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Evaluation of antioxidant capacity of *Aidia borneensis* leaf infusion, an endemic plant in Brunei Darussalam

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Abstract

We investigated the total antioxidant capacity of *Aidia borneensis* leaf infusion, a Bornean endemic plant, which is traditionally consumed as a home-remedy beverage in Brunei Darussalam. The antioxidant capacity of the infusion of *A. borneensis* leaves was evaluated by 2,2-diphenyl-1-picryhydrazyl (DPPH) radical-scavenging ability. We found that the infusion shows a relatively high antioxidant capacity, and it was attributed to its high phenolic, flavonoid, and flavanol contents which were evaluated by Folin–Ciocalteu reagent, colorimetric assay, and aluminum chloride colorimetric method, respectively. By comparing its total antioxidant capacity, we estimated that the infusion of *A. borneensis* leaves is in the middle rank among twelve different commercially available *Camellia sinensis* teas. Our findings would have significant implications on *A. borneensis* products from Brunei Darussalam and on the feasibility of establishing this new beverage among the commercially available conventional *C. sinensis* and herbal teas.

1. Introduction

Antioxidants are compounds that inhibit the formations of free radicals and lipid peroxidations, which are responsible for many diseases and aging process in the human body (Ostrowska *et al*., 2001; Karimi *et al*., 2011; Gonbad *et al*., 2015). Among the antioxidants, polyphenol derivatives such as phenolic, flavonoid, and flavanol contents naturally found in plants are the most beneficial (Salah *et al*., 1995; Zandi and Gordon, 1995; Kim *et al*., 2003; Karimi *et al*., 2011). In addition to those polyphenols, the existences of phytochemical constituents, such as ascorbic acid, tocopherols, and carotenoids in consumable plants enhance their total antioxidant capacity (Chatterjee *et al*., 2013). Therefore, entire leaves either raw, cooked or processed into beverages are commonly consumed. Several research on herbal plants have suggested that their vitamins A, C, and E, carotenoids, polyphenolics and flavonoid contents also have antioxidant capacities (Karimi *et al*., 2011). Thus, they have medicinal values in the treatment of many human diseases, including cancer, cardiovascular diseases, and inflammatory diseases (Krishnaiah *et al*., 2011). For instance, the herbal tea infusions (also known as tisanes) made from fresh or dried flowers, leaves, seeds or roots are also widely consumed worldwide. These herbal tea infusions are believed to have medicinal effects, especially for their stimulant, relaxant, or sedative properties (Kara, 2009).

In case of beverages, many studies have confirmed that leaves of tea (*Camellia sinensis* which belongs to *Theaceae* family) possess considerable antioxidant capacity (Yen and Chen, 1995; Robinson *et al*., 1997; Rice-Evans, 1999; Gardner *et al*., 2007; Pekal *et al*., 2012; Gonbad *et al*., 2015; Hajiaghaalipour *et al*., 2016). It has also been demonstrated that flavonoid compounds such as epigallocatechin gallate, epicatechin gallate, epigallocatechin, and epicatechin accumulated in tea leaves at all their fermentation levels act as effective scavengers of free radicals (Salah *et al*., 1995). More interestingly, the polyphenols extracted from tea infusions in ethanol solution have stronger antioxidant, anticarcinogenic, and antimutagenic properties, but they are less toxic as compared with the synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and dl-α-tocopherol (Chen and Wan., 1994). Therefore, *C. sinensis* tea has been considered to

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have potential health-promoting properties, due to its ability to reduce the risks of coronary heart disease (Stensvold *et al*., 1992; Emerit, 1994; Halliwell, 1996; Hertog *et al*., 1997), stroke incidence (Keli *et al*., 1995; Peters *et al*, 2001), chronic inflammation (De Bacquer *et al*., 2006), and cancer incidence (Arab and Il'yasova, 2003; Chung *et al*, 2003).

Driven by such high health benefits of natural antioxidants, exploration on new potential plants possessing high antioxidant capacity is of interest (Ariffin *et al*., 2011). Through such exploration, one could expect that there will be more varieties of healthy and good quality beverages in addition to the existing *C. sinensis* teas. In this regard, we have focused our research efforts on plants locally grown in Brunei Darussalam, where endemic plant species are remarkably plentiful. Those plants are uncultivated, but many of them have been utilized in traditional medicines to treat many diseases. Several of those important medicinal plants have been reported by Goh *et al*. (2017), but extensive researches on those plants are currently pursued. One of those plants is *Aidia borneensis*, a Bornean endemic plant and locally known as *Sambah bagangan*, belonging to *Rubiaceae* family (Goh *et al*., 2017). In general, this family includes perennial evergreen plants, which require humid and warm environmental geographic regions. *A. borneensis* grows up to about 2 m high, as depicted in Figure 1A. This plant thrives well on hilly and drained soils under the shadow of big trees, and it has the unique pinkish-red color of young leaves and green color of matured leaves, as shown in Figure 1B-C.

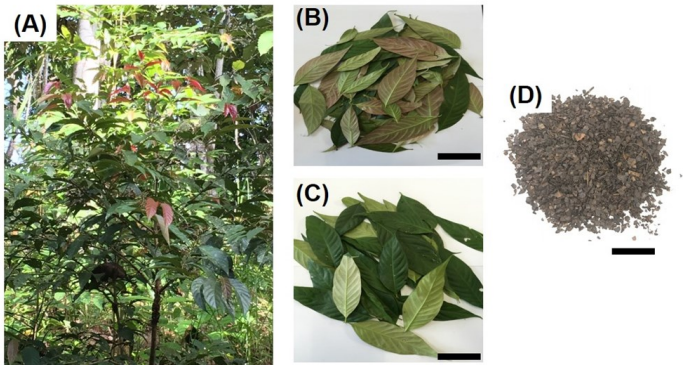

Figure 1. Representative images of (A) the plant, (B) young leaves, (C) matured leaves, and (D) the commercial tea of *A. borneensis*. The scale bars of 10 cm are shown in the images B-D.

Traditionally, infusion of *A. borneensis* leaves are treated as beverages, where a decoction is prepared from the crude or dried leaves. It is consumed as a hot drink and it is believed to be able to relieve body aches, tiredness, and gastric pains (Goh *et al*., 2017). Intensive researches characterizing and quantifying the beneficial constituents as well as principle substances in this particular plant leaves are indispensable. In the present study, we have evaluated the antioxidant capacity of infusion *A. borneensis* leaves (Figure 1D).

In order to assess relative potential of infusion of *A. borneensis* leaves, we have also compared its total antioxidant capacity to those found in twelve different commercially available *C. sinensis* black, Pu Er, and herbal teas. We should consider that this is a comparison without assessing numerous factors that may affect chemical constituents in the commercial teas according to cultivation type, growth conditions, horticultural practices, and different treatments by the tea manufacturer. The relationship between the total antioxidant capacity and total phenolic, flavonoid, flavanol, catechin, and caffeine contents in the infusion of *A. borneensis* leaves were also investigated. This is the first report on the antioxidant capacity and chemical constituents in *A. borneensis* leaf infusion. Our findings would have significant implications on *A. borneensis* tea and on its feasibility among the conventional commercially available *C. sinensis* and other herbal teas.

2. Materials and methods

2.1 Chemicals and equipment

Dried leaves or tea of *A. borneensis* was obtained from the local factory (3MPK Herbal Tea, Kampong Kiudang, Brunei Darussalam). All chemicals in this work were obtained from different sources; namely 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid monohydrate, quercetin, and catechin hydrate were purchased from Sigma-Aldrich (St. Louis, MO, USA), Folin-Ciocalteu reagent, sodium carbonate, sodium nitrite, aluminium chloride, sodium acetate, and methanol were purchased from Merck (Darmstadt-Germany), and caffeine were obtained from Fluka (Buchs, Switzerland). All these chemicals were mainly in analytical grade, except for catechin hydrate (in HPLC grade), and they were used as received without any further purifications. Distilled water was used throughout the sample preparations and measurements. All absorption measurements were performed on a single-beam UV-visible spectrophotometer (Optizen model 1412V, Korea). In all measurements, the sample was contained in a 2 mL disposable cuvette with 1.2 cm optical path, and the absorbance was recorded at a specific wavelength.

2.2 General procedure of sample preparations

Infusion of *A. borneensis* leaves was prepared using an aqueous extraction according to a standard procedure, similarly to typical household tea preparation. Briefly, 2 g of granular dried leaves of *A. borneensis* (Figure 1D) was soaked in 200 mL distilled water while stirring thoroughly with a glass rod, allowing their constituents to infuse into the solution. The infusion was then passed through a filter paper (Whatman Grade 1) with the pore size of 11 µm, and the filtrate was kept at room temperature to cool down to 20–25°C. For chemical analyses, depending on each specific assay the infusion was diluted with distilled water to achieve concentration of the substances roughly within 10–4000 ppm.

2.3 The effects of brewing temperature and brewing time

To evaluate the effect of brewing temperature and time on the antioxidant capacity, we have prepared an infusion by soaking 2 g of dried leaves of *A. borneensis* directly into 200 mL distilled water. For the effect of brewing temperature, the distilled water was set to be at 10, 20, 40, 60, 80, and 100°C for a constant brewing time at 3 min. On the other hand, the effect of brewing time was inspected from the samples prepared in the distilled water at 80°C with various brewing time in the range of 1–120 min.

2.4 Determination of total antioxidant capacity by DPPH assay

The total antioxidant activity of the infusion of *A. borneensis* leaves was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to the modified procedures reported by Delgado-Andrade *et al*. (2005). Here, a 200 µL of the

$$\%\ inhibition\ of\ DPPH\ activity = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100\%$$

infusion with various concentrations within 10–1000 ppm was added into freshly prepared 1 mL DPPH (50 ppm) in methanol. The mixture was allowed to stand in the dark at room temperature for 30 min. The monochromatic absorption of the mixture then was recorded at 517 nm against methanol, and a DPPH solution without infusion was used as a blank or control solution. The DPPH free radicals in the solution are scavenged by an antioxidant or a free radical species, resulting in a decrease in the intensity of its absorption band. Consequently, lower absorption intensity of the mixture indicated higher free radical scavenging activity (Yen *et al*., 1997), and DPPH free radical scavenging activities of the infusions were calculated according to where $A_{control}$ is the absorbance of the control solution and A_{sample} is the absorbance of the infusion. We have plotted a curve of % inhibition of DPPH activity against concentration of the infusions (data not shown), and from the plot we deduced IC_{50} value which was attributed to the concentration of the infusions required for 50% inhibition. Radical scavenging activities of well-known antioxidants, namely gallic acid monohydrate and quercetin, have also been measured for comparison or calibration. Thus, the total antioxidant activity of the infusions associated with that of gallic acid calibration curve is considered as gallic acid equivalents (GAE) and it is expressed as in ppm standard DPPH equivalent.

2.5 Determination of total phenolic content

Total phenolic content (TPC) in the infusions was determined according to Folin-Ciocalteu method based on the procedure used by Chan *et al*. (2007). For this TPC determination, 300 µL of diluted infusions were introduced into sample vial followed by 1.2 mL sodium carbonate (7.5% w/v) and 1.5 mL Folin-Ciocalteu reagent (diluted by 10 fold) in distilled water. The color of the mixture turned from yellow to blue, and this mixture was allowed to stand for 30 min at room temperature. Absorption of the mixture then was recorded at a single wavelength at 765 nm. The calibration curve of gallic acid was plotted in the range of 25–200 ppm. The TPC of the infusion was calculated from linear regression of the calibration curve. Thus, TPC was expressed as gallic acid equivalents (GAE) in mg/100 g dry sample.

2.6 Determination of total flavonoid content

Total flavonoid content (TFC) in the infusion was measured using a slightly modified colorimetric assay according to the procedure reported by Damiani *et al*. (2014). Briefly, 100 µL of the infusions was dissolved in 1.35 mL distilled water in a sample vial. Into the solution, 50 µL sodium nitrite (5% w/v) were added, followed by 50 µL aluminium chloride (10% w/v). The mixture was mixed well-using vortex (Model V-1 plus, Biosan, Latvia) and it was allowed to stand at room temperature in the dark for 10 min. Similarly, we have appropriately prepared standard ethanolic solutions of catechin hydrate in the same way. Absorption of the mixture and the standard solutions was recorded at a single wavelength at 415 nm. The TFC of the infusion was determined from linear regression of a calibration curve, which was obtained from catechin hydrate with different concentrations in the range of 25–1000 ppm.

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Therefore, the TFC of the infusion was expressed as catechin equivalent in mg/100 g dry sample.

2.7 Determination of total flavanol content

Total flavanol (TF) content in the infusions was determined using the aluminium chloride colorimetric method according to the procedure reported by Iqbal *et al*. (2015) with slight modifications. Briefly, the infusion (1.5 mL) was put in a test tube, followed by addition of 1 mL aluminium chloride (2% w/v) in distilled water. Then, 3 mL sodium acetate (5% w/v) was added, and the mixture was mixed well using vortex. The mixture then was centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 440 nm. In this measurement, ethanolic quercetin solutions which were prepared similarly to the samples of the infusions were used as a standard, and the calibration curve was obtained from the absorbance of the standard solutions with different concentrations in the range of 1–100 ppm. The TF of the infusion was determined from linear regression of the calibration curve, and it is expressed as quercetin equivalent concentration in mg/100 g dry sample.

2.8 Data analysis

In this work, appropriate treatments were carried out throughout all the experiments. All measurements on the infusions and control solutions have been performed at least in triplicate and all data have been analyzed. The data presented in this report represent the average values.

3. Results and discussion

3.1 Total antioxidant capacity of A. borneensis leaf infusion

The total antioxidant capacity of the infusion of *A. borneensis* leaves has been analyzed by quantifying their DPPH free radical scavenging. The IC_{50} of 2 g dried leaves of *A. borneensis* infused in 200 ml water was measured to be within 312 to 805 ppm, equivalent to 1440 to 3730 mg gallic acid per 100 g dry sample of *A. borneensis* leaves. This indicates that the infusion of *A. borneensis* leaves is a significant source of antioxidants. According to the DPPH free radical scavenging, the total antioxidant capacity depends on the brewing time and temperature of the infusions, as described below.

3.2 Effect of brewing time

By plotting IC_{50} of the infusion against brewing time, as shown in Figure 2A, we demonstrate that its total antioxidant capacity increases gradually with the

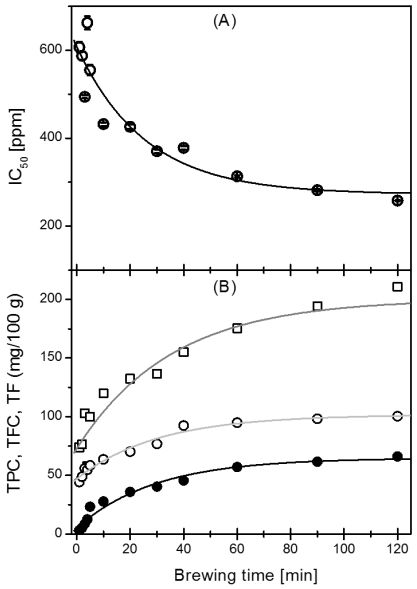

Figure 2. The effect of different infusion time on; (A) the total antioxidant activity, and (B) on the TPC (□), TFC (○), and TF (●) contents of *A. borneensis* tea. The solid lines are the best single exponential fits for the data. In the plot, the TPC has been scaled down by factor of 10 for the sake of clarity.

brewing time. The similar trend has also been observed in all *C. sinensis* teas (Ryan and Petit, 2010; Fernando and Soysa, 2015; Nikniaz *et al*., 2016). This may not be surprising because there are more phytochemicals and polyphenol derivatives could be permeated from the leaves into the solution at longer infusion time. However, we should note that at brewing temperature, 80°C, more than 50% of the total antioxidant capacity was achieved during the first 3 min infusion time. The total antioxidant capacity increases exponentially with infusion time and it is saturated at infusion times longer than 1 hour, indicating that the optimum extraction capacity is reached. This implies that water could effectively extract the antioxidants from *A. borneensis* leaves, and most of them should pass through the openings or interstices on the leaves in the early time of infusion. We may consider that this phenomenon should be similar to the cases of a liquid passing through a membrane due to different concentrations, where its concentration increases exponentially. Therefore, the concentrations of the antioxidants in the infusion may obey first- or second-order kinetics (Peleg, 1988; Lafka *et al*., 2013; Fernando and Soysa, 2015). To clarify this issue, we have fitted our data with a single exponential function. As shown in Figure 2A, the single-exponential fitting gives an infusion rate constant of the antioxidants to be 0.040 ± 0.005 min^{-1}. The validity of this consideration is unambiguously supported by the infusion-time dependence of TPC, TFC, and TF content of *A. borneensis* leaves, where their infusion rate constant is also within the range of 0.035–0.040 min^{-1}, as shown in

Figure 2B. These findings further indicate that the total antioxidant of *A. borneensis* leaves is clearly related to its TPC, TFC, and TF contents. We may also note that TPC, TFC, and TF contents, which are considered to be an important criterion of tea quality, were measured to be in the range of 739–2110, 440–1005, and 3.0–66.5 mg per 100 g dried leaves of *A. borneensis*, respectively.

3.3 Effect of brewing temperature

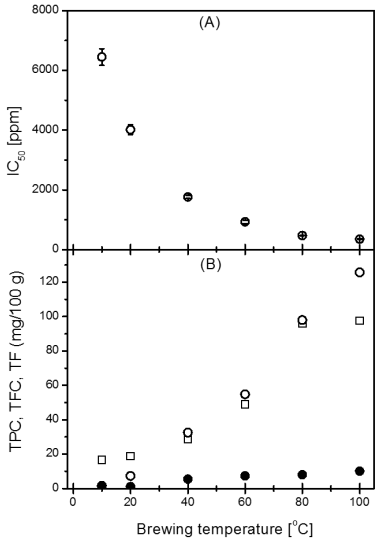

Figure 3. The effect of different infusion temperature on; (A) the total antioxidant activity, and (B) on the TPC (□), TFC (○), and TF (●) contents of *A. borneensis* tea (B). In the plot, the TPC has been scaled down by factor of 10 for the sake of clarity.

Figure 3A shows that the total antioxidant capacity of *A. borneensis* leaves increases abruptly with brewing temperature (in the range of 10–100°C). A similar tendency has also been observed for *C. sinensis* black and green teas, where infusion with greater antioxidant capacity was obtained at longer brewing time and higher temperatures (Sharpe *et al*., 2016). This finding implies that more phytochemicals and polyphenol derivatives from the *A. borneensis* leaves are infused into the water due most probably to the existences of more openings and interstices on the leaves, increasing the diffusion rate at higher brewing temperature. We found that more than 90% of the total antioxidant capacity was achieved at temperature higher than 60°C, indicating efficient releases of the phytochemicals and polyphenol derivatives from *A. borneensis* leaves into the brewing solution due most probably to larger numbers of openings, interstices, denaturation, or disruption of their cells at temperature higher than 60°C.

Accordingly, in Figure 3B, we show that TPC, TFC, and TF contents in the infusion of *A. borneensis* leaves also increase abruptly with brewing temperature. Therefore, we could draw a conclusion that the total antioxidant capacity is related to the concentrations of phytochemicals and polyphenol derivatives in the *A. borneensis* leaf infusion, as discussed above. Considering that TF content in the infusions to scavenge DPPH is one or two orders lower when compared with those of TPC and TFC (see Figures 2B and 3B), thus polyphenols and flavonoids should be the major constituents responsible for the total antioxidant capacity of *A. borneensis* leaves.

It is noteworthy that although caffeine does not belong to polyphenols, it has a capability to inhibit lipid peroxidation (Brezova *et al*., 2009). Thus, one may consider that caffeine content could have a contribution in the antioxidant capacity of *A. borneensis* leaves. In this sense, we have measured its caffeine content, and we found that it is negligibly small (data not shown). Therefore, the contribution of caffeine in the total antioxidant capacity of *A. borneensis* leaves is not important. Such negligible contribution of caffeine in the total antioxidant capacity has also been discussed for *C. sinensis* black tea infusions (Fernando and Soysa, 2009).

3.4 Antioxidant capacity of several commercially available teas

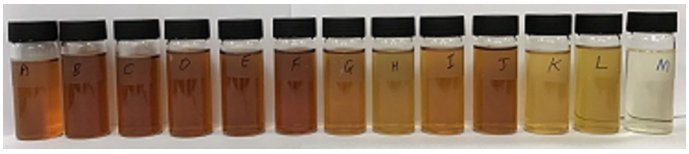

Figure 4. The images of aqueous infusions of commercially available *A. borneensis* of 3MPK (I) and *C. sinensis* black teas of various brands (A, B, C, D, E, F, G, H, J, K, L, and M) brewed for 3 min at 80°C.

We should note that most of the health benefits of tea are based on its total antioxidant activity. The total antioxidant activity of the infusion of *A. borneensis* leaves, therefore, was compared with those of twelve different *C. sinensis* black, herbal, and Pu Er teas commercially available. With the same brewing time and temperature, color of the infusions clearly varies depending on their brands, as shown in Figure 4. We found that the total antioxidant activity, either in IC_{50}, gallic acid equivalent antioxidant capacity (GEAC), or quercetin equivalent antioxidant capacity (QEAC), is also significantly different in the various brands of teas. As listed in Table 1, the minimum IC_{50} (143 ppm) was observed in the tea infusion of brand **A**, while the maximum value (4078 ppm) was found in the infusion of brand **M**. This indicates that the total antioxidant capacity of black tea brand **A** is approximately 30-fold higher than that of brand **M**. We should note that

infusion of *A. borneensis* leaves (brand **I**) has an IC_{50} of 592 ppm, equivalent to approximately 4-fold lower than that of brand **A** and it is 7-fold higher than of brand **M**. This means that the health benefits of *A. borneensis* infusion are in the middle rank among the twelve *C. sinensis* and herbal teas evaluated in the present study.

Table 1. Antioxidant activity of aqueous infusions of commercially available *A. borneensis* and *C. sinensis* black teas brewed for 3 min at 80°C.

Tea brand	IC_{50}	QEAC	GEAC
A	143 ± 7	11097 ± 611	8179 ± 450
B	222 ± 14	7140 ± 440	5262 ± 324
C	248 ± 2	6377 ± 63	4700 ± 47
D	265 ± 14	5985 ± 303	4412 ± 223
E	271 ± 10	5831 ± 221	4298 ± 163
F	297 ± 19	5335 ± 345	3932 ± 254
G	410 ± 22	3860 ± 212	2845 ± 156
H	535 ± 39	2967 ± 223	2187 ± 164
I	592 ± 27	2675 ± 122	1972 ± 90
J	641 ± 32	2471 ± 127	1821 ± 94
K	673 ± 41	2353 ± 140	1735 ± 103
L	3364 ± 152	470 ± 21	347 ± 15
M	4078 ± 42	388 ± 4	286 ± 3

We should note that, in addition to the brewing conditions (brewing time and temperature), the total antioxidant capacity of teas should vary greatly depending on a wide variety of factors such as the location of plantation, harvesting season, drying method, and manufacturing. Although there have been several literatures comparing the total antioxidant capacity and polyphenolic contents of commercial teas from different various brands, those factors are simply neglected *(Chan et al*., 2007; Taheri *et al*., 2011; Carloni *et al*., 2013). In particular, black teas are produced by fermentation with a number of parameters which can also modify their antioxidant capacity. For instance, polyphenol oxidases released from the disrupted cells can react with catechins, thus during black tea manu-facturing, the most considerable changes occur in black teas is the concentration of catechins. Therefore, it is obvious that there is a significant difference in the total antioxidant capacity found in commercial black teas from various brands. However, we should mention that, regardless of that wide variety of factors determining tea quality, the infusion of *A. borneensis* leaves which was processed traditionally is among those teas with considerable total antioxidant capacity. In order to explore the effect of processing, a study on the antioxidant capacity of *A. borneensis* leaves with different pretreatments is currently being pursued.

4. Conclusion

In summary, we have investigated the total antioxidant capacity of *Aidia borneensis* leaf infusion, a Bornean endemic plant, by measuring its DPPH free radical scavenging activity. We found that the antioxidant capacity of the infusion increases with brewing time and temperature, and we have attributed the total antioxidant capacity of the infusion to their TPC, TFC, and TF contents. We have also assessed relative potential of the infusion of *A. borneensis* leaves by comparing its total antioxidant capacity with twelve different *C. sinensis* black, herbal, and Pu Er teas commercially available. We found that the infusion of *A. borneensis* leaves is in the middle rank among the twelve commercially available teas. Therefore, the present preliminary study has highlighted significant implications on *A. borneensis* leaves and on the feasibility of establishing this new potential plant among commercially available conventional *C. sinensis* tea, which has been known as a rich source of antioxidants.

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