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Phytochemical profile of *Orthosiphon aristatus* extracts after storage: Rosmarinic acid and other caffeic acid derivatives

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ABSTRACT

Background: *Orthosiphon aristatus* (Blume) Miq. is a medicinal herb which is traditionally used for the treatment of diabetes and kidney diseases in South East Asia. Previous studies reported higher concentration of antioxidative phytochemicals, especially rosmarinic acid (ester of caffeic acid) and other caffeic acid derivatives in this plant extract than the other herbs such as rosemary and sage which are usually used as raw materials to produce rosmarinic acid supplement in the market.

Purpose: The phytochemical profile of *O. aristatus* was investigated at different storage durations for quality comparison.

Methods: The phytochemicals were extracted from the leaves and stems of *O. aristatus* using a reflux reactor. The extracts were examined for total phenolic and flavonoid contents, as well as their antioxidant capacities, in terms of radical scavenging, metal chelating and reducing power. The phytochemical profiles were also analyzed by unsupervised principal component analysis and hierarchical cluster analysis, in relation to the factor of storage at 4 °C for 5 weeks.

Results: The leaf extract was likely to have more phytochemicals than stem extract, particularly caffeic acid derivatives including glycosylated and alkylated caffeic acids. This explains higher ratio of total phenolic content to total flavonoid content with higher antioxidant capacities for the leaf extracts. Rosmarinic acid dimer and salvianolic acid B appeared to be the major constituents, possibly contributing to the previously reported pharmacological properties. However, the phytochemical profiles were found changing, even though the extracts were stored in the refrigerator (4 °C). The change was significantly observed at the fifth week based on the statistical pattern recognition technique.

Conclusion: *O. aristatus* could be a promising source of rosmarinic acid and its dimer, as well as salvianolic acid B with remarkably antioxidant properties. The phytochemical profile was at least stable for a month stored at 4 °C. It is likely to be a good choice of herbal tea with comparable radical scavenging activity, but lower caffeine content than other tea samples.

Introduction

Orthosiphon aristatus (Blume) Miq. is a medicinal herb in the family Lamiaceae. This herb is widely found in the South East Asian countries such as Malaysia, Thailand and Vietnam. It is called as Misai Kucing and Kumis Kucing in Malaysia and Indonesia, respectively (Majid and Basheer, 2010). It has such a local name because of its flower having many long exerted filaments like cat's whiskers. The herb has traditionally been used to treat bladder inflammation, diabetes, rheumatism, arthritis, gout and kidney diseases.

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]; HPLC, high performance liquid chromatography; UPLC-MS/MS, ultra performance liquid chromatography tandem mass spectrometer; TPC, total phenolic content; TFC, total flavonoid content; RE, rutin equivalent; CAE, caffeic acid equivalent; EDTA, ethylenediaminetetraacetic acid; IC_{50}, effective concentration at 50% inhibition; EMS, enhanced mass spectra; IDA, information dependent acquisition; EPI, enhanced product ion; PCA, principal component analysis

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Till to date, phytochemicals such as polyphenols (sinensetin, eupatorin, 3′-hydro-5,6,7,4′-tetramethoxyflavone), diterpenes (norstaminolactone A, norstaminols B and C, secoorthosiphols A–C and orthosiphols R–T) and triterpenes (ursolic acid, oleanolic acid, betulinic acid, hydroxybetulinic acid, maslinic acid, α-amyrin and β-amyrin), as well as phenolic acids (rosmarinic acid, chlorogenic acid and isorinic acid) have been identified in *O. aristatus* by researchers (Awale et al., 2002; Olah et al., 2003; Akowuah et al., 2004; Hossain and Ismail, 2010). Phenolic compounds, especially rosmarinic acid is one of the most widely investigated phytochemicals in *O. aristatus*. This is probably because of its significant pharmacological activities such as anticancer (Hossan et al., 2014), anti-hyperglycemia (Zhu et al., 2014), anti-allergenic response (Zhu et al., 2014), anti-human immunodeficiency virus (Mazumder et al., 1997; Tewtrakul et al., 2003; Dubois et al., 2008) and suppression of ultraviolet A-induced reactive oxygen species production (Psotova et al., 2006).

Rosmarinic acid was first isolated by Sumaryono et al. (1991) from *O. aristatus*. It is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, approximately covered for 67% of the total identified phenolic compounds in aqueous methanol extract of the plant (Sumaryono et al., 1991). Rosmarinic acid can be also found in other medicinal plants of the family Lamiaceae. The herbs include *Rosmarinus officinalis* (rosemary, 7.2–11.0 mg/g), *Salvia officinalis* (sage, 8.5–39.3 mg/g), *Thymus vulgaris* (thyme, 4.5–23.5 mg/g), *Melissa officinalis* (balm, 13.3–36.5 mg/g), *Lavendula angustifolia* (lavender flower, 1.7–2.0 mg/g), *Prunella vulgaris* (self-heal, 21.7 mg/g), *Mentha spicata* (spearmint, 7.1–58.5 mg/g) and *Origanum vulgare* (Oregano, 25.0 mg/g) (Wang et al., 2004; Başkan et al., 2007; Shekarchi et al., 2012; Miron et al., 2013). Somehow, *O. arisatus* (53.0–299.0 mg/g) appeared to have the highest rosmarinic acid content among the herbal plants (Akowuah et al., 2004).

In the present study, the phytochemical profiles of the leaves and stems of *O. arisatus* were analyzed and evaluated for their antioxidant capacities. Caffeic acid derivatives are likely to be the dominant phytochemicals attributed to the previous reported phamacological activities. A high sensivity hyphenated analytical tool of liquid chromatography tandem mass spectrometry was used to generate mass spectra which were statistically analyzed by unsupervised multivariate data analysis techniques of principal component analysis and hierarchical cluster analysis. The quality of the plant extracts was examined based on data clustering of phytochemicals stored for 5 weeks.

Materials and methods

Chemicals and plant samples

High purity of chemicals; ferrous chloride (98%), potassium ferricyanide III (99%), potassium persulfate (≥99%), ferric chloride hexahydrate (97%), trichloroacetic acid (≥99%) and antioxidant reagents; Folin–Ciocalteu reagent (1.9–2.1 N), 1,1-diphenyl-2-picrylhydrazyl (DPPH, 97%), 2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS, ≥ 98%), ethylenediaminetetraacetic acid (EDTA, 99.995%) and ferrozine (3-(2-pyridyl) − 5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate, 97%) were obtained from Sigma-Aldrich (St. Louis, MO). The standard chemicals; ascorbic acid (99%) and rutin (97%) were purchased from Acros Organics (Pittsburgh, PA). Sodium carbonate (99.99%) was purchased from Merck (Darmstadt, Germany), whereas aluminium chloride (98.5%) and HPLC grade of solvents (methanol and acetonitrile) were sourced from Fisher Scientific (Pittsburgh, PA). Ultrapure water (18.2 MΩ − cm) was produced from a water purification system (Arium® Sartorius, Goettingen, Germany).

The plant sample of *O. arisatus* (stems and leaves) was purchased from the company, Fidea Resources (Selangor, Malaysia) and authenticated by the Forest Research Institute Malaysia with the voucher specimen SBID 001/13. The tea samples such as black tea (Lipton, Unilever, UK), Oolong tea (Thanyaporn, Thailand), green tea (Thanyaporn, Thailand), mulberry leaves (Thanyaporn, Thailand), roselle (Thanyaporn, Thailand), dandelion root (Traditional Medicinals Inc., CA) and *Pluchea indica* were purchased from the local market of Bangkok, Thailand. All samples were dried and ground into fine powder before extraction.

Phytochemical extraction

The leaf and stem extracts of *O. aristatus* were prepared from reflux extraction using 50% v/v methanol for 60 min. The ratio of solvent and plant material was 10. The extracts (A) were subjected to UPLC-MS/MS for untargeted mass screening. The similar extracts were kept in the fridge (4 °C) up to 5 weeks. Samples were drawn for untargeted mass screening after 2 (B), 3 (C) and 5 (D) weeks.

Total phenolic and flavonoid content of leaf and stem extracts

The total phenolic content (TPC) of the leaf and stem extracts was analyzed using Folin–Ciocalteu reagent. A 1 ml extract (12.5 mg/ml) was mixed with 50% Folin–Ciocalteu reagent (50 µl) and 2% sodium carbonate (2 ml). The solution was mixed thoroughly and incubated for 30 min. The absorbance of the solution was measured using a UV–Vis spectrophotometer (UV-1800, Kyoto, Japan) at 720 nm. Rutin (6.55–32.79 mg/l) was used to build the calibration curve. The TPC was expressed as milligram of rutin equivalent (RE) in a gram of dry weight extract. All experiments were carried out in triplicate unless stated otherwise.

The total flavonoid content (TFC) of the leaf and stem extracts was determined based on the formation of flavonoid-aluminium complex. A 1 ml extract (12.5 mg/ml) was mixed with 1 ml methanolic 2% aluminium chloride. The complex was formed after 15 min of incubation, and then measured at 430 nm spectrophotometrically. Rutin (6.67–33.33 mg/l) was used to construct the calibration curve. The TFC was expressed as milligram of rutin equivalent (RE) in a gram of dry weight extract.

Antioxidant capacities of leaf and stem extracts

The radical scavenging capacity of the leaf and stem extracts was determined using radical DPPH• assay. A 2 ml DPPH• solution (0.1 mM) was added into sample (1 ml) which was prepared at different concentrations ranged from 100 to 500 µg/ml. The absorbance was measured at 520 nm after 30 min of incubation. Rutin was used as positive control in all antioxidant assays unless stated otherwise. The ability to scavenge the DPPH• was calculated using Eq. (1), where $A_{control}$ and A_{sample} are the absorbance of control and sample, respectively. The concentration of sample which was required to scavenge 50% of DPPH• (IC_{50}) was determined in this study.

$$\text{Scavenging capacity}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

Another scavenging capacity of the extracts was determined using radical ABTS cation decoloration assay. The $ABTS^{\bullet+}$ solution was prepared by mixing ABTS (7 mM) with potassium persulfate (2.45 mM) in a volume ratio of 1:1. The solution was stored in a dark place 6 h, and then diluted with ethanol till the absorbance of the solution was 0.70 ± 0.02 at 734 nm before experiment. The experiment was carried out by adding 0.2 ml extract (12.5 mg/ml) to 1.8 ml diluted $ABTS^{\bullet+}$ solution. The absorbance was recorded after 20 min of incubation. The affinity of sample to quench free radicals was evaluated according to Eq. (1).

The chelating capacity of both extracts was measured using ferrous ion (Fe^{2+}) chelation assay. Sample (0.1 ml) with the concentrations ranged from 50–250 µg/ml was mixed with 2 mM ferrous chloride, $FeCl_2$ (0.1 ml), and then topped up with distilled water (1.6 ml). The reaction was initiated after the addition of 5 mM ferrozine (0.2 ml). The

Table 1
Total phenolic, flavonoid content and antioxidant capacity of leaf and stem extracts from *O. aristatus*.

Assay	Standard chemical		Plant extracts (Week 1)		Plant extracts (Week 5)	
	Rutin	Caffeic acid	Leaf	Stem	Leaf	Stem
Total phenolic content (mg RE/g extract)	–	–	22.25 ± 0.79	13.01 ± 2.23[a]	14.14 ± 0.48[a]	6.17 ± 1.79[c]
Total phenolic content (mg CAE/g extract)	–	–	41.32 ± 0.47	35.46 ± 0.50[a]	36.56 ± 0.34[a]	31.45 ± 2.42
Total flavonoid content (mg RE/g extract)	–	–	1.76 ± 0.21	1.17 ± 0.11	1.45 ± 0.41	1.06 ± 0.25
DPPH (IC_{50}, mg/ml)	0.080 ± 0.008[a,b,c,d]	0.008 ± 0.002[a,b,c,d]	0.145 ± 0.030	0.318 ± 0.033[a]	0.158 ± 0.004	0.355 ± 0.006[c]
ABTS (IC_{50}, mg/ml)	0.164 ± 0.015[a,b,c,d]	0.017 ± 0.001[a,b,c,d]	1.143 ± 0.056	1.194 ± 0.108	1.051 ± 0.009[a]	1.345 ± 0.100[b,c]
Iron chelating (IC_{50}, mg/ml)	0.403 ± 0.077[a,b,c,d]	*0.024 ± 0.001[a,b,c,d]	0.192 ± 0.012	0.201 ± 0.038	1.069 ± 0.120[a]	1.808 ± 0.060[b,c]
FRAP (IC_{50}, mg/ml)	**0.005 ± 0.001[a,b,c,d]	0.011 ± 0.000[a,b,c,d]	0.013 ± 0.001	0.03 ± 0.002[a]	0.036 ± 0.001[a]	0.102 ± 0.001[b,c]

RE is rutin equivalent.
CAE is caffeic acid equivalent.
DPPH (1,1-diphenyl-2-picrylhydrazyl) is denoted for free radical (DPPH•) scavenging activity.
ABTS (2,2′-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) is denoted for cation radical ($ABTS^{\bullet+}$) scavenging activity.
FRAP (ferric reducing antioxidant power) is denoted for reducing power to reduce ferric ions to ferrous ions
* The value is based on ethylenediaminetetraacetic acid (EDTA) as the standard chemical
** The value is based on ascorbic acid as the standard chemical
[a] Significant difference ($p < 0.05$) in comparison to week 1 leaf
[b] Significant difference ($p < 0.05$) in comparison to week 1 stem
[c] Significant difference ($p < 0.05$) in comparison to week 5 leaf
[d] Significant difference ($p < 0.05$) in comparison to week 5 stem

absorbance of the solution was spectrophotometrically measured at 562 nm after incubation for 10 min. The formation of ferrozine–Fe^{2+} complex was determined and expressed as the required concentration of extracts to chelate 50% of ferrous ions (IC_{50}). EDTA was used as positive control.

The reducing power of the leaf and stem extracts was analyzed based on the method of ferric (III) reduction. A 1 ml extract (20–100 µg/ml) was dissolved in 2.5 ml sodium phosphate buffer (0.2 M, pH 6.6) before adding 1% w/v potassium ferricyanide III (2.5 ml). After the mixture was incubated at 50 °C for 20 min, 2.5 ml trichloroacetic acid (10% w/v) was added into the mixture. The upper layer of the solution (2.5 ml) was diluted with 2.5 ml distilled water before adding 0.1% w/v ferric chloride solution (0.5 ml). The absorbance of the solution was spectrophotometrically measured at 700 nm. Ascorbic acid was used as positive control

UPLC-MS/MS

The UPLC, Waters Acquity (Milford, MA) system was coupled with a triple quadrupole-linear ion trap tandem mass spectrometer (Applied Biosystems 4000 Q TRAP; Life Technologies Corporation, Carlsbad, CA) with an electrospray ionisation (ESI) source. A C18 reserved phase Acquity column (150 × 4.6 mm, 1.7 µm) was used in this study.

The mobile phase consisted of solvent A (water with 0.1% formic acid and 5 mM ammonium formate) and solvent B (acetonitrile with 0.1% formic acid). The UPLC gradient was: 0–5 min, 10% B; 5–15 min, 10–90% B; 15–20 min, 90% B; 20–25 min, 90–10% B; 25–30 min, 10% B for final washing and column equilibration for the next run. The flow rate of UPLC was 0.15 ml/min. The injection volume was 5 µl. All samples were filtered with 0.2 µm nylon membrane filter prior to injection.

The mass spectra were acquired in the range of m/z 100–1500 with a 20 ms ion accumulation time. The mass spectrometric data were acquired in both positive and negative ion modes. The capillary and voltage of the ESI source were maintained at 400 °C and 5.5 kV (positive mode) or 4.5 kV (negative mode), respectively. Nitrogen was used as ion source gas for nebulisation, 40 psi; for drying solvent, 40 psi; curtain gas, 10 psi and collision gas, high. The declustering potential and collision exit energy were set at 40 V and 10 V, respectively. The scan rate was 1000 amu/s. Data acquisition and data processing were performed using Analyst 1.4.2.

The scan mode of enhanced mass spectra (EMS) was used to screen the samples and information dependent acquisition (IDA) with two parallel enhanced product ion (EPI) scan at different collision energies ranging from 10 to 50 V. The method of multiple reaction monitoring with three transition ions, namely m/z 195 > 163, m/z 195 > 138 and m/z 195 > 110 were used to identify caffeine the tea samples (Wang et al., 2008).

Data processing and interpretation

MarkerView 1.1 (Applied Biosystems/MDS Sciex) was used to perform sample classification using unsupervised principal component analysis (PCA). R-language (version 2.11.1) was used to construct heatmap and cluster analysis for visual sample classification. The compounds were putatively identified based on the literature data and matched with the high resolution mass spectral database (European MassBank, Norman Association) which could be accessed from http://massbank.eu/MassBank/. The statistical paired two sample *t*-test was used to compare the significant difference of week 1 and week 5 samples, as well as standard chemicals and samples at 95% confidence level.

Results and discussion

Polyphenols and antioxidant capacity

In the present study, reflux extraction produced 13.3% and 9.5% yield of crude extracts from the leaves and stems of *O. aristatus,* respectively. The results found that TPC and TFC of leaf extracts were higher than stem extracts (Table 1). The result was in good agreement with the finding of Farhan et al. (2012) who reported higher total phenolic content and antioxidant activity in the leaves of *O. aristatus.* High TPC and TFC contributed to high antioxidant capacities in terms of the free radical scavenging, iron chelating and ferric reducing power. Lower values of IC_{50} for leaf extracts required lower concentration of samples to exhibit 50% of the inhibitory activity. This can be seen from the reducing power of the leaf extract which was comparable with caffeic acid ($p > .05$). The leaf and stem extracts showed higher iron chelating activity than rutin ($p < .05$), but lower than EDTA ($p < .05$). This indicates that both extracts are rich in phenolic metabolites acting as siderophores to facilitate the formation of iron complex (Chobot and

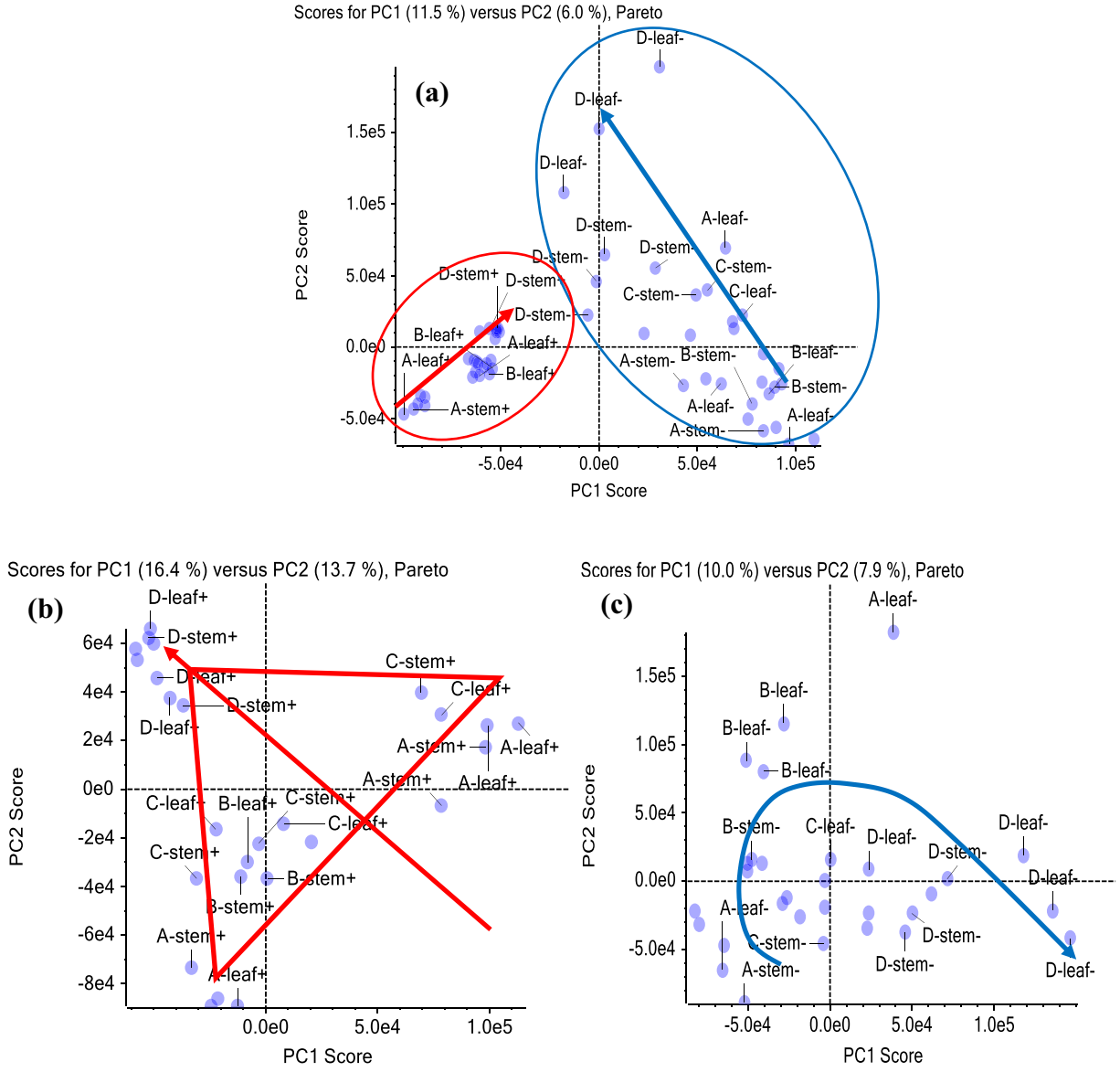

Fig. 1. (a) Score plot of metabolites ionized at both positive and negative modes from the fresh (A) and aged extracts stored for 2 weeks (B), 3 weeks (C) and 5 weeks (D); (b) score plot of metabolites ionized at the positive mode only; and (c) score plot of metabolites ionized at the negative mode only.

Hadacek, 2010). Somehow, the antioxidant activities were dropped after 5 weeks of storage with higher IC_{50} values. Phytochemicals that were used to chelate iron, to scavenge free radicals and to reduce ferric ions could be degraded after storage. Previously, the antioxidant activity of caffeic acid derivatives was found in ascending order from caffeic acid, danshensu (hydroxylated caffeic acid), rosmarinic acid (caffeic acid + danshensu) to salvianolic acids, mainly due to the increasing number of catechol moieties in the compounds (Zhao et al., 2008). Therefore, the leaf extracts of *O. aristatus* might have higher content of caffeic acid derivatives than the stem extracts.

Cluster analysis on phytochemicals

The phytochemicals of plant tissues, namely leaves and stems of *O. aristatus* were extracted using 50%v/v methanol. The extracts were subjected to accurate mass screening using UPLC-MS/MS in both positive and negative modes. The metabolites were analysed by PCA using Pareto scaling. There were 12,692 m/z values defined using a minimum spectral peak width of 0.3 amu, a retention time tolerance of 0.1 min and a mass tolerance of 0.1 amu. The score plot of the first two principal components only covers for 17.5% of the total variance (Fig. 1(a)). The plot shows significant difference between positive and negative ions, but no significant difference between leaf and stem extracts. The insignificant difference of metabolite profiles between leaf and stem extracts can be seen from Fig. 1(b) and (c) for positive and negative ion modes, respectively. The metabolite profiles of the extracts were changed in the direction as indicated by the colored arrows in Fig. 1, when the extracts were kept from week 1 to week 5. The metabolites of week 5 shows the most variance because they are located far away from other groups of samples as presented in Fig. 1(b) and (c).

Many phytochemicals, mostly organic acids, phenolic acids and flavonoids have been identified in the negative mode of UPLC-MS/MS. In particular, caffeic acid (3,4-dihydroxycinnamic acid) derived compounds including glycosylated and alkylated caffeic acids are likely to be the major phytochemicals in the extracts of *O. aristatus*. The compounds are putatively identified based on their fragmentation patterns. Their relative concentrations in term of peak areas are plotted in the bar chart as shown Fig. 2. Consequently, only the caffeic acid derivatives were further analyzed using another unsupervised multivariate data analysis technique by integrating heat-map and hierarchical cluster

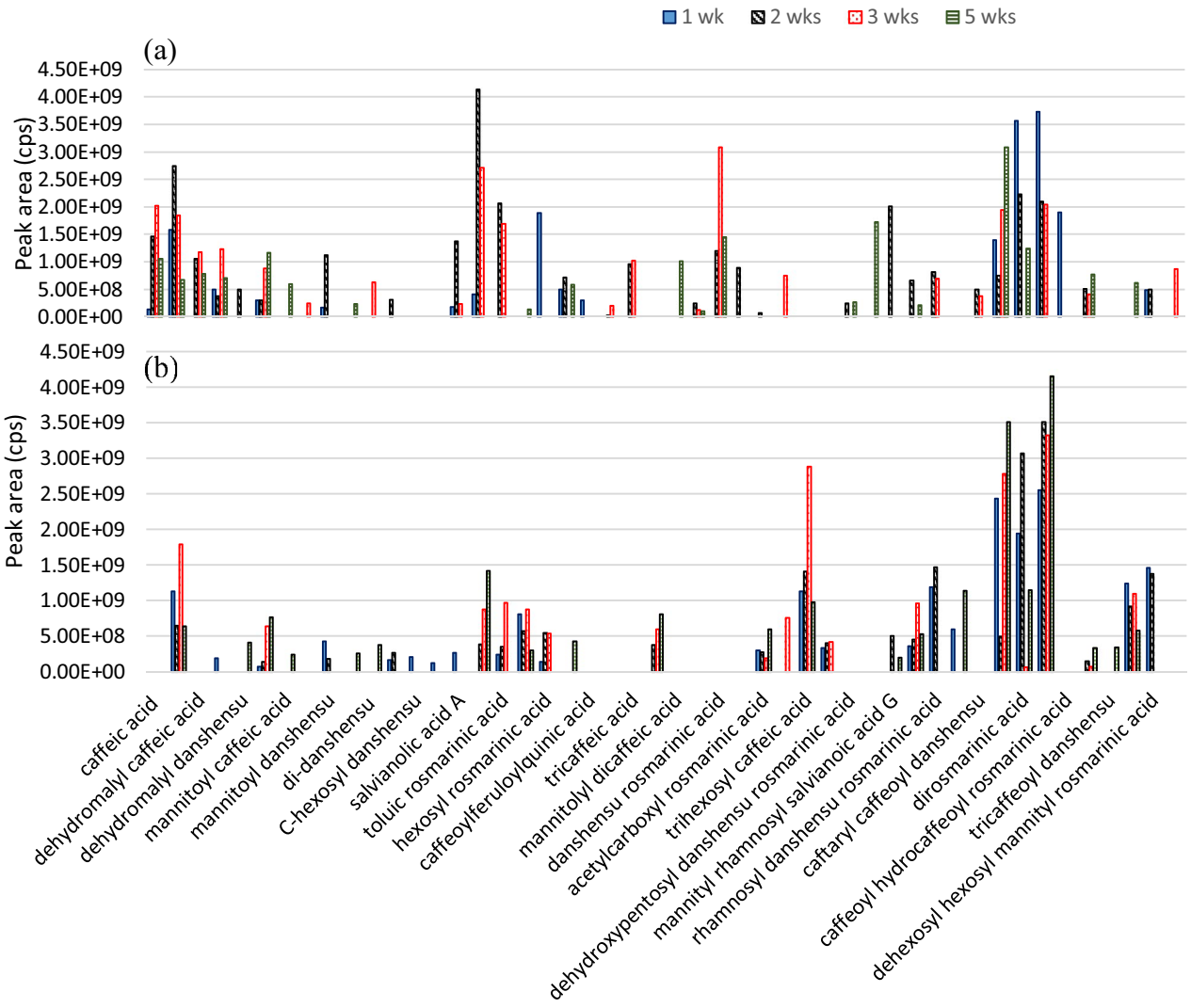

Fig. 2. Caffeic acid derived phytochemicals from the leaf (a) and stem (b) extracts of *O. aristatus*.

analysis as shown in Fig. 3. This two dimensional data representation clearly indicates the difference between plant leaves and stems in term of their phytochemical content. The leaf extracts were found to have more caffeic acid derivatives than the stem extracts. It is noticed that caffeic acid, dehydromalyl caffeic acid, tricaffeic acid, dimannitoyl caffeic acid, caftaryl caffeoyl danshensu, danshensu rosmarinic acid and hexosyl dirosmarinic acid were not detected in the stem extracts (Figs. 2 and 3). The absence of these caffeic acid derived compounds most probably had reduced the antioxidant capacity of stem extracts (Table 1). As the samples were stored for 5 weeks, the changes were observed in the phytochemical content and their profiles. There were significant decreases in the amount of caffeic acid, danshensu, salvianolic acid A and dirosmarinic acid after stored for 5 weeks as indicated in Fig. 2.

Rosmarinic acid, salvianolic acid B, tricaffeoyl hydrocaffeoic acid, danshensu caffeic acid cinnamyl ester and danshensu appeared to be the highest amount of caffeic acid derived compounds. Similarly, researchers from Thailand also reported the antioxidant activity of *Orthosiphon grandifloras* was mainly contributed by caffeic acid, danshensu, rosmarinic acid and salvianolic acid B and other caffeic acid derivatives (Nuengchamnong et al., 2011). It is interesting to highlight that rosmarinic acid and salvianolic acids were also reported to be the major caffeic acid derivatives in the Chinese *Salvia* species, or commonly called as Danshen (Da et al., 2015). Most probably, both *O. aristatus* and *Salvia* species belong to the family Lamiaceae. The high biological activities of Danshen was found to be related to these caffeic acid derivatives. Therefore, the finding revealed that the wide application of *O. aristatus* as a traditional herb by indigenous people for disease treatment could be attributed to the presence of caffeic acid derivatives, particularly rosmarinic acid and salvianolic acids in *O. aristatus* (Fig. 3).

Comparison of Orthosiphon aristatus and other tea samples

The scavenging activity of *O. aristatus* was also compared with other tea samples by using DPPH assay expressed in IC_{50} value (Fig. 4). Interestingly, the scavenging activities of *O. aristatus* either in the form of leaves (65.6 μg/ml) or the mixture of leaves and stems (79.2 μg/ml) were comparable with green tea (44.6 μg/ml), Oolong tea (28.7 μg/ml) and black tea (77.4 μg/ml). Green tea, Oolong tea and black tea belong to the aerial parts of *Camellia sinensis* which were harvested and processed using different techniques of un-fermentation, partial fermentation and full aerobic fermentation, respectively. It seemed that partial fermentation could increase the antioxidant capacity of tea leaves in Oolong tea samples significantly. The higher scavenging activity was in line with higher ratio of TPC/TFC for the tea samples including *O. aristatus*. However, the tea samples showed about three hundred times higher caffeine content than other plant samples. Therefore, *O. aristatus*

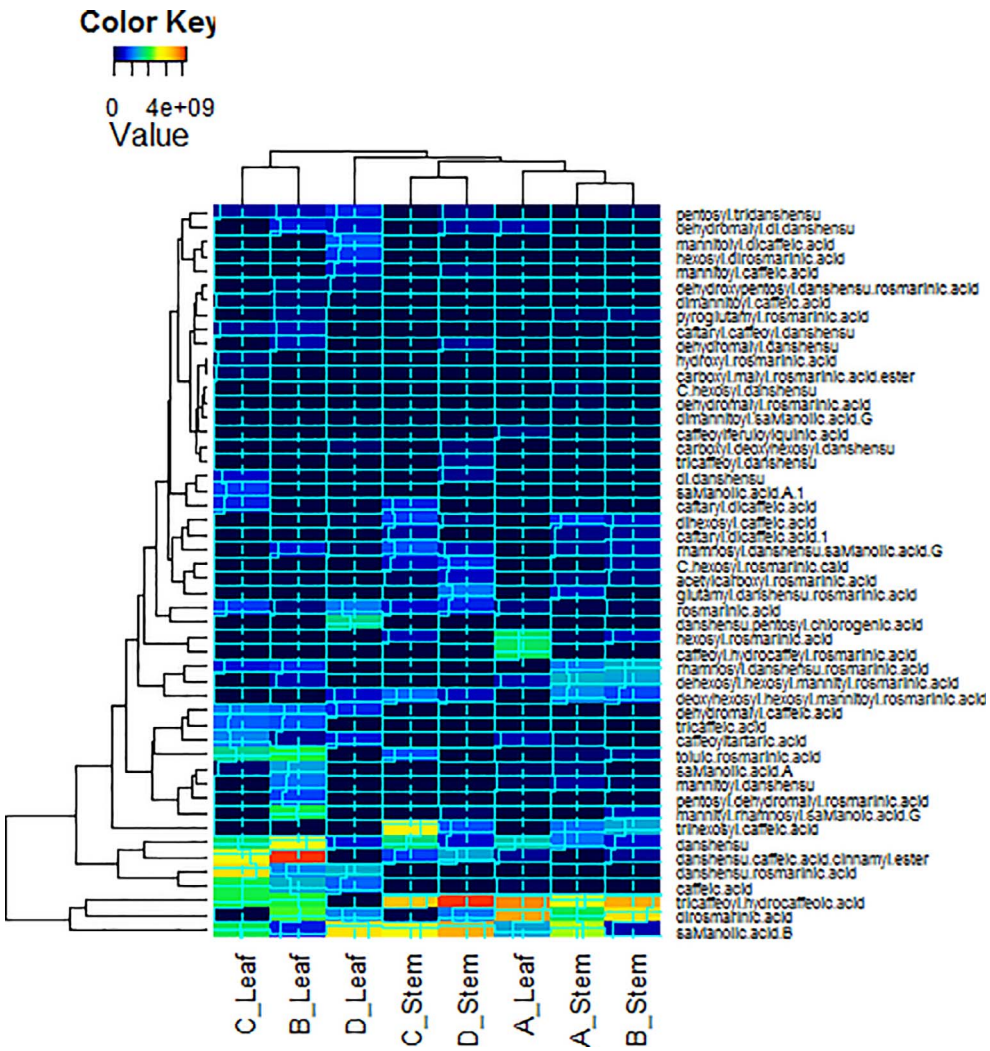

Fig. 3. Heat-map and dendrogram of caffeic acid derivatives identified from the leaves and stems of *O. aristatus* at the first week (A), second week (B), third week (C) and fifth week (D).

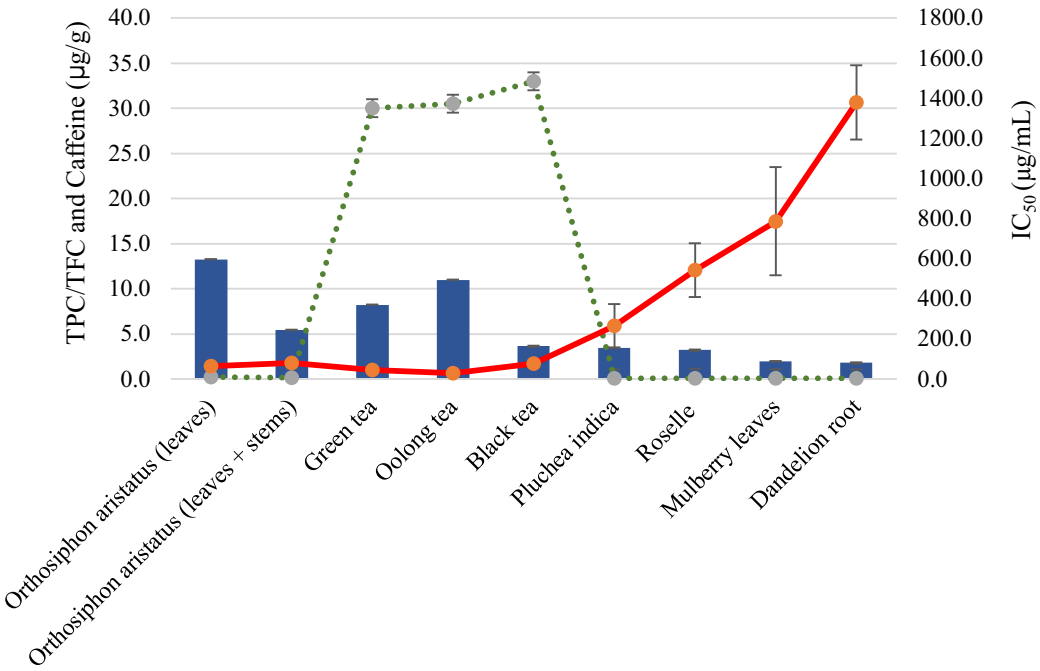

Fig. 4. TPC/TFC ratio (bar chart), antioxidant activity (solid line, IC_{50}) and caffeine content (dot line) of different tea samples.

could be the tea of choice with high antioxidant capacity, especially for those who are having caffeine allergic problem.

Conclusion

There were differences in the phytochemical profile of the leaves and stems of *O. aristatus*, particularly for caffeic acid derivatives. The difference was also noticed as the plant extracts stored for few weeks at 4 °C, especially at the fifth week of storage. The presence of caffeic acid derivatives, particularly rosmarinic acid and salvianolic B were found to be the major phytochemicals. This explains high antioxidant capacity of *O. aristatus* compared to other plant samples which are commonly used as herbal tea for health improvement. The low caffeine content of *O. aristatus* also suggests that this herb could be another good herbal tea of choice.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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