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Effect of Fermentation on the Composition of *Centella asiatica* Teas

Chew Shio Heong, Kaur, Bhupinder, Nurul Huda, Alias A. Karim and Ariffin Fazilah
Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11700 Minden, Penang, Malaysia

Corresponding Author: Ariffin Fazilah, Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11700 Minden, Penang, Malaysia Tel: +6(04) 657 5208 Fax: +6(04) 657 3678

ABSTRACT

Centella asiatica leaves were exposed to fermentation/oxidation for varying amounts of time: no fermentation (0 min), partial fermentation (120 min) and full fermentation (24 h). The chemical composition of the teas was determined and compared with commercial *Camellia sinensis* teas. The results of proximate analysis showed *Centella asiatica* herbal teas contained significantly higher amounts of protein, fat and ash than *Camellia sinensis* teas. Compared to *Camellia sinensis* teas, all *Centella asiatica* tea infusion extracts contained significantly more total free amino acids (24.87-54.44 mg l-glutamic acid equivalent g^{-1}) but significantly less total free polysaccharides (24.33-31.52 mg glucose equivalent g^{-1}) and were caffeine free. High thiamine, riboflavin, niacin and ascorbic acid contents were found in all *Centella asiatica* teas, but biotin was found only in fully fermented *Centella asiatica* tea (CAFF). Colour measurements demonstrated that *Centella asiatica* infusions generally had lowered a (greenness) and b (yellowness) values than *Camellia sinensis* teas. All infusions exhibited low turbidity levels (less than 10%), except for CAFF. However, the *Centella asiatica* teas exhibited significantly lower total phenolic (3.53-6.22 mg gallic acid equivalent g^{-1}), total flavonoid (1.81-2.54 mg quercetin equivalent g^{-1}) and total anthocyanin (0.99-1.49 mg catechin equivalent g^{-1}) contents than *Camellia sinensis* teas and thus had lower antioxidant capacities (DPPH: 21.86-32.64 μm trolox equivalent g^{-1} and FRAP: 25.86-43.09 μm trolox equivalent g^{-1}) than *Camellia sinensis* teas. Partially-fermented *Centella asiatica* (120 min) showed no significant change in antioxidant properties, but its total free polysaccharide content increased and it produced the darkest infusion.

Key words: *Centella asiatica*, herbal tea, chemical properties, water soluble vitamins, antioxidant properties

INTRODUCTION

In recent years, there has been an increased effort to find foods and beverages with high antioxidant contents and health-promoting properties. Herbs traditionally used in folk medicine have attracted consumer interest because of their long historical consumption and ready acceptability. Herbal teas or tisanes have gained popularity all over the world due to their antioxidant activity and fragrance (thought to exert a calming effect on the mind) (Aoshima et al., 2007). According to the World Health Organization (Zhang, 2002), over 80% of the world's population relies on largely plant-based traditional medicine for primary healthcare needs. In Malaysia, the herbal market was valued at US $10 billion in 2008 and is predicted to grow at an annual rate of 8 to 15% (Malaysian National News Agency, 2008).

Most Malaysians are habitual herbal tea consumers and believe that herbal teas are safe to consume, assist in health promotion, boost energy level, prevent diseases and have cosmetic properties (Hassali *et al*., 2009). Herbal teas can be easily prepared from any part of a plant, including the roots, flowers, seeds, berries and bark. The preparation of an herbal infusion, which may consist exclusively of one or more herbs, is simple and can be performed by means of decoction, infusion or maceration (Apak *et al*., 2006).

Centella asiatica (L.) Urban, synonym *Hydrocotyle asiatica*, is locally known as pegaga and belongs to the plant family Apiaceae (Umbelliferare). The herb is well known and is called various names all over the world, including gotu kola, Mandukaparni, Brahmi and Indian pennyworth. The herb is native to both tropical and subtropical countries such as China, India, South America, Madagascar and Malaysia. *Centella asiatica* is famous in Ayurvedic medicine for the treatment of leprosy, insanity, asthma, ulcers, eczema, skin tuberculosis, wounds, stomach aches, arthritis, varicose veins and high blood pressure; it is also known as a memory enhancer (Hargono *et al*., 1999). The medicinal values reported lead this plant to a higher demand in pharmaceutical industries (Raghu *et al*., 2007). The major components of the herb are β-amyrin ursolic acid group: The asiatic acid, asiaticoside, madecassic acid and madecassoside (Pick Kiong *et al*., 2005). However, previous studies also showed that *Centella asiatica* has a high level of phenolic compounds and these compounds are believed to be responsible for the antioxidative activity (Hussin *et al*., 2009). The herb was reported to have abundant phenolic compounds that are majorly from flavonoids such as quercetin, catechin, epicatechin, rutin, luteolin, myricetin, kaempferol and naringenin (Hussin *et al*., 2009; Mustafa *et al*., 2010). The phenolic hydroxyl group in flavonoids is found to be a strong antioxidant capable of effectively scavenging reactive oxygen species (Cao *et al*., 1997). The nutritional value of *Centella asiatica* is promising, as it is rich in carotenoids and vitamins B and C (Paramageetham *et al*., 2004); the herb is commonly used as porridge for feeding pre-school children in Sri Lanka in order to combat nutritional deficiencies (Cox *et al*., 1993).

Centella asiatica is widely available in the market in the form of tea, soft drinks and syrup. However, the tea is normally prepared by a simple drying technique and usually mixed with black tea to enhance a darker color of infusion which *Centella asiatica* lacking off. For that reason fermentation process in this study is believed to improve the quality of herbal tea in terms of color, flavor and taste (Du Toit and Joubert, 1998; Heck and Mejia, 2007). As there is a dearth of information on the use of fermented *Centella asiatica* teas in beverage production, the main objective of this research was to explore the feasibility of *Centella asiatica* as an herbal tea produced by three types of fermentation: No fermentation (CANF), partial fermentation (CAPF) and full fermentation (CAFF). Since *Camellia sinensis* tea was always used as reference to compare with herbal teas (Almajano *et al*., 2008; Buyukbalci and El, 2008; Ho *et al*., 2010) the chemical properties, water soluble vitamin content, sensory properties (color and turbidity) and antioxidant capacity of the products were studied and compared with commercial *Camellia sinensis* teas: Green Tea (GT), Oolong Tea (OT) and Black Tea (BT).

MATERIALS AND METHODS

Plants, materials and chemicals: Fresh *Centella asiatica* leaves and commercial *Camellia sinensis* teas was obtained from a local market in Penang, Malaysia during January to March 2009. Folin-Ciocalteu's phenol reagent, sodium carbonate, aluminum chloride, sodium hydroxide, caffeine, iron (III) chloride and phenol were purchased from Merck (Darmstadt, Germany). Gallic

acid, sodium nitrite, quercetin, vanillin, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, TPTZ (2,4,6-tripyridyl-s-triazine), ninhydrin, DL-panthenol, nicotinamide, pyridoxine, biotin and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO., USA). All other chemicals used were of analytical grade.

Non-fermented (unprocessed) *Centella asiatica* (CANF) tea: *Centella asiatica* leaves were washed under running tap water. The cleaned leaves were dried in a hot air oven at 100°C until the moisture content was less than 6.5%. The dried leaves were kept in an air tight container.

Partially-fermented (CAPF) and fully-fermented (processed) (CAFF) *Centella asiatica* tea: The *Centella asiatica* cleaned leaves were withered in a locally obtained forced air oven at 30°C for 2 h. The withered leaves were manually twisted and torn for 20 min. The crumpled leaves were allowed to undergo partial fermentation for 120 min. For CAFF, a similar procedure was repeated, but the fermentation time was prolonged to 24 h. The fermented leaves were dried in a hot air oven at 100°C until the moisture content was less than 6.5%. The dried matter was kept in an air tight container.

Tea infusion preparation: For all experiments, 1 g of tea leaves was weighed into a beaker. Hot distilled water (100 mL) was then added and allowed to infuse the leaves for 10 min. The infusions were filtered through Whatman filter paper No. 1 prior to analysis.

Proximate analysis: Determination of the moisture, crude protein, crude fat and crude ash contents were performed according to the AOAC, (1993). The protein conversion factor used was 6.25.

Determination of total free amino acids: The total free amino acids were determined using the method described by Yao *et al.* (2006a). Tea infusions (1 mL), 0.5 mL of phosphate buffer solution and 0.5 mL of 2% ninhydrin solution containing 0.8 mg mL^{-1} of tin chloride were placed into a 25 mL volumetric flask. The mixture in the volumetric flask was then heated in a boiling water bath for 15 min. The flask was quickly cooled down, and the volume was adjusted to 25 mL with distilled water. After the solution was left standing for 10 min, the resulting blue-purple products were read at 570 nm using a spectrophotometer (Shimadzu UV 1240). Results were expressed as mg L-glutamic acid equivalent/100 mL infusion. The measurement was performed in triplicate.

Determination of total free polysaccharides: The polysaccharide content was determined by the phenol-sulphuric colorimetric method (Cuesta *et al.*, 2003). Tea infusions 0.5 and 0.6 mL of 5% phenol solution were added into each test tube, followed by 0.3 mL of concentrated sulphuric acid. Each tube was mixed well and kept at room temperature for 30 min. The resulting dark brown solution was measured at 490 nm using a spectrophotometer (Shimadzu UV 1240). Results were expressed as mg glucose equivalent/100 mL infusion. Measurements were performed in triplicate.

Water soluble vitamin determination

Vitamin B determination using LC/MS: An Agilent LC-MS system 1100 (USA) was equipped with a binary pump, degasser, wellplate sampler and thermostatted column compartment directly connected with a positive ESI mass spectrometer system. The optimization step was carried out in

flow injection mode using a scan range of m/z 100-900. The separation was performed on a ZORBAX RRHT SB-Aq (100×3.0 mm, 1.8 μm) with a mobile phase of (A) 20 mM ammonium formate and 0.1% formic acid in water and (B) 20 mM ammonium formate and 0.1% formic acid in methanol. The gradient elution was programmed as follows: 0-8 min, 10% B; 8-8.1 min, 55% B; 8.1-10 min, 10% B at a flow rate of 0.5 mL min^{-1} with an injection volume of 10 μL. Nitrogen gas was used as the nebulising gas (30 psig) and as the drying gas (10 L min^{-1}). The drying temperature was kept at 350°C. The capillary exit was 1850 V.

Vitamin C determination using HPLC: Tea infusions (10 mL each) were extracted using an equal volume of 4.5% (w/v) metaphosphoric acid solution. Samples were filtered through a 0.45-μm membrane filter in aliquots of 20 μL for each tea infusion. A Jacob HPLC system (Jasco, Tokyo, Japan) equipped with a Jasco PU-2080 Plus Intelligent HPLC Pump, a Jasco AS-2055 Plus Intelligent Sampler, a Jasco UV-2077 PLUS 4-λ Intelligent UV/Vis detector and a Jasco ChromNAV version 1.11.02 (Build 4) was used. The separation was performed with a Hypersil ODS C18 column (250×4.6 mm, 5 μm) (Thermo Scientific, Waltham, MA, USA) fitted with a Hypersil ODS guard column. The deionised water mobile phase was adjusted with metaphosphoric acid to pH 2.2 at a flow rate of 1.0 mL min^{-1} and detected at 276 nm (Polydera *et al*., 2003). Results were expressed as mg ascorbic acid/100 mL infusion.

Caffeine determination using HPLC: The tea infusions were diluted with deionised water and filtered through a 0.45-μm membrane filter and injected into a Jasco HPLC system (Jasco, Tokyo, Japan). The separation was performed on a Hypersil ODS C18 column (250×4.6 mm, 5 μm) (Thermo Scientific, Waltham, MA, USA) fitted with a Hypersil ODS guard column containing a mobile phase of 25 methanol: 75 deionised water at a flow rate of 1.0 mL min^{-1} and detected at 276 nm. Results were expressed as mg caffeine/100 mL infusion.

Colour measurements: A Konica Minolta spectrophotometer CM-3500d (Minolta, Kyoto, Japan) with illuminant D65 was used to measure the CIE L*, a* and b* colour space of tea infusions (L* [lightness (0 = black, 100 = white)], a* (-a = greenness, +a = redness) and b* (-b = blueness, +b = yellowness)). Results were obtained using SpectraMagic™ computer software version 3.61 G (Cyber Chrome, Inc., Minolta Co. Ltd).

Turbidity: The turbidity of tea infusions was measured according to the method described by Morton and Murray (2001) using a spectrophotometer (Shimadzu UV 1240) at 800 nm. The percent transmittance (T%) was recorded, and (100-T%) was used as a measure of turbidity.

Antioxidant activity

Total Phenolic Content (TPC): The total phenolic content of each tea infusion was measured using the method described by Kahkonen *et al*. (1999). The tea infusion (3 mL) was added to 1.5 mL of Folin-Ciocalteu's phenol reagent (10% v/v) and allowed to react for 5 min. Next, 1.2 mL of 7.5% w/v sodium carbonate was added to the reaction mixture and incubated for 30 min. The resulting blue complex was measured at 765 nm and TPC was expressed as mg gallic acid equivalents (GAE)/100 mL infusion. Measurements were performed in triplicate.

Total Flavonoid Content (TFC): Total Flavonoid Content (TFC) was assayed as described by Zhishen *et al.* (1999). In brief, 0.5 mL of the tea infusion was mixed with 2 mL distilled water and 0.15 mL, 20% (w/v) sodium nitrite and left to stand for 5 min. Then, 0.3 mL of 10% w/valuminum chloride was added to this mixture. After 6 min, 2 mL of 1M sodium hydroxide and 0.2 mL of distilled water were added. The absorbance was read at 510 nm. The results were expressed as mg quercetin equivalents (QE)/100 mL infusion; measurements were performed in triplicate.

Total proanthocyanidin content (TAC): The total proanthocyanidin content of the tea infusions was measured using the method described by Sun *et al.* (1998). First, 2.5 mL of 1% (w/v) vanillin in methanol and 2.5 mL of 9.0 N hydrochloric acid in methanol were added to 1 mL of tea infusion. After incubation at 30°C for 20 min, the absorbance was measured at 500 nm using a spectrophotometer (Shimadzu UV 1240) and expressed as mg catechin equivalents (CE)/100 mL infusion. Measurements were performed in quintuplicate.

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity: The DPPH free radical scavenging activity of each tea infusion was determined according to the method described by Leong and Shui (2002). A 0.1 mM solution of DPPH in methanol was prepared. An aliquot of 0.1 mL of tea infusion was added to 2.9 mL of methanolic DPPH solution and kept in the dark for 30 min. Absorbance was assayed at 517 nm using a spectrophotometer (Shimadzu UV 1240). Trolox solution was used to perform the calibration curves. Results were expressed as μmol Trolox equivalents/100 mL infusion. The measurement was performed in triplicate.

Ferric Reducing Antioxidant Potential (FRAP) assay: The ability to reduce ferric ions was measured according to the method described by Benzie and Strain (1996). The FRAP reagent was prepared using 300 mM sodium acetate buffer at pH 3.6, 20 mM iron chloride and 10 mM 2,4,6-tripyridyl-s-triazine dissolved in 40 mM hydrochloric acid at a ratio of 10:1:1 (v:v:v). The reagent was incubated in a water bath at 37°C for 5 min before use. The initial reading of the reagent was measured at 593 nm using a Shimadzu UV 1240 spectrophotometer. An aliquot of 0.1 mL of tea infusion was then added to 2.9 mL of FRAP reagent and kept in the dark for 30 min. Trolox solution was used to create the calibration curves. Results were expressed as μmol Trolox equivalents/100 mL infusion. Measurements were performed in triplicate.

Statistical analysis: Experimental data were analysed using Statistical Package for the Social Sciences (SPSS) 17.0 for Windows® (SPSS Inc.). A one-way ANOVA procedure followed by a Duncan test was used to determine the significant difference ($p<0.05$) between treatment means. A Pearson correlation analysis was performed to determine the correlationship between the polyphenols and the antioxidant capacity of tea infusions.

RESULTS AND DISCUSSION

Three types of *Centella asiatica* teas (CANF, CAPF and CAFF) were successfully prepared in this study. The tea infusions were yellow.

Controlling moisture content is an important factor in tea preservation, particularly for the inhibition of microbial growth. Owuor (2003) suggested that the moisture content of tea products should be below 6.5%. However, tea leaves with a moisture content of 2.5% or less may evidence a smoky taste (Owuor, 2003). Based on the results obtained (Table 1), the moisture contents of

Table 1: Chemical properties of *Centella asiatica* herbal teas and *Camellia sinensis* teas

				Centella asiatica tea		
Properties	Green tea	Oolong tea	Black tea	Non-fermented	Partially-fermented	Fully-fermented
Moisture content	6.13±0.03^{c}	6.49±0.07^{b}	7.47±0.03^{a}	6.42±0.12^{b}	6.41±0.12^{b}	5.88 ±0.09^{d}
Protein[1]	27.50±0.96^{cd}	25.49±0.79^{d}	27.47±0.36^{c}	29.22±0.64^{a}	30.35±0.37^{a}	30.50±1.85^{a}
Fat[1]	0.15±0.06^{d}	0.27±0.04^{c}	0.39±0.11^{c}	0.60±0.07^{a}	0.54±0.08^{b}	0.50±0.01^{b}
Ash1	5.91±0.06^{b}	5.93±0.11^{b}	5.45±0.13^{c}	9.50±0.10^{a}	9.25±0.21^{a}	9.43±0.22^{a}
Total free amino acids[2]	37.70±4.68^{b}	31.41±5.46^{bc}	34.62±4.75^{bc}	51.71±6.35^{a}	54.44±7.66^{a}	24.87±6.80^{c}
Total free polysaccharides[3]	39.52±0.46^{a}	31.64±0.86^{b}	30.17±0.22^{c}	24.33 ± 0.67^{e}	31.52±0.80^{b}	28.45 ± 0.77^{d}
Caffeine[4]	21.29±2.18^{a}	12.72±1.68^{b}	24.35±0.52^{a}	ND	ND	ND

[1]Values in dry weight basis. [2]Total free amino acids in mg L-glutamic acid equivalent/ 100 mL infusion. [3]Total free polysaccharides in mg glucose equivalent/ 100 mL infusion. [4]Caffeine content in mg/ 100 mL infusion; ND. Not detected. Values marked by different letters in each row are significantly different ($p<0.05$); Data are Mean±SD, n = 9

CANF, CAPF and CAFF were 6.42, 6.41 and 6.42%, respectively; for the three types of *Camellia sinensis* teas, GT, OT and BT, the moisture contents were 6.13, 6.49 and 7.47%, respectively. All the teas analysed fell within the suggested limit, with the exception of BT.

There was no significant difference observed in all types of *Centella asiatica* teas during protein analysis. The protein contents of *Centella asiatica* teas were significantly higher than those of *Camellia sinensis* teas. The protein content remained constant in all *Centella asiatica* teas. This was in accordance with the results of Tsai *et al.* (1990) who reported that the nitrogen content of tea leaf was not affected by harvesting and fermentation process. A similar trend was found for ash contents, which remained unchanged in the three samples (CANF, CAPF and CAFF). This may be due to the stability of minerals present in ash, as the temperature used for drying was 100°C. The fat content of CANF tea was significantly higher than CAPF and CAFF teas. The fat content of all *Centella asiatica* teas was also significantly higher than that of *Camellia sinensis* teas. The fat content reduced with fermentation time, which could have been due to the release of volatile fat content from the samples during fermentation.

Free amino acid content is regarded as an important criterion for tea quality assurance and contributes to overall quality in terms of taste, flavour and colour (Yao *et al.*, 2006a). In CANF and CAPF tea infusions, the total free amino acid released showed no significant change but was significantly higher than that released by CAFF tea and *Camellia sinensis* teas. There were no significant differences for all *Camellia sinensis* teas. The dramatic decrease in total free amino acid content in CAFF may have been due to the breakdown of protein during the 24 h fermentation time (Yao *et al.*, 2006b). GT had the highest total free polysaccharide content, followed by OT and BT. these contents were significantly higher than those of CANF and CAFF. However, the polysaccharide content of CAPF was not found to be significantly different from that of OT. However, it was significantly higher than that of BT.

No caffeine was found in any *Centella asiatica* tea. In contrast, the caffeine contents in GT, OT and BT were 21.29, 12.72 and 24.35 mg/100 mL, respectively. This indicates that *Centella asiatica* teas possess great potential for the growing caffeine-free tea market. Recently, decaffeination has become popular for minimising caffeine content in various sources, including tea and coffee. The caffeine content in beverages should be minimised due to caffeine-related side effects, including anxiety, nausea, jitteriness, nervousness, a decrease in heart rate and an increase blood pressure and the risk of cardiovascular disease (Temple, 2009).

Table 2: Water soluble vitamins contents of *Centella asiatica* herbal teas and *Camellia sinensis* teas

				Centella asiatica tea		
Vitamins	Green tea	Oolong tea	Black tea	Non-fermented	Partially-fermented	Fully-fermented
B[1], thiamine1	0.13±0.00[d]	ND	ND	10.20±0.53[c]	41.11±1.14[a]	27.84±3.57[b]
B[2], riboflavin1	7.95±0.50[d]	9.12±0.39[d]	3.60±0.09[e]	47.64±7.27[c]	189.20±4.19[a]	160.04±2.24[b]
B3, niacin1	672.98±24.13[c]	621.80±13.06[c]	426.31±14.37[d]	1179.85±91.32[a]	786.78±19.79[b]	269.16±47.13[e]
B6, pyridoxine1	9.52±0.63 b	9.74±0.49[b]	15.89±2.03[a]	4.33±0.16[c]	5.11±0.21[c]	15.27±2.90[a]
B7, biotin1	ND	ND	ND	ND	ND	15.51±1.70
C, ascorbic acid[2]	0.19±0.04[bc]	0.10±0.01[d]	0.09±0.00[d]	0.31±0.02[a]	0.27±0.09[ab]	0.17±0.01[c]

[1]Values in μg/100 mL infusion. [2]Values in mg/100 mL infusion. ND. Not detected. Values marked by different letters in each row are significantly different ($p<0.05$). Data are Mean±SD, n = 3

Vitamins are organic compounds present in trace amounts in our diet. Individual vitamins have specific functions to promote health and life. The amount of water-soluble B and Cvitamins present in tea infusions was determined. Vitamin B is made up of a complex group and consists of eight vitamins: vitamin B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), vitamin B_6 (pyridoxine), B_7 (biotin), B_9 (folic acid) and vitamin B_{12} (cyanocobalamine).

The amount of thiamine in *Centella asiatica* teas was significantly higher than in GT. However, thiamine was not detected in OT or BT. CAPF had a significantly higher amount of thiamine than CAFF and CANF. *Centella asiatica* teas also contained significantly more riboflavin than *Camellia sinensis* teas (Table 2). There was no significant difference between GT and OT and both teas had a significantly higher riboflavin content than BT. Niacin was the predominant B-vitamin type present in both tea types (*Centella asiatica* and *Centella sinensis).* Niacin content was found to decrease along with fermentation time. CANF exhibited the highest niacin content (1179.85 μg/100 mL); niacin contents were 786.78 and 269.16 μg/100 mL in CAPF and CAFF, respectively. At the same time, CANF and CAPF had higher niacin content than GT, OT and BT. The pyridoxine content in both tea types increased with fermentation time. *Camellia sinensis* teas were shown to have a higher pyridoxine content than CANF and CAPF. The pyridoxine content of CAFF was not significantly different from that of BT and it was higher than those of GT and OT. Biotin was found only in CAFF. Pantothenic acid, folic acid and vitamin B_{12} were not detected in any of the tea infusions. The absence of folic acid may be due to its sensitivity to sunlight, air, light and the heat from the boiling water (Leskova *et al.*, 2006). Vitamin B_{12} (cyanocobalamine) was reported as not present in black teas (Pasha and Reddy, 2005). The high concentration of thiamine, riboflavin and pyridoxine present in *Centella asiatica* teas, especially CAPF, suggested that it is a potential isotonic or energy drink.

The amount of vitamin C or ascorbic acid in *Centella asiatica* teas was generally higher than in *Camellia sinensis* teas and CANF had the highest amount. Ascorbic acid was found to decrease with fermentation degree, indicating that ascorbic acid oxidised during fermentation. Similar results were also shown by *Camellia sinensis* teas.

Tea colour and turbidity are important sensory qualities. The CIE Lab and turbidity values for *Centella asiatica* and *Camellia sinensis* tea infusions are given in Table 3. The *L* value decreased (from 99.42 to 98.38) with the degree of fermentation in *Centella asiatica* teas. A similar trend was found in *Camellia* sinensis teas. The L values showed that the infusions of both major tea types were bright and clear. The greenish value (-α) of GT and BT was significantly higher than all the *Centella asiatica* teas, although the -α of CANF was higher than that of OT. However, the b values of all the *Camellia sinensis* teas were greater than those of the *Centella asiatica* teas. This implies

Table 3: Color parameters and turbidity of *Centella asiatica* herbal teas and *Camellia sinensis* teas

				Centella asiatica tea		
Properties	Green tea	Oolong tea	Black tea	Non-fermented	Partially-fermented	Fully-fermented
L	99.27±0.05^{b}	98.89±0.01^{c}	96.22±0.11^{e}	99.42±0.03^{a}	99.43±0.08^{ab}	98.38±0.23^{d}
a	-0.44±0.03^{d}	-0.25±0.03^{b}	-1.91±0.01^{e}	-0.34±0.01^{c}	-0.32±0.05^{b}	-0.07±0.04^{a}
b	3.76±0.31^{c}	4.65±0.03^{b}	19.99±0.54^{a}	1.83±0.04^{e}	2.15±0.37^{d}	2.27±0.46^{d}
Turbidity1	2.74±0.19^{a}	3.43±0.38^{b}	2.82±0.25^{a}	2.45±0.21^{a}	5.15±1.14^{c}	60.17±3.53^{d}

[L: (0 = black, 100 = white); +a: Redness, -a: Greenness; +b: Yellowness, -b: Blueness]. [1]Turbidity in (100-T%). Values marked by different letters in each row are significantly different ($p<0.05$), Data are Mean±SD, n = 12

that *Camellia sinensis* teas were darker than *Centella asiatica* teas. In *Centella asiatica* teas, fermentation significantly improved the redness and yellowness of the infusions.

Turbidity is the optical property that describes the scattering and absorption of light as it travels through a tea infusion, making the infusion look cloudy or smoky (Bhuyan, 2007). The turbidity values of the three types of *Camellia sinensis* teas were generally lower than *Centellaasiatica* teas, except for that of CANF, which was significantly lower than that of OT and not significantly different from that of GT and BT. In *Centella asiatica* teas, turbidity increased with fermentation time with the following trend: CANF<CAPF<CAFF. Results showed that the turbidity values of both tea types were generally lower than 10%, indicating a low level of turbidity. However, CAFF exhibited a high level of turbidity. The breakdown of compounds in leaves during fermentation probably allowed these compounds to diffuse easily, therefore increasing the turbidity value of the infusion. A high level of turbidity in beverages such as herbal tea is known to decrease their aesthetic value, as mentioned by Hutchings (1999) and Harbourne *et al*. (2009).

The TPC, TFC and TAC of *Centella asiatica* and *Camellia sinensis* infusions were determined and are given in Fig. 1. Polyphenols are aromatic secondary metabolites widely found in herbs and associated with colour, sensory qualities, nutritional and antioxidant properties of food (Gupta and Prakash, 2009). All *Camellia sinensis* teas had higher total polyphenol contents than *Centella asiatica* teas. BT had the highest TPC, followed by GT and OT, then CANF, CAPF and CAFF. There was no significant difference between the TPCs of CANF and CAPF, but both were significantly higher than that of CAFF. This suggests that prolonged fermentation time broke down the polyphenols in *Centella asiatica* teas. Teas with a high TPC also exhibited high TFC and TAC values. However, BT had a higher TPC than GT but had a lower TAC than GT. Herbal teas contain no caffeine and therefore have lower TPC contents than *Camellia sinensis* teas (Aoshima *et al*., 2007). Moreover, the *Camellia sinensis* teas also exhibited high polyphenol contents (17-25% dry weight) contributed mainly by catechins, theaflavins, thearubigins and theabrownins. About 45% of these tea constituents can be infused into hot water (Yao *et al*., 2006a).

In order to obtain a reliable result, two antioxidant assays, a DPPH free radical scavenging assay and a FRAP assay, were used to determine the antioxidant activities of tea infusions (Fig. 2).

Extracts that contain a high amount of TPC also generally exhibit high antioxidant activity. In our study, all *Camellia sinensis* teas with high TPC values showed high antioxidant activities. GT had the highest $TEAC_{DPPH}$ value, followed by BT and OT, then CANF, CAPF and CAFF. A similar trend was also demonstrated in $TEAC_{FRAP}$. The higher antioxidant activity of GT in comparison with other teas, including OT and BT, was probably due to its high proantocyanin

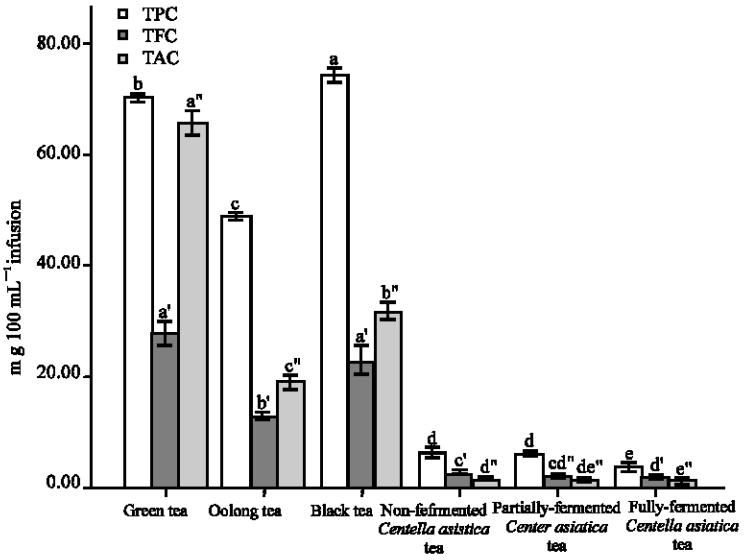

Fig. 1: Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and total proanthocyanin content (TAC) of *Centella asiatica* herbal teas and *Camellia sinensis* teas. TPC (Total phenolic content) in mg gallic acid equivalent/100 mL infusion, TFC (Total flavonoid content) in mg quercetin equivalent/100 mL infusion, TAC (Total proanthocyanin content) in mg catechin equivalent/100 mL infusion, Values marked by different letters are significantly different ($p<0.05$). Data are Mean±SD, n = 9; n=15 for TAC

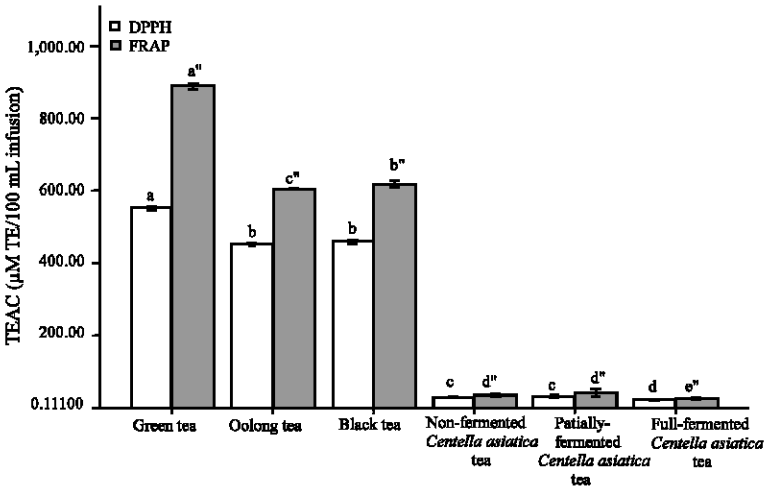

Fig. 2: Total antioxidant activity of *Centella asiatica* herbal teas and *Camellia sinensis* teas. TEAC = Trolox equivalent antioxidant capacity, DPPH = 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, FRAP = Ferric reducing antioxidant potential assay. Values marked by different letters are significantly different ($p<0.05$). Data are Mean±SD, n = 9

content (65.62 mg/100 mL infusion). These results are in accordance with the findings of Aoshima *et al.* (2007) and Atoui *et al.* (2005) who reported that green and black teas have higher

Table 4: Pearson's correlation coefficients of antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC) and total antocyanin content (TFC)

	TPC	TFC	TAC	TEACDPPH
TFC	0.971			
TAC	0.868	0.932		
TEACDPPH	0.914	0.83	0.769	
TEACFRAP	0.953	0.949	0.928	0.935

Significant at $p<0.01$

antioxidant activity rates than herbal teas like sage, mint, chamomile, ginkgo and peppermint. Furthermore, the high caffeine content of *Camellia sinensis* contributed to a higher TEAC. The antioxidant ability of caffeine was reported to be higher than ascorbic acid and displayed a strong ability to scavenge the hydroxyl radical and singlet oxygen at a constant rate (Devasagayam *et al*., 1996). The high amount of catechins derivative present in *Camellia sinensis* also contributed to the high rate of antioxidant activity (Yao *et al*., 2006).

A correlation analysis was performed to determine the correlation between polyphenol content and the antioxidant capacity of tea infusions (Table 4). A strong relationship between total phenolic content and total flavanoid content ($r = 0.971$) showed that flavonoids were the major polyphenols present in both types of teas compared to antocyanin ($r = 0.868$). Polyphenols are a complex group that consists mainly of flavonoids, phenolic acids and hydroxycinnamic acids. The major polyphenols in *Camellia sinensis* are catechins and its derivatives (Yao *et al*., 2006), and quercetin, catechin, epicatechin, rutin, luteolin, myricetin, kaempferol and naringenin in *Centella asiatica* (Hussin *et al*., 2009; Mustafa *et al*., 2010) are compounds from flavonoids. As such, polyphenol type showed a clear relationship with $TEAC_{DPPH}$; a high correlation was found between $TEAC_{DPPH}$ and TPC ($r = 0.914$), followed by TFC ($r = 0.830$) and TAC ($r = 0.769$). A similar trend was evident regarding polyphenols and $TEAC_{FRAP}$. The phenolic hydroxyl group in flavonoids was found to be a strong antioxidant capable of effectively scavenging reactive oxygen species (Cao *et al*., 1997). Moreover, a strong correlation was observed in $TEAC_{DPPH}$ and $TEAC_{FRAP}$. The strong correlation between $TEAC_{DPPH}$ and $TEAC_{FRAP}$ in edible tropical plants has also been reported by Wong *et al*. (2006). This indicated that compounds able to reduce DPPH radicals are also capable of reducing ferric ions.

In conclusion, the present study showed that *Camellia sinensis* teas have TPC, TFC, TAC and antioxidant properties superior to those of *Centella asiatica* teas. Nevertheless, the CANF and CAPF possess great potential as herbal teas as a result of their higher physiochemical property values, soluble vitamin contents and lack of caffeine when compared to *Camellia sinensis* teas. CAPF exhibited no significant difference in antioxidant properties but had higher free polysaccharide total and infusion yellowness compared to CANF. However, prolonged fermentation (full fermentation) inverted these effects.

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