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Biochemical and functional properties of indigenous Australian herbal infusions

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Abstract:

The phytochemical profile, organic acid content, minerals, various antioxidant assays and consumers acceptability of indigenous Australian herbal infusions namely gulban (*Melaleuca citrolens*), anise myrtle (*Syzygium anisatum*), and lemon myrtle (*Backhousia citriodora*) were compared with a commercial green tea (*Camellia sinensis*). Total phenolic content and catechin derivatives were higher in green tea as compared to indigenous herbal infusions ($P < 0.05$). Phytochemical profiles showed high levels of caffeine in green tea, but, it was not found in herbal infusions ($P < 0.05$). Australian indigenous herbal infusions were a good source of calcium and magnesium compared to green tea ($P < 0.05$). Oxalic acid was higher in green tea, whereas gulban and anise myrtle infusions were rich in citric acid ($P < 0.05$). Antioxidant activities of green tea and gulban herbal infusions were comparable ($P \geq 0.05$). Overall liking scores were higher for herbal infusions compared to green tea ($P < 0.05$). Indigenous Australian herbal infusions particularly gulban has a potential to become a successful commercial herbal beverage.

Keyword: *Melaleuca citrolens*, *Syzygium anisatum*, *Backhousia citriodora*, *Camellia sinensis*, tea, Australian herbs

1. Introduction

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Tea is the most widely consumed refreshing drink in the world, after water. Tea is the hot water extract of *Camellia sinensis* L. leaves and leaf nodes. Tea is widely cultivated in China followed by India, Kenya, Sri Lanka, Turkey, Vietnam and Iran (Alasalvar et al., 2013). Tea can be categorized into three groups such as non-fermented (green tea), semi-fermented (oolong tea) and fermented (black tea). Consumer interest in green tea and herbal infusions has been increasing due to their health benefits. Herbal infusions or beverages are made from hot water extracts of any part of the medicinal plants including fresh or dry leaves, fruits, flowers and roots. Green and herbal infusions are popular due to their pronounced biochemical activities such as antioxidant, anti-inflammatory and anti-carcinogenic (Chatterjee et al., 2012; Gorjanovic et al., 2012). The chemical composition of green tea includes phenolic compounds, purine alkaloids, terpenoids, fatty acids, essential oils, amino acids, minerals and metals (Schwalfenberg et al., 2013; Zhao et al., 2011). The major phenolic compounds present in green tea belong to the catechin group which includes (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCg), and epicatechin gallate (ECG).

Herbal infusions and medicines have been known to alleviate disease and promote health. The preparation of herbal infusions depends on tradition and perceived health benefits. Herbs can be steeped several times and can be served with addition of lemon juice, honey or other sweeteners. Most of the herbal infusions contain well known compounds such as simple polyphenols, catechins, flavonoid, flavonoid glycosides, anthocyanins, aglycone, theaflavins and chalcones (Mazina et al., 2015). Different herbal infusions are available and their bioactive content is mostly affected by plant species, cultural habits, horticultural practices and geographical regions (Moodley et al., 2015). Moreover, sensory properties of these herbal infusions depend on the selection of plant organs such as leaves, fruits, flowers and roots and their polyphenol content.

Australia has some of the oldest and largest unexploited rainforests with unique biodiversity. However, the Australian Aboriginal community frequently uses such plants, herbs

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and fruits in their food and drink. Australian indigenous foods have traditionally played an important role in the diet of Aboriginal people. Gulban, anise myrtle and lemon myrtle are some of the Australian indigenous bush food plants with unique tastes and flavor, which are part of the daily diet for Indigenous people. However, anise myrtle and lemon myrtle are probably the most commercialized native herbs and used by the bush food industry (Konczak et al., 2010). Anise and lemon myrtle have been widely used for herbal infusions (Chan et al., 2010; Sakulnarmrat et al., 2013). *Melaleuca Spp.* including *M. alternifolia*, *M. bracteata*, *M. ericifolia*, *M. quinquenervia*, *M. squamohpic*, *M. uncinata* showed antimicrobial activity against pathogenic microorganism (Wilkinson and Cavanagh, 2005). However, gulban from Australia has not been studied. The use of *M. citrolens* leaves as herbal infusion remains traditional knowledge of Aboriginal people. To introduce this Australian indigenous herbal infusion, knowledge of its biochemical composition, functional and organoleptic characteristics of gulban infusions will help determine the commercial viability of this plant. Therefore, the aim of this study was to evaluate the biochemical composition, antioxidant activities and sensory characteristics of indigenous Australian bush foods, gulban, anise myrtle and lemon myrtle herbal infusions in comparison with well-known commercial green tea.

2. Materials and Methods

2.1. Tea sample collection and preparation

Green tea (GT) leaves (TRADITION fresh green tea, Good Young Co. Ltd., Taipei, Taiwan) were purchased from the local market in Brisbane (QLD, Australia). Anise myrtle (AM) and lemon myrtle (LM) leaves were supplied by Australian Rainforest Products Pty. Ltd.

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(Lismore, NSW, Australia). Gulban (GB) was a gift from Laura Egan (Enterprise Learning Projects, Katherine, NT, Australia) and Traditional Owner of Alawa Country, Northern Territory, and identified as *Melaleuca citrolens* by the Queensland Herbarium (Brisbane, QLD, Australia). The dry samples were ground into fine powder using a Retsch mixer mill (MM 400, Retsch GMBH. Haan, Germany). Ground samples (1 g) were brewed with boiled water (40 ml) for 5 min and extracts were decanted. All samples were extracted in triplicates and analyzed in duplicate for chemical assays.

2.2. Biochemical Analyses

2.2.1. Determination of total phenolic content

The total phenolic content in herbal infusions and green tea samples was determined using the Folin-Ciocalteu reagent assay method (Singleton and Rossi, 1965). Total phenolics were calculated from a calibration curve using standard gallic acid solutions (20-100 mg/l) used under the same conditions, and concentrations were expressed as mg gallic acid equivalents (GAE)/g sample.

2.2.2. Identification and quantification of phytochemical component using ultra-high performance liquid chromatography (UHPLC)

The identification and quantification of phytochemical component of herbal infusions were done using the method of Zhao et al. (2011) with slight modification using an UHPLC system. The system consisted of a Dionex UltiMate 3000 (Thermo Scientific, Waltham, MA, USA) equipped with a quaternary solvent delivery system, a column manager, and a sample manager. The instrument was fitted with an Acuity UHPLC® BEH Shield 1.7 μm , 2.1 x 100

mm (Waters, Milford, MA, USA). Chromatographic separation was carried out with mobile phase A (distilled water containing 0.1% formic acid) and mobile phase B (acetonitrile containing 0.1% formic acid). The linear gradient program was as follows: 0-12 min, 5-26% B; 12-14 min, 26-65% B. The flow rate was 0.5 ml/min, the column temperature was 40°C, the auto-sampler temperature was 15°C and the injection volume was 1 µl. Various standards (Sigma-Aldrich, St. Louis, MO, USA) were also run through UHPLC. Individual phenolic compounds in the samples were quantified using a calibration curve of the corresponding standard phenolic compound. Compounds (usually small peaks) not having a standard were ignored.

2.2.3. Quantitative analysis of organic acids

Herbal infusions and green tea samples were studied using high performance liquid chromatography (HPLC) analysis for the detection and quantification of organic acids. The system consisted of a Shimadzu HPLC LC-10A series system, consisting of a quaternary solvent delivery system, auto sampler, column oven and UV detector (Shimadzu Corporation, Kyoto, Japan). The separation was done at 30°C on a Synergi Hydro-RP column (4 µm, 4.6 x 250 mm). The mobile phase consisting of 20 mM potassium phosphate, pH 2.9 at a flow rate of 0.7 ml/min (Alasalvar et al., 2012). The sample injection volume was 10 µl, and the chromatogram was obtained at 220 nm. The standard calibration curves of citric, fumaric, glucuronic, malic, oxalic and tannic acids were obtained using HPLC grade pure acids (Sigma Aldrich). Individual acids in samples were quantified using a calibration curve of the corresponding standard acid.

2.3. Mineral and heavy metal analysis

Mineral and heavy metal analysis was done at Symbio Alliance Pty Ltd., (Brisbane, QLD, Australia) on dry powders of herbal and green tea leaves using inductively coupled optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. Elemental (Na, K, Zn, Ca, Fe, and Mg) testing was done using Symbio method code ESI02. Symbio method code ESM02 was used for heavy metal (Hg, Pb, Cd, As, Al, and Cr) analysis. The results of the tests are traceable to the Australian standards and expressed in mg/kg of dry sample.

2.4. Determination of antioxidant activity

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging activity was determined using the method of Nirmal and Panichayupakaranant (2015). Radical scavenging potential was expressed as IC_{50} value which represents the sample concentration at which 50% of the radicals are scavenged.

2.4.2. Reducing power

The reducing power was determined by using potassium ferricyanide and ferric chloride as substrates as described by Nirmal and Panichayupakaranant (2015). Higher absorbance of the reaction mixture indicated greater reducing power of the sample.

2.5. Sensory analysis

Sensory evaluation of herbal infusions and green tea samples were done using a 9-point hedonic scale as mentioned by Adnan et al. (2013) with slight modification. Briefly, herbal and

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tea samples were steeped with boiled water (1 g/40 ml) for 5 min. The liquid was collected into different vacuum flasks and served hot in a small plastic cup covered with a lid. All samples were labeled using a random three digit code. Sensory evaluation was done in a sensory laboratory at Health and Food Science Precinct, Department of Agriculture and Fisheries, Brisbane, Australia. Fifteen well trained and regular consumers of tea were selected as participants for sensory evaluation. Participants included male and female with ages from 22 to 45 years. . The participants were asked to evaluate each sample for the sensory attributes appearance, color, flavor, taste and overall acceptability. Each attribute was scored using the following 9 point scale: 9 = Like extremely; 8 = Like very much; 7 = Like; 6 = Like slightly; 5 = Neither like nor dislike; 4= Dislike slightly; 3 = Dislike moderately; 2 = Dislike; 1 = Dislike extremely, which for statistical purposes was considered to be linear.

2.6. Statistical analyses

All samples were extracted in triplicates and analyzed in duplicates for chemical assays, except the samples used for sensory analyses. Results were expressed as the mean \pm standard deviation (SD). Analyses of variance (ANOVA) were done and mean comparisons were done using the post-hoc Tukey's (HSD) and Duncan's range tests. $P < 0.05$ were considered statistically significant. Analysis was done using a XLSTAT package (Microsoft Excel, Redmond, WA, USA).

3. Results and discussion

3.1. Total phenolic content and major phenolic compounds

The total phenolic content of indigenous Australian herbal infusions is shown in Table 1. The total phenolic content ranged from 131 to 170 mg GAE/ g sample ($P < 0.05$). The highest total phenolic content was observed in green tea followed by gulban, anise myrtle and lemon myrtle herbal infusions ($P < 0.05$). The variation of total phenolic content in different tea leaves could be attributed to different species, quality of leaf, cultivars and agricultural conditions. Nirmal and Benjakul (2011) reported a total phenolic content of 118 mg GAE/g for a green tea sample, when extracted with hot water (80°C) for 20 min. Khokhar and Magnusottir (2002) measured green teas from different countries including India (106 mg GAE/g dry matter), China (87.0 mg GAE/g dry matter) and Japan (65.8 mg GAE/g dry matter). Anise myrtle (55.9 mg GAE/g dry matter) had higher total phenolic content than lemon myrtle (31.4 mg GAE/g dry matter), when extracted with 80% aqueous methanol/ 1% HCl (v/v) (Konczak et al., 2010). Chan et al. (2010) investigated the TPC in the various tropical and temperate herbal teas collected from different countries and found that lemon myrtle and green tea collected from Malaysia had TPC value of 7.56 and 14.1 mg GAE/g dry matter, respectively. The present result showed higher total phenolic content in anise myrtle (149 mg GAE/g sample) and lemon myrtle (131 mg GAE/g sample) as compared to those previously reported by Konczak et al. (2010). This could be due to different solvent extraction affinities for different phenolic compounds in the samples.

Quantitative analysis results of major phenolic compounds in indigenous Australian herbal infusions are shown in Table 2. In general, green tea sample showed the presence of higher phenolic compounds as compared to indigenous Australian herbal infusions ($P < 0.05$). When compared among indigenous Australian herbal infusions, the higher amount of phenolic compounds was observed in lemon myrtle followed by anise myrtle and gulban. This indicated that gulban infusions might have other phenolic compounds that were not tested. Catechin derivatives were found to be highest in green tea and lowest in gulban ($P < 0.05$). Among the phenolic compounds, caffeine was found at a significantly higher amount in the green tea.

However, no caffeine was found in any of the indigenous Australian herbal infusions. Caffeoylquinic acid and hesperitin were found only in gulban herbal infusions. The green tea variety *Fanning Belas* from China contained active ingredients mainly catechins (191 g/kg dry leaf) and caffeine (36 g/kg dry leaf) (Perva-Uzunalić et al., 2006). Caffeine content was highest in green tea (38 - 42.4 g/kg) as compared to black tea (23.4 - 43.3 g/kg) samples commercialized in Pakistan (Adnan et al., 2013). The tea composition is affected by climate, season, tea variety and age of the leaves (Perva-Uzunalić et al., 2006). Konczak et al. (2010) reported that anise myrtle and lemon myrtle extracts contained quercetin and hesperitin aglycones as major compounds which include a rhamnoside, a pentoside, a hexoside and rutinoside as identified using LC/MS.

3.2. Minerals and heavy metals content

Minerals and heavy metals are bio-accumulated and bio-transferred by natural sources. Heavy metals above their normal level are a threat for both plant and animal life. Six different minerals (Na, K, Zn, Ca, Fe and Mg) and heavy metals (Hg, Pb, Cd, As, Al and Cr) of the herbal infusions and green tea were measured and the results are shown in Table 3. In general, minerals and heavy metals of all tea samples varied significantly. The significant variation of metal content of different tea samples can be correlated with the differences in soil properties, species, climatic conditions, harvesting time, geographical origin and use of fertilizer (Adnan et al., 2013; Alasalvar et al., 2013). K, Ca and Mg were significantly higher and major minerals were present in all infusions and tea sample. Similar results were reported for the fresh and processed *Buddleja saligna* teas from Southern Africa (Moodley et al., 2015). K was significantly higher in green tea as compared to Australian herbal infusions. Ca was higher in gulban and lemon myrtle infusions in comparison with green tea and anise myrtle infusions. Whereas, Mg was higher in gulban and anise myrtle as compared to green tea and lemon myrtle samples. Lemon myrtle

followed by green tea were observed with higher content of Fe. The Fe content of lemon myrtle (168 mg/kg sample) was similar to *Buddleja saligna* green tea (167 mg/kg sample) from Southern Africa (Moodley et al., 2015). Gulban and anise myrtle infusions are good sources of Na, K, Ca and Mg. However, lemon myrtle has Fe and green tea has Zn in addition. The tested minerals content of anise myrtle and lemon myrtle were more or less similar with the earlier report of Konczak et al. (2009). Negligible amounts of tested heavy metals, except Al were observed in all the samples. Al was observed in higher amount in green tea (706 mg/kg) followed by lemon myrtle (68.6 mg/kg), anise myrtle (25.6 mg/kg) and gulban (13.4 mg/kg). Naturally high Al content foods include tea, potatoes, spinach and the daily intake limit of Al in Australia is between 1.9-2.4 mg per day (WHO, 1998).

3.3. Organic acids content

Organic acids are synthesized in plants as a result of the incomplete oxidation of photosynthetic products and represent the stored form of fixed carbon (Igamberdiev and Eprintsev, 2016). Six different organic acids (citric, fumaric, glucuronic, malic, oxalic and tannic) were quantified in herbal infusions and tea samples (Table 4). Citric acid was detected only in gulban (5.1 ± 0 g/kg) and anise myrtle (10.3 ± 0 g/kg) samples, whereas malic acid was found only in green tea (1.2 ± 0 g/kg). Oxalic acid was detected in green tea (9.3 ± 1 g/kg), gulban (2.0 ± 0 g/kg) and anise myrtle (4.3 ± 0 g/kg), but not in lemon myrtle ($P < 0.05$). The presence of oxalic acid was significantly higher in green tea samples in comparison with gulban and anise myrtle ($P < 0.05$). HPLC analysis indicated that oxalic and malic acids are the major acids present in green tea samples compared to Australian herbal infusions ($P < 0.05$). Fumaric, glucuronic and tannic acid were not found in any of the tested samples. These results are in agreement with Harbowy et al. (1997) where they reported that green tea is a significant source of oxalic and malic acid along with citric, shikimic and quinic acids. Anise myrtle and gulban

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are good sources of citric acid ($P < 0.05$). However, none of the above organic acids were detected in lemon myrtle tea at the given extraction conditions. The composition of organic acids in plants varies depending on the species, age of the plant and the tissue type (Lopez-Bucio et al., 2000). Organic acids also are important during nutrient deficiencies, for metal tolerances and for plant-microbe interactions at the root-soil interface (Lopez-Bucio et al., 2000). Therefore, the presence of organic acids is of importance for plant physiology. However, organic acids in green leaves and fruits are known for their sour, tart and astringency notes in foods or beverages. Therefore, organic acids have a role in the sensory characteristics of herbal infusions and tea samples.

3.4. Antioxidant activities

The antioxidant properties of samples were determined using DPPH radical scavenging activity and reducing power. The IC₅₀ value of samples for DPPH radicals are shown in Table 1. The scavenging potential of samples is represented by the percentage discoloration of DPPH radical, which is attributed to the hydrogen donating ability of test compounds. The lower IC₅₀ value of test samples indicated the higher antioxidant potential. In general, standard gallic acid was the most powerful DPPH radical scavenger with the lowest IC₅₀ value (3.9 µg/ml) compared to all samples ($P < 0.05$). When compared among the samples, green tea showed the highest activity with the lowest IC₅₀ value, whereas lemon myrtle had lower activity with higher IC₅₀ value ($P < 0.05$). Polyphenols from herbal infusions have a role in antioxidant activity (Bekdeser et al., 2014).

Figure 1 shows the reducing power of samples. Generally, increases in the concentration of samples showed increased reducing power in a dose dependent manner ($P < 0.05$). Reducing power of the samples is associated with the presence of reductones, which are capable of breaking the free radical chain by donating a hydrogen atom (Nirmal and Panichayupakaranant,

2015). At lower concentrations there were no significant differences between the samples. However, at higher concentration, green tea and gulban showed higher reducing power as compared to anise myrtle and lemon myrtle infusions ($P < 0.05$). These results are in agreement with the DPPH radical scavenging activity of each sample. Antioxidant activities are in agreement with the total phenolic content of samples. Rusaczek et al. (2010) studied the TPC and antioxidant properties of teas and herbal infusions including green tea, black tea, pu-erh tea, white tea, lemon balm, peppermint and chamomile. They noticed that TPC and antioxidant properties were considerably lower in herbal infusions compared to *Camellia sinensis* teas ($P < 0.05$). Benzie and Szeto (1999) reported that different types of tea showed different antioxidant power and their antioxidant capacity was strongly correlated with the total phenolic content of the tea.

3.5. Sensory attributes of herbal infusions and tea

Sensory evaluation can provide direct measurement of perceived intensities of tea attributes. Table 5 shows the liking score of commercial green tea and Australian indigenous herbal infusions. The appearance and color liking scores were higher for lemon myrtle and anise myrtle and lower for gulban infusion ($P < 0.05$) (Figure 2). The flavor score was higher for gulban in comparison with commercial green tea and lemon myrtle. Quality and consumer acceptability of teas are affected by the content of catechins, caffeine, amino acids, theaflavins and other volatile components. (Adnan et al., 2013) The gulban was appreciated because of its flavor and taste characteristics ($P < 0.05$). The highest overall acceptability score of gulban, lemon myrtle and anise myrtle infusions could be related with other volatile components and lower bitterness due to absence of caffeine compared to green tea ($P < 0.05$). Flavor is the most important sensory attribute for tea evaluation and flavor is comprised of the taste from nonvolatile compounds (e.g., organic acids, sugars and amino acids) and aromas from volatile compounds (e.g.,

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aldehydes, alcohols, furan, ketones and aromatic compounds) (Alasalvar et al., 2012). Overall liking scores of indigenous Australian herbal infusions were higher than green tea due to flavor and taste characteristics, which are influenced by the biochemical composition of the herbal leaves.

4. Conclusion

Indigenous Australian herbal infusions including gulban, anise myrtle and lemon myrtle had lower phenolic content than the commercial green tea. Gulban infusions showed similar antioxidant capacity as commercial green tea. However, anise and lemon myrtle showed slightly lower antioxidant activity as compared to green tea and gulban. Indigenous Australian herbal infusions were a good source of minerals mainly Ca and Mg. High levels of citric acid were observed in gulban and anise myrtle herbal infusions. Moreover, caffeine was not found in indigenous Australian herbal infusions. Interestingly, flavor, taste and overall acceptability scores were higher for indigenous Australian herbal infusions when compared with green tea. Therefore, indigenous Australian herbs have the potential to be used for commercial herbal infusions.

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Conflict of Interests:

None.

References:

- Adnan, M., Ahmad, A., Ahmed, A., Khalid, N., Hayat, I., & Ahmed, I. (2013). Chemical composition and sensory evaluation of tea (*Camellia sinensis*) commercialized in Pakistan. *Pakistan Journal of Botany*, 45(3), 901-907.
- Alasalvar, C., Pelvan, E., Ozdemir, K. S., Kocadagli, T., Mogol, B. A., Pasli, A. A., Ozcan, N., Ozcelik, B., & Gokmen, V. (2013). Compositional, nutritional, and functional characteristics of instant teas produced from low- and high-quality black teas. *Journal of Agriculture and Food Chemistry*, 61(31), 7529-7536.
- Alasalvar, C., Topal, B., Serpen, A., Bahar, B., Pelvan, E., & Gokmen, V. (2012). Flavor characteristics of seven grades of black tea produced in Turkey. *Journal of Agriculture and Food Chemistry*, 60(25), 6323-6332.
- Bekdeser, B., Durusoy, N., Ozyurek, M., Guclu, K., & Apak, R. (2014). Optimization of microwave-assisted extraction of polyphenols from herbal teas and evaluation of their *in vitro* hypochlorous acid scavenging activity. *Journal of Agriculture and Food Chemistry*, 62(46), 11109-11115.
- Benzie, I. F. F., & Szeto, Y. T. (1999). Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *Journal of Agriculture and Food Chemistry*, 47(2), 633-636.
- Chan, E. W. C., Lim, Y. Y., Chong, K. L., Tan, J. B. L., & Wong, S. K. (2010). Antioxidant properties of tropical and temperate herbal teas. *Journal of Food Composition and Analysis*, 23(2), 185-189.

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- Chatterjee, P., Chandra, S., Dey, P., & Bhattacharya, S. (2012). Evaluation of anti-inflammatory effects of greenta and black tea: A comparative *in vitro* study. *Journal of Advanced Pharmaceutical Technology and Research*, 3(2), 136-138.
- Gorjanovic, S., Komes, D., Pastor, F. T., Belscak-Cvitanovic, A., Pezo, L., Hecimovic, I., & Suznjevic, D. (2012). Antioxidant capacity of teas and herbal infusions: Polarographic assessment. *Journal of Agriculture and Food Chemistry*, 60(38), 9573-9580.
- Harbowy, M. E., Balentine, D. A., Davies, A. P., & Cai, Y. (1997). Tea chemistry. *Critical Reviews in Plant Sciences*, 16(5), 415-480.
- Igamberdiev, A. U., & Eprintsev, A. T. (2016). Organic acids: The pools of fixed carbon involved in redox regulation and energy balance in higher plants. *Frontiers in Plant Science*, 7, 1042.
- Khokhar, S., & Magnusdottir, S. G. M. (2002). Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *Journal of Agriculture and Food Chemistry*, 50, 565-570.
- Konczak, I., Zabarar, D., Dunstan, M., Aguas, A., Roulfe, P., & Pavan, A. (2009). Health benefits of Australian native foods - an evaluation of health-enhancing compounds. *RIRDC*, 9(133), 1-52.
- Konczak, I., Zabarar, D., Dunstan, M., & Aguas, P. (2010). Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. *Food Chemistry*, 122(1), 260-266.
- Lopez-Bucio, J., Nieto-Jacobo, M., F., Ramirez-Rodriguez, V., & Herrera-Estrella, L. (2000). Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Science*, 160, 1-13.
- Mazina, J., Vaher, M., Kuchtinskaja, M., Poryvkina, L., & Kaljurand, M. (2015). Fluorescence, electrophoretic and chromatographic fingerprints of herbal medicines and their comparative chemometric analysis. *Talanta*, 139, 233-246.

- Moodley, T., Amonsou, E. O., & Kumar, S. (2015). Nutritional quality and acceptability of *Buddleja saligna* herbal tea. *Journal of Food Science and Technology*, 52(11), 7519-7524.
- Nirmal, N. P., & Benjakul, S. (2011). Use of tea extracts for inhibition of polyphenoloxidase and retardation of quality loss of Pacific white shrimp during iced storage. *LWT - Food Science and Technology*, 44(4), 924-932.
- Nirmal, N. P., & Panichayupakaranant, P. (2015). Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. *Pharmaceutical Biology*, 53(9), 1339-1343.
- Perva-Uzunalić, A., Škerget, M., Knez, Ž., Weinreich, B., Otto, F., & Grüner, S. (2006). Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. *Food Chemistry*, 96(4), 597-605.
- Rusaczonek, A., Swiderski, F., & Waszkiewicz-Robak, B. (2010). Antioxidant properties of tea and herbal infusions - a short report. *Polish Journal of Food and Nutrition Sciences*, 60(1), 33-35.
- Sakulnarmrat, K., Fenech, M., Thomas, P., & Konczak, I. (2013). Cytoprotective and pro-apoptotic activities of native Australian herbs polyphenolic-rich extracts. *Food Chemistry*, 136(1), 9-17.
- Schwalfenberg, G., Genuis, S. J., & Rodushkin, I. (2013). The benefits and risks of consuming brewed tea: Beware of toxic element contamination. *J Toxicol*, 2013, 370460.
- Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.
- WHO. (1998). Aluminium in drinking water. In *Guidelines for Drinking Water Quality* 2 ed., vol. 2). Geneva: World Health Organization.

Wilkinson, J. M., & Cavanagh, H. M. (2005). Antibacterial activity of essential oils from Australian native plants. *Phytotherapy Research*, 19(7), 643-646.

Zhao, Y., Chen, P., Lin, L., Harnly, J. M., Yu, L. L., & Li, Z. (2011). Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS. *Food Chemistry*, 126(3), 1269-1277.

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Tables:

Table 1: Total phenolic content and DPPH radical scavenging activities (IC₅₀ value) of Australian indigenous herbal infusions

Tea	Total phenolic content (mg GAE/g sample)	DPPH radical scavenging activity
		IC ₅₀ value (µg/ml)
Green	170 ^a + 3	20.4 ^c
Gulban	156 ^b + 5	21.3 ^c
Anise myrtle	150 ^c + 10	24.4 ^b
Lemon myrtle	131 ^d + 1	33.7 ^a
Gallic acid	NA	3.9 ^d

Mean + SD (n=6). Mean with different letters in columns are significantly different (P<0.05).

NA= Not applicable

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Table 2: Quantitative analyses of major phenolic compounds in Australian indigenous herbal infusions

Compound (mg/kg)	Sample			
	Green	Gulban	Anise myrtle	Lemon myrtle
Catechins derivative				
Catechins	22 + 1	8.1 + 1	46 + 1	11.2 + 0
Epicatechin	120 + 10	0.3 + 0	NF	21.8 + 0
Epigallocatechin	180 + 10	0.3 + 0	0.1 + 0	5.1 + 1
epigallocatechin gallate	470 + 10	NF	NF	0.8 + 0
Galocatechin	32 + 5	5.4 + 1	1.6 + 0	2.5 + 0
Other phenolics				
Gallic acid	215 + 5	88 + 6	48 + 4	140 + 10
Gallyoylquinic acids 4	82 + 6	32 + 3	NF	NF
Caffeine	20000 + 1000	NF	NF	NF
Caffeic acid	1.8 + 0	NF	NF	NF
Caffeoylquinic acid	NF	12 + 1	NF	NF
Chlorogenic acid	4.9 + 0	1.3 + 0	1 + 0	4.7 + 1
Hesperitin	NF	0.1 + 0	NF	NF
Kaempferol	0.5 + 0	0.1 + 0	0.1 + 0	T
Rutin	16 + 1	NF	19 + 1	NF

Mean + SD (N=6), NF = Not found; T= Trace

Table 3: Minerals, heavy metals and ash content of Australian indigenous herbal infusions

Analyses	Samples			
	Green	Gulban	Anise myrtle	Lemon myrtle
Minerals (mg/kg)				
Sodium (Na)	34	1700	610	340
Potassium (K)	16,900	8140	5520	9580
Zinc (Zn)	22.5	16.9	16.3	12.7
Calcium (Ca)	3800	11000	3100	15000
Iron (Fe)	112	49.6	43.0	168
Magnesium (Mg)	1740	2890	2820	1880
Heavy metals (mg/kg)				
Mercury (Hg)	<0.01	0.015	0.021	0.026
Lead (Pb)	0.27	0.13	0.18	0.17
Cadmium (Cd)	0.026	<0.016	0.019	<0.01
Arsenic (As)	0.058	0.055	0.035	0.063
Aluminium (Al)	706	13.4	25.6	68.6
Chromium (Cr)	0.41	0.53	0.50	1.33
Ash (%)	5.2	5.4	4.5	6.0

Table 4: Organic acid content of Australian indigenous herbal infusions

Acids	Samples (g/kg)			
	Green	Gulban	Anise myrtle	Lemon myrtle
Citric	ND	5.1 + 0	10.3 + 0	ND
Fumaric	ND	ND	ND	ND
Glucuronic	ND	ND	ND	ND
Malic	1.2 + 0	ND	ND	ND
Oxalic	10 + 1	2.0 + 0	4.3 + 1	ND
Tannic	ND	ND	ND	ND

Mean + SD (N=6). Mean with different letters in columns are significantly different (P<0.05).

ND = Not detected

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Table 5: Nine point hedonic sensory scores of green tea, gulban, anise myrtle and lemon myrtle infusions

Tea	Sensory attributes				
	Appearance	Color	Flavor	Taste	Overall
Green	6.4 ^b + 2	6.0 ^c + 2	5.2 ^c + 2	5.1 ^b + 2	5.6 ^b + 2
Gulban	5.9 ^c + 2	5.3 ^d + 2	6.8 ^a + 2	6.6 ^a + 1	6.6 ^a + 1
Anise myrtle	6.8 ^b + 1	6.9 ^b + 1	6.1 ^b + 1	5.8 ^b + 1	6.1 ^a + 1
Lemon myrtle	7.3 ^a + 1	7.4 ^a + 1	5.8 ^c + 1	5.6 ^b + 1	6.2 ^a + 1

Mean + SD (n=15). Mean with different letters in columns are significantly different (P<0.05).

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Figure legends:

Figure 1. Reducing power of herbal infusions at different concentrations. Mean + SD (N=6)

Mean with different letters with the same concentration are significantly different ($P < 0.05$).

GT; Green tea, GB; Gulban, AM; Anise myrtle, LM; Lemon myrtle.

Figure 2. Photograph of herbal infusions after having been brewed with boiled water (1g/40 ml) for 5 min.

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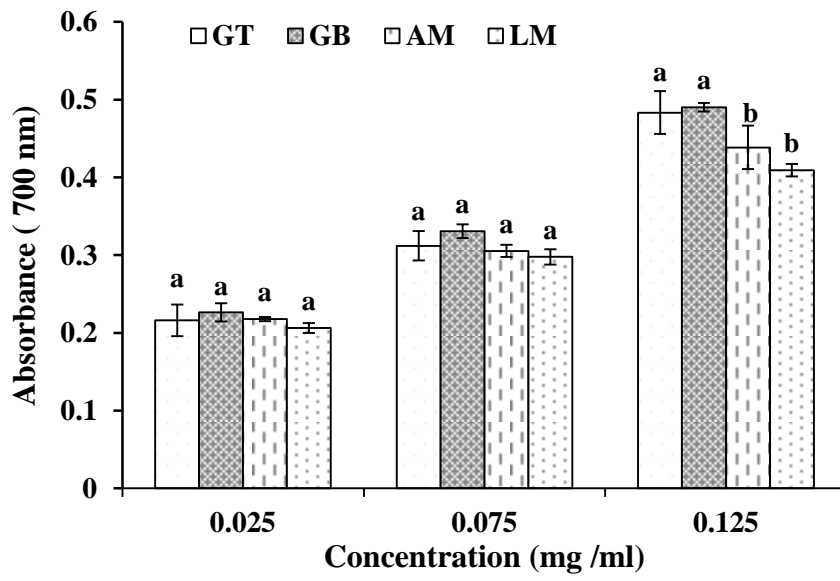


Figure 1.



Figure 2.

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Highlights:

- Gulban, anise myrtle and lemon myrtle are the Australian indigenous bush food plants.
- Australian indigenous herbal infusions are a good source of Ca, Mg and citric acid.
- Caffeine was not found in Australian indigenous herbal infusions.
- Overall liking score was higher for Australian indigenous herbal infusions compared to green tea.

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