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Phenolics Comparison between Twinning and Celestial Peppermint Teas using HPLC-DAD

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Abstract
Twinings and Celestial pure peppermint herbal tea is made from 100% select peppermint leaves and is naturally caffeine and gluten-free and parts of their differences lie in the packaging. The HPLC profile of Twinning peppermint and celestial peppermint teas showed that both the teas samples contain caffeic acid, ellagic acid, *p*-coumaric acid, rosmarinic acid and rutin while celestial peppermint tea had an extra phenolic compound called quercetin. Quercetin was known for its anti-inflammatory and anti-cancer properties, and these properties could be found in celestial peppermint tea compared to the twinning counterpart.
Keywords: Twinning, Celestial, Peppermint, Tea, Phenolic

1. Introduction

Phenolic compounds functions have been the subject of a great number of agricultural, biological, chemical and medical studies. These compounds form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids. Hydroxycinnamic acid compounds are (often) produced as simple esters with glucose or hydroxy carboxylic acids. Plant phenolic compounds are diverse in molecular structure, and are characterized by hydroxylated aromatic rings (Mandal *et al.,* 2010).

Peppermint (*Mentha piperita*) is a medicinal plant of the *Labiatae* family, and it possibly originated in Eastern Asia. The plant is native to the Mediterranean and Asia, but today, peppermint is grown in temperate regions around the world. Peppermint has been used extensively in herbal medicines, and it's believed that peppermint is efficient in the immune system and in fighting secondary infections (Schuhmacher *et al.*, 2003). Peppermint has biological activities, such as anti-bacterial, anti-fungal, and anti-oxidant properties (Schuhmacher *et al.,* 2003). It has been reported that mint genera have adverse effects on induction of oxidative stress (Akdogan *et al.,* 2003). The medicinal properties of the plant are related to its chemical constituents which were reported to be containing volatile oil (0.1 – 1%): menthol (29 – 48%) as the major constituent, menthone (20- 31%), menthofuran (6 – 8%), menthyl acetate (3 – 10%), limonene (0-25%), bitter substances, caffeic acid, flavonoids (12%), polymerized polyphenol (19%), carotenes, tocopherols, betaine, choline and tannins (Rodriguez-Porcel *et al*., 2003; Paul *et al*., 2011). Twinings and Celestial Pure Peppermint Herbal Tea is made from 100% select peppermint leaves and is naturally caffeine and gluten-free and parts of their differences lie in the packaging.

Methods

Chemical, apparatus and general procedures

All chemical were of analytical grade. Acetonitrile, phosphoric acid, acetic acid, caffeic acid, ellagic acid, *p*-coumaric acid and rosmarinic acid were purchased from Merck (Darmstadt, Germany). Rutin and quercetin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

HPLC-DAD

Twinning peppermint and celestial peppermint teas were injected onto reversed phase Phenomenex C_{18} column (4.6 mm x 250 mm) packed with 5 µm diameter particles. The mobile phases were 0.5% (v/v) aqueous phosphoric acid (solvent A) and 1% (v/v) acetic acid in acetonitrile (solvent B). The binary elution system was as follows: 2% B at initial 5 min to wash the column, a linear gradient was 8% B (15 min), 12% B (25 min), 24% B (50 min). After 50 min, the organic phase concentration was brought back to 2% (B) and lasted 10 min for column equilibration. Flow rate of 0.6 mL/min and injection volume 40 µl (Khaliq *et al.,* 2015) with slight modifications. The sample and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.500 mg/mL. Quantifications were carried out by integration of the peaks using the external standard method, at 325 nm for caffeic acid, ellagic acid and *p*-coumaric acid; 330 nm for rosmarinic acid and 366 for rutin and quercetin. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Calibration curve for ellagic acid: Y = 12573x + 1427.6 (r = 0.9998); caffeic acid: Y = 11856x + 1394.7 (r = 0.9996); *p*-

coumaric acid: Y = 13648x + 1095.7 (r = 0.9995), rosmarinic acid: Y = 11682x + 1257.3 (r = 0.9999), rutin: Y = 13289x + 1045.1 (r = 0.9997) and quercetin: Y = 14025x + 1349.3 (r = 0.9995). All chromatography operations were carried out at ambient temperature and in triplicate.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated based on the standard deviation of the responses and the slope using three independent analytical curves, as defined by Boligon *et al.* (2015). LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

Statistical analysis

Differences between groups of HPLC were assessed by an analysis of variance model and Tukey's test. The level of significance for the analyses was set to $p < 0.05$. These analyses were performed by using the free software R version 3.1.1. (R Core Team, 2014).

Results

HPLC analysis

The HPLC profile of Twinning peppermint and celestial peppermint teas were acquired, HPLC analysis is shown in Fig. 1. The samples contains other minor compounds in addition to caffeic acid (retention time-t_R = 21.96 min, peak 1), ellagic acid (t_R = 22.35 min, peak 2), *p*-coumaric acid (t_R = 28.71 min, peak 3), rosmarinic acid (t_R = 32.45 min, peak 4), rutin (t_R = 39.68 min, peak 5) and quercetin (t_R = 45.73 min, peak 6).

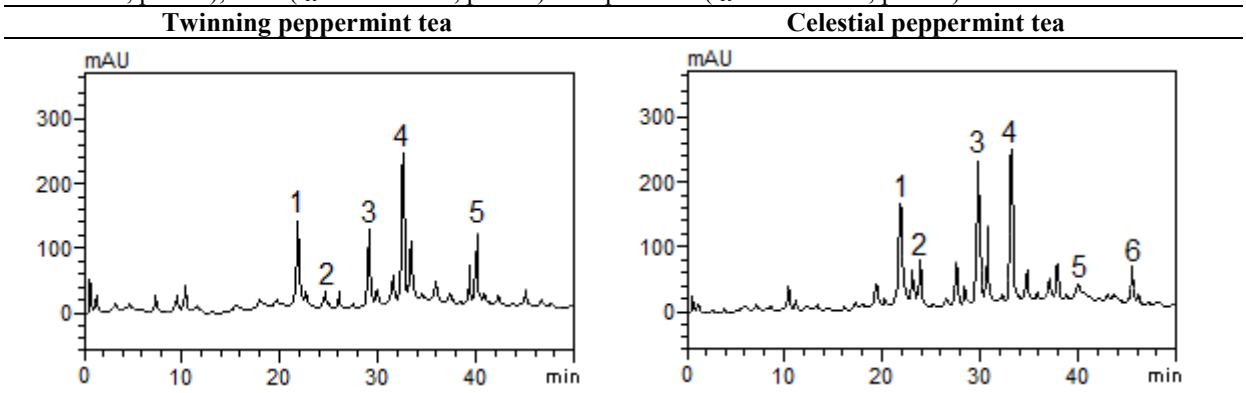

Figure 1 – Representative high performance liquid chromatography profile of Twinning peppermint and celestial peppermint teas. Caffeic acid (peak 1), ellagic acid (peak 2), *p*-coumaric acid (peak 3), rosmarinic acid (peak 4), rutin (peak 5) and quercetin (peak 6).

Table 1 – Components of twinning peppermint celestial peppermint teas.

Compounds	Twinning peppermint	Celestial peppermint	LOD	LOQ
	mg/g	mg/g	μg/mL	μg/mL
Caffeic acid	3.15 ± 0.01[a]	3.78 ± 0.02[a]	0.008	0.025
Ellagic acid	0.49 ± 0.03[b]	1.43 ± 0.03[b]	0.017	0.056
p-Coumaric acid	2.96 ± 0.01[c]	5.96 ± 0.01[c]	0.023	0.078
Rosmarinic acid	5.81 ± 0.02[d]	6.01 ± 0.04[c]	0.011	0.037
Rutin	2.93 ± 0.04[c]	0.75 ± 0.01[d]	0.009	0.030
Quercetin	-	1.40 ± 0.01[b]	0.025	0.084

Results are expressed as mean ± standard deviations (SD) of three determinations.
Averages followed by different letters differ by Tukey test at $p < 0.05$

Discussion

Phenolics and flavonoids possess diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant (Ghasemzadeh *et al.,* 2011), cytotoxic and antitumor, antispasmodic and antidepressant activities (Lim *et al.,* 2006). The HPLC profile of Twinning peppermint and celestial peppermint teas showed that both the teas samples contain caffeic acid, ellagic acid, *p*-coumaric acid, rosmarinic acid and rutin. In addition, celestial peppermint tea had an extra phenolic compound called quercetin, which is an added advantage to the sample. Quercetin has been shown to have potent anti-inflammatory activity and antitumor properties including the inhibition of cancer cells proliferation and migration (Lim *et al.,* 2006).

Conclusion

It can be concluded from this study that celestial peppermint tea had an additional unique phenolic content than twinning counterpart.

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