ($p < 0.5$) extractability of such phytoconstituents than in 8:2 extraction. The aqueous extract of *Bacopa monnieri* has demonstrated increase in TS in a concentration dependent manner (Fig. 1D). Moreover, significantly higher extractability of total saponin in 8:1 extraction than that in 8:2 extraction may probably be due to longer heating time of the former than the latter (Fig. 1D). As different groups of phytochemicals possess antioxidant activity, information on TAC of an extract might be more helpful than that on specific antioxidant species (Gunathilake and Ranaweera, 2016). The results revealed concentration dependency of TAC of aqueous extract of *Lunuwila*, regardless of the extraction ratio (Fig. 1 E). Moreover, significantly higher ($p<0.5$) extractability of phytoconstituents contributing to TAC was evident in the case of 8:1 extraction than that of 8:2 (Fig. 1E).

Significantly higher DPPH-RSA, LPA, TS and TAC of decoctions prepared by heating dehydrated *Lunuwila* in distilled water in 8:1 than that in 8:2 was evident (Table 1). Even

Table 1. DPPH-RSA, FRP, LPA, TS and TAC of decoctions of dehydrated *Lunuwila* prepared in 8:1 and 8:2.

Decoction	DPPH radical scavenging activity (% inhibition)	Reducing power (mg AAE/mL extract)*	Lipid peroxidation activity (% inhibition)	Total saponins %	Total antioxidant capacity (mg AAE/mL extract)*
8:1	92.2 ± 0.5^{a}	8.85 ± 0.09^{a}	61.6 ± 1.1^{a}	73.4 ± 1.1^{a}	8.23 ± 0.6^{a}
8:2	89.1 ± 0.6^{b}	7.34 ± 0.96^{a}	50.8 ± 0.4^{b}	48.4 ± 1.2^{b}	4.08 ± 0.9^{b}

Values are presented as mean ± SD, n = 3
Means within the same column bearing the same superscript are not significantly different ($p>0.05$)
* mg ascorbic acid equivalent per mL extract

though 2-fold concentration is expected between 8:1 and 8:2 extracts, 3.47, 21.41, 51.62 and 104.14% increase in DPPH-RSA, LP, TS and TAC respectively, was evident. Moreover, there was no significant difference between FRP between 8:1 and 8:2 extracts. Therefore, phytoconstituents of dehydrated *Lunuwila* could be extracted in 8:1 or 8:2 and used as an ingredient in manufacturing beverages, even though the practice in traditional medicine is to extract in 8:1. Moreover, extracting in 8:2 is more cost effective due to comparatively lower requirement of energy for heating. Yield of the two extracts were 0.110 ± 0.017 g/mL (8:1) and 0.078 ± 0.007 g/mL (8:2). Even though extraction in 8:1 resulted in 15% more yield than that in 8:2, considering the energy efficiency, extraction of dehydrated *Bacopa monnieri* in 8:2 could be recommended for spray drying followed by microencapsulation for manufacturing nutraceuticals or any other similar applications.

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