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Chemical composition and antioxidant and antifungal properties of *Mentha x piperita* L. (peppermint) and *Mentha arvensis* L. (cornmint) samples

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Abstract

Essential oils and infusions from commercial peppermint sachets (CPS), and non-commercial genuine peppermint (NCP) and cornmint (NCC) samples were analyzed by GC/MS and LC/MS. Minimum inhibitory concentration (MIC) of mint oils against *Fusarium moniliforme*, *Aspergillus niger* and *Aspergillus fumigates* was determined. Antioxidant potential was monitored by total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and soybean oil oxidation tests. CPS and NCC oils had lower menthofuran content than NCP. Mint oils did not show a uniform standard of antifungal activity and they had the modest reducing ability. CPS and NCC infusions showed higher IC50 and lower TPC than NCP ones. In the soybean oxidation test, mint oils presented prooxidant behavior. CPS infusions showed antioxidant potential significantly (P<0.05, Tukey) lower than that from NCC and NCP infusions. NCP infusions were more efficient in delaying propagation reaction than NCC ones. This may be attributed to higher amount of rosmarinic acid and hesperidin in NCP.

1. Introduction

Available epidemiological and experimental evidence suggest that infusion–and essential oil derived from peppermint leaves have potential health benefits. Several biological effects, including antifungal activity and relief of irritable bowel syndrome and allergic respiratory symptoms, are usually assigned to essential oil or to some of its terpenic constituents (e.g. p -menthanes, 1,8-cineole) (Khanna *et al.,* 2014; Balakrishnan, 2015). On the other hand, complementary treatment of dyspepsia, flatulence, intestinal colic, both gall bladder and biliary tract spasms and oxidative stress has been attributed to consumption of peppermint infusions which contain biologically active components, such as rosmarinic acid and flavonoids, primarily eriocitrin, luteolin and hesperidin and their glycosides (Lv *et al*., 2012; Riachi and De Maria, 2015).

Plant-derived antioxidant and antifungal properties have raised appreciable interest among consumers, food scientists, and producers. Firstly, the scope of interest comes from numerous observational and experimental studies relating plant food consumption to the lower risk of developing cancer and cardiovascular disease (Oyebode *et al.*, 2014; Abu-Reidah *et al.*, 2015). Although the free-radical theory of ageing (FRTA) remains unproven, etiology of chronic diseases and cancer appears to emerge from different pathways involving metabolic flux changes after genetic perturbations due to exogenous and endogenous factors, including oxidative stress (Barja, 2014). Secondly, there are serious concerns about risks and safety of the chronic human consumption of synthetic compounds traditionally used as preservatives, for example, antifungals. This concern is reflected in the growing interest in evaluating the availability of natural plant

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products as an alternative to the use of synthetic food additives (Uribe *et al.*, 2016).

Commercial sachets and/or essential oils of peppermint have commonly been used for human consumption. Consumers are likely to have favorable attitudes toward the use of genuine commercial dried products (eg sachets) in the hope that they have similar properties to fresh green plant. However, literature reports on chemical composition and antioxidant and antifungal properties of CPS are really scarce. Further, very little or nothing is known about whether and to what degree the properties of fresh plants are found in sachets. A factor which could potentially influence the quality of CPS is the addition of cornmint, a less-expensive mint plant which is frequently used as peppermint adulterant (Boren *et al.*, 2015). Peppermint contains menthofuran in levels ≥ 0.4%, while this monoterpene is not detected or is detected in levels ≤ 0.01% in cornmint (Benn, 1998; Boren *et al.*, 2015). The addition of cornmint could drastically change intrinsic properties of CPS what would make it exceedingly walk away from the biological properties of the fresh plant. The purpose of this study was to analyze and compare antioxidant and antifungal properties, and phenolic and essential oil profiles in both CPS, NCP and NCC samples.

2. Materials and methods

2.1 Chemicals

DPPH, Folin - Ciocalteu's reagent, gallic acid, methanol, absolute ethanol, rosmarinic and chlorogenic acids, flavonoid standards, menthol, menthone, menthofuran, pulegone, 1,8 cineole and other terpene standards were purchased from Sigma - Aldrich Chemical Co. (Missouri, USA). Acetonitrile (HPLC grade) and isooctane (UV grade) were supplied by Merck (Darmstadt, Germany). All other solvents and reagents used in the analysis were of analytical grade. Fresh refined soya oil was purchased in a Brazilian supermarket.

2.2 Sachets and fresh plants

Five different brands of CPS samples (sachets 1-5) were acquired in Brazilian supermarkets. Samples of the same brand were acquired from different batches. NCP and NCC leafs were from *Mentha x piperita* L. and *Mentha arvensis* L., respectively, cultivated in the UFFS (Chapecó, Santa Catarina, Brazil) by Dr. Geraldo Ceni Coelho. Leaves were collected from plants between the bud stage and pre-flowering and carefully dried and pressed. Vouchers were examined by Dr. José Luis Fernández Alonso (Consejo Superior de Investigaciones Científicas / CSIC, Madríd, España) to verify the identity of non-commercial dried plants used in the present study. Each voucher specimen of *Mentha x piperita* L. (MA 888548) and of *Mentha arvensis* L. (MA 888550) was deposited in the Real Jardín Botánico at CSIC by Dr. José Luis.

2.3 Tisane preparation and essential oil extraction

Tisanes (aqueous extracts) were obtained from 2.0 g of each CPS and of each sample of NCP and NCC leaves which were subjected to drying and grinding in similar conditions to CPS. To obtain infusions of studied samples, boiling distilled water (100 mL) was poured over the plant material, mixed, capped and filtered under gravity after 15 min.

CPS, NCP and NCC samples (100 g in 700 mL distilled water) were used for essential oil extraction by Clevenger's apparatus (hydrodistillation). Essential oil extraction by hydrodistillation was continued at 97^{o}C for 4 hours. The essential oil extracts were dried under vacuum with anhydrous sodium sulphate.

2.4 Chromatography

2.4.1 Essential oil

A Shimadzu GC-17A/QP-505 quadrupole mass spectrometer equipped with a 30 m x 0.25 mm apolar fused-silica capillary column with a film thickness of 0.25 mm and with an own data system and 1 cyclic scan (Shimadzu, Kyoto, Japan) was employed. The equipment was operated at an ion source temperature of 240^{o}C and a mass range of m/z 20-300. Oven temperature program was 50-240^{o}C with the rate at 3^{o}C min^{-1} maintaining 240^{o}C for 20 min. Injector temperature was 240^{o}C. Identification of the volatiles in mint essential oils was based on a comparison of mass spectra of unknown compounds against NIST library data for the GC/MS, and of Kövatz index (KI) from literature. Definitive identification was obtained by comparison with commercial standards.

2.4.2 Phenolics

Phenolics in mint tisanes were analyzed by HPLC-UV based on the previous work (Fecka and Turek, 2007). An Agilent 1200 HPLC system with two pumps (type 64) and a diode array UV detector (280 nm) and an auto-sampler (injection volume of 20 µL) and a 5 µm C18 column (250 x 4 mm, i.d.) (Agilent, California, USA) was used for quantitative analyses. Chromatographic conditions were as follows: flow rate

of 0.9 mL min^{-1} with solvent A, 5% formic acid in acetonitrile; solvent B, 5% formic acid in water; commencing with 10% A in B, rising to 40% after 25 min and then to 70% after 30 min.

For identification of phenolics was used a MicrOTOF II© Bruker Daltonic GmbH series mass spectrometer (Bruker, California, USA) with an electrospray ionization system. The flow rate was set to 0.3 ml min^{-1} and the column was a Poroshell® EC-C18, 2.7 µm (100 x 2.1 mm ID) type from Agilent (California, USA). The injection volume was 1.0 µl. The solvent program was: solvent A, 5% formic acid in acetonitrile; solvent B, 5% formic acid in water, commencing with 15% A in B, rising to 35% A after 25 minutes, then to 70% A after 27 minutes and rising to 100% A after 35 minutes. Definitive identification was based on comparison with mass spectra from the literature and by use of commercial standards. The limit of quantitation (LoQ) was achieved for each analyte. The concentration of the analyte below the LoQ was considered trace amount.

LoQ: hesperidin = 10 µg mL^{-1}; rosmarinic acid = 10 µg mL^{-1}; eriocitrin = 10 µg mL^{-1}; chlorogenic acid = 25 µg mL^{-1}; luteolin-7-o-glucoronide = 10 µg mL^{-1}

2.5 DPPH radical scavenging activity

The reduction of the DPPH by the mint tisanes and essential oils was measured at 515 nm on a UV/visible light spectrophotometer (SP 2000 UV, Bel, Colorado, USA), according to the procedure described by Derwich *et al.* (2011), with some modifications. The decrease in absorbance at 515 nm was determined after 1 h for all samples. Methanol was fixed to zero of the spectrophotometer. The absorbance of the DPPH radical without antioxidant, the control, was also measured.

2.6 TPC

TPC of both mint tisanes and essential oils was measured according to the Folin - Ciocalteu method (Dewanto *et al.*, 2002), with some modifications. Briefly, 1g of mint tisane (1g 100 mL^{-1}) or 50 mg of mint oil was diluted in methanol to 25 mL in a volumetric flask. An aliquot of 200 µL of tisane or essential oil solution was mixed with 2 ml of 4 % (w/v) sodium carbonate solution and 2.5 mL of Folin – Ciocalteu's reagent. After standing for 2 h in dark environment at room temperature for color development, absorbance was measured at 750 nm using the Bel spectrophotometer described in Section 2.5. A gallic acid standard (0.1 g 100^{-1} mL) solution was used. Results were expressed as mg gallic acid equivalents (GAE) per g dry-matter.

2.7 Oxidative stability of soya oil

Oxidation in soya oil was monitored by the increase in the ultraviolet absorption based on previous method (Luzia *et al.*, 1994), with some modifications. Conjugated dienes were measured at 233 nm ($E^{1\%}_{1\ cm}$) in the Bel spectrophotometer described in Section 2.5. Mint essential oil (20 mg) or mint tisane (100 mg) was dissolved in 4 mL of absolute ethanol and afterward added to the oil (20 g) in 100 mL beakers and mixed with a magnetic stirrer for 1 min. Beakers were covered externally with aluminium foil and subjected to accelerated oxidation in the dark at 55±2°C for 15 days. The position of each beaker inside the oven was consistent throughout storage. Aliquots (100 µL) were removed at 5-days intervals (0, 5, 10, 15 days) to monitor the rate of oxidation in the oil and dissolved in isooctane and made up to the 100 mL mark in a volumetric flask. The contents of each beaker were thoroughly mixed with a magnetic stirrer for 1 min before removing the aliquot. Control sample contained the same amount of ethanol as was used to dissolve mint fractions.

2.8 Antifungal activity

The minimal inhibitory concentration (MIC) of each mint essential oil against *Fusarium moniliforme*, *Aspergillus niger* and *Aspergillus fumigatus* was determined using the broth microdilution method recommended previously (Clinical and Laboratory Standards Institute, 2008). MICs were determined for all species with the adjusted inoculum suspension of 3 X 10^3 cells mL^{-1} in RPMI 1640 medium (Sigma, Missouri, USA) containing various concentrations of mint oil (2 – 5 mg mL^{-1}) directly diluted in culture medium. Cell growth was determined visually in 96-well plates by turbidity and subsequent color change of resazurin used as an indicator of viability (blue indicated inhibition of microorganism growth and pink indicated microorganisms growth). The MIC was defined as the concentration required to inhibiting the growth of the strains relative to controls, which were grown in the absence of mint oils. All the measurements were performed in triplicate, and two independent experiments were performed with similar results.

2.9 Statistical analysis

The data were reported as mean ± standard deviation (SD) (n = 5). The mean results from the forced oxidation of soya oil test were submitted to analysis of variance (ANOVA) with Tukey's test, (GraphPad Prism 6.0 software, California, USA). Statistical significant was

declared at $P < 0.05$.

3. Results and discussion

3.1 Essential oil and phenolics profile

In the present study, essential oil yields of NCC (4%, v/w) and NCP samples (2.5%, v/w) were consistent with those (1.2-3.9%, v/w) reported elsewhere (Chen *et al.*, 2011). CPS, on the other hand, provided low oil yields (0.5-0.7%, v/w). Although oil yield is susceptible to genetic and environmental variations and different cultivation practices (Rohloff *et al.*, 2005) the lower yield also could be explained by the presence of higher macroscopic-impurity contents and other interfering plant materials. CPS contained 5 to 13% of macroscopic impurities (sticks and stones), whereas NCP and NCC samples contained less than 1%. More than 90% of the total relative peak area of mint oils was represented by p-menthanes (Table 1), which agreed with results found elsewhere (Chen *et al.*, 2011). They were mainly represented in CPS by menthol (> 37%), wherein NCP samples mainly contained menthone (31%). Differences in the concentration of individual p-menthanes could be attributed to leaf maturity and/or particular harvest stage. In general, menthol is abundant in older leaves, whereas younger leaves have relatively more menthone (Clark and Menary, 2006). Peppermint plants harvested at the flowering stage usually have a higher concentration of menthol than plants in the bud stage, which produce a large amount of menthone, indicating an early harvest (Rohloff, 2005). In NCC oil, the most abundant p-menthane was isomenthol (> 80%) (Table 1). It also contained more 3-octanol and isopulegol than peppermint oil. Variations in pattern of menthol, 3-octanol, and isopulegol were in accordance with previous data reported elsewhere (Benn, 1998; Boren *et al.*, 2015). Interestingly, menthofuran was detected in levels up to 0.34% in CPS, while this monoterpene was not detected in NCC. In contrast, NCP samples had considerably higher menthofuran levels (11.95%). In general, menthofuran is found in peppermint in levels from 0.4% to 14.6% and so it could be an original marker to identify authentic peppermint oil (Boren *et al.*, 2015). A total ion chromatogram of NCP sample is shown in Figure 2.

Distribution of some phenolics in CPS, NCC and NCP tisanes is shown in Table 2. Hesperidin, a flavone glycoside, was a major compound in all the samples analysed, while rosmarinic acid and luteolin-*7-O-β*-glucuronide were majority compounds only in CPS and NCP tisanes. Eriocitrin, a flavanone glycoside, was a minor compound, while chlorogenic acid was found only in trace amounts. These findings agreed in part with reports described elsewhere (Fecka and Turek, 2007; Farnad *et al.*, 2014; Riachi and De Maria, 2015). Individual phenolic composition from the CPS tisanes showed a great variation. These differences could be attributed to different edaphoclimatic conditions, which influence the plant secondary metabolism and to particular harvest stage. However, it was possible to observe a pattern of reducing hesperidin content in CPS tisanes when compared to NCP tisanes.

3.2 In vitro antioxidant and antifungal properties

TPC and DPPH tests have often been considered to be easy-to-use indicators of the reducing ability of a food. However, based on previous report (Amorati *et al.*, 2013), the best way to assess the antioxidant potency is to accomplish the rate of oxidation of polyunsaturated lipids. Nevertheless, combining results from DPPH and TPC with the lipid oxidation test leads to a full assessment of either antioxidant or prooxidant behaviour of essential oils and tisanes. There is a close correlation between radical scavenging activity and TPC of extracts obtained from various natural sources (Erkan *et al.*, 2008). Further, a direct correlation between DPPH e TPC tests (r^2 = 0.97, p-value < 0.05) has been found in peppermint (Uribe *et al.*, 2016).

TPC and DPPH values of mint oils are shown in Table 3. In DPPH assay, reducing ability of NCP and NCC essential oils was by about 3-fold and 1.5-fold, respectively, higher than that of CPS oils. The IC50 of CPS oils found here partially agreed with those found in other studies, such as (Kizil *et al.*, 2010) (IC50 = 60) and (Derwich *et al.*, 2011) (IC50 = 57), but was *circa* 12 -fold higher than that reported by Tsai *et al.* (2013) (IC = 4.5). Despite the wide variation observed with DPPH test, it is possible to observe modest scavenging potential. This finding is in accordance with low levels of TPC found in all analyzed CPS, NCP and NCC essential oils (Table 3).

Reducing ability of NCP and NCC tisanes was approximately 36% and 22%, respectively, higher than that of CPS tisanes (Table 3). NCP tisane, on the other hand, had higher (*circa* 21%) reducing ability than NCC one. NCP tisanes, but no CPS, had similar IC50 values to those found elsewhere (IC = 13.6) (Grul'ová *et al.*, 2012) and (IC = 11.3) (Farnad *et al.*, 2014). In general, mint infusions are rich in flavonoids and phenolic acids, as can be easily verified by the higher values on the TPC test (Table 3), and therefore they have larger reducing ability than mint oils.

Table 1. Essential oil composition of non-commercial peppermint (NCP) and cornmint (NCC) samples and commercial peppermint sachets (CPS)

Compounds	KI	*M. piperita*	*M. arvensis*	S.1	S.2	S.3	S.4	S.5
α-Pinene	959	0.46	tr.	tr.	tr.	tr.	tr.	tr.
Myrcene	968	0.76	tr.	tr.	tr.	tr.	tr.	tr.
3-Octanol	977	tr.	0.53	0.20	0.30	0.10	0.30	0.04
Octanol	978	0.43	Tr.	0.02	0.15	0.20	tr.	0.02
Limonene	1024	1.13	0.63	0.01	tr.	tr.	tr.	0.03
1,8-cineole	1030	3.89	tr.	0.42	1.21	0.30	1.60	1.31
Sabinene hydrate	1072	0.32	tr.	tr.	tr.	tr.	tr.	tr.
Linalool	1099	tr.	0.44	0.13	0.22	0.12	0.30	0.19
Isopulegol	1155	tr.	0.32	tr.	tr.	tr.	tr.	tr.
Menthone	1162	31.43	4.93	14.90	21.22	15.31	6.20	6.73
Isomenthone	1171	2.35	1.62	4.04	6.64	4.24	0.10	4.22
Menthofuran	1173	11.95	tr.	0.34	0.26	0.16	0.10	0.32
Neomenthol	1175	tr.	1.71	3.50	3.86	2.83	4.60	0.74
Menthol	1186	14.08	84.52	44.71	37.08	50.70	35.50	38.17
Isomenthol	1189	12.00	tr.	tr.	0.51	tr.	2.30	1.18
α-Terpineol	1204	tr.	tr.	tr.	tr.	tr.	tr.	tr.
Pulegone	1244	10.47	0.35	3.01	2.76	2.18	1.10	1.31
Piperitone	1259	0.41	0.76	0.05	1.84	0.03	0.30	0.09
Caryophyllene	1424	tr.	0.77	0.08	0.05	0.01	0.10	0.41
Menthol acetate	1426	2.28	tr.	7.12	6.99	4.58	4.10	4.15
Germacrene	1485	tr.	0.46	tr.	tr.	tr.	tr.	tr.

tr - trace amount; Results are expressed in % area; KI - Kóvatz index; S.1 – S.5 = commercial peppermint sachets (CPS).

Table 2. Phenolic compounds of the aqueous extract (tisane) from non-commercial peppermint (NCP) and cornmint (NCC) samples and commercial peppermint sachets (CPS).

Phenolic compounds	*M. piperita*	*M. arvensis*	S.1	S.2	S.3	S.4	S.5
Hesperidin	18.64 ± 0.16	9.51 ± 0.44	tr	1.49 ± 0.05	8.72 ± 0.76	1.50 ± 0.29	12.81 ± 0.84
Rosmarinic acid	4.91 ± 0.18	tr	5.32 ± 0.29	5.54 ± 0.06	9.53 ± 0.14	2.15 ±0. 02	4.28 ± 0.29
Eriocitrin	1.55 ± 0.08	tr	1.89 ± 0.1	tr	1.99 ± 0.1	tr	1.62 ± 0.04
Chlorogenic acid	tr	tr	tr	tr	tr	tr	tr
Luteolin 7-*O*-*β*-glucuronide	3.68 ± 0.01	tr	7.48 ± 0.01	tr	9.37 ± 0.01	tr	9.34 ± 0.01

tr – trace amount; Results expressed as mg 100mL^{-1} tisane; S.1 – S.5 = commercial peppermint sachets (CPS).

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Some of the differences in IC50 values between results obtained here and those from literature arise because single-point measurements of absorbance can vary from laboratory to laboratory. Thus, results should only be compared when obtained under similar settings. The observed differences in IC 50 values could also be satisfactorily explained on the basis of compositional changes in mint leaves exposed on different edaphoclimatic conditions.

Edible oils containing methylene interrupted dienes or polyenes show a shift in their double bond position during oxidation due to isomerization and formation of conjugated dienes which is easily accompanied by an intense absorption at 233 nm (Wanasundara *et al.*, 1995). The effect of mint essential oils on UV absorption at 233 nm of refined soybean oil samples over the 15-days storage period at 55°C is shown in Figure 1a. Although some degree of unpredictability could arise from the variability of the composition of the oxidizing matrix, it was minimized by careful monitoring of the oxidation of pure soybean oil (control sample) and soybean oil treated with mint oils. A 5-day induction period was noted for soybean oil. After, it had a typical lipid oxidation behavior showing propagation stage. The gain of dienes values (DV) in the soybean samples treated with mint oils was significantly (Tukey test, $P<0.05$) higher than that from control sample. Soybean samples treated with CPS oils showed DV significantly ($P<0.05$) higher than those treated with NCP and NCC oils. On the other hand, soybean samples treated with NCC oil exhibited a significantly ($P<0.05$) lower DV than those treated with NCP oil. In general terms, mint oils exhibited prooxidant behaviour throughout the accelerated dark oxidation assay which was carried out in soybean oil. The prooxidant behaviour observed during forced oxidation test (Figure 1a) contrasted with the modest antioxidant potential observed in DPPH assay (Table 3). This inconsistency is attributed to DPPH ability to react not only with reactive oxygen species but also with unsaturated hydrocarbons (eg α-pinene) by abstraction of a hydrogen atom from C–H bonds. The hydrogen atom transfer is thermodynamically feasible because of dissociation enthalpy of the weakest C–H bond of α-pinene parallels with N–H bond of DPPH (80.7 and 78.9 kcal mol^{-1}, respectively) (Amorati *et al.*, 2013). Therefore, DPPH discolouration would be unable to truly reflect the antioxidant behaviour of peppermint oil. On the contrary, DPPH reduction could be caused by abstraction of a hydrogen atom from α-pinene favouring the formation of prooxidant species. Cornmint oil, on the other hand, did not contain α-pinene but it had linalool. A previous study has characterized the linalool-O_2biradical intermediate state, which constitutes a branching point for the further oxidation reactions pathways (Bäcktorp *et al.*, 2006). Linalool oxidation products may favour isomerization and formation of conjugated dienes during accelerated dark oxidation of soybean oil treated with cornmint oil. Previous findings on the antioxidant behaviour of mint oils are conflicting. Peppermint essential oil subjected to Rancimat assay exhibited prooxidant behavior (Politeo *et al.*, 2006). In contrast, it showed antioxidant action in a linoleic acid emulsion system through decreasing DV formation (Schmidt *et al.*, 2009). In fact, monoterpenes, such as linalool and α-pinene have shown prooxidant action, whereas thymol and unsaturated terpenes which have a cyclohexadiene structure (eg γ-terpinene) appear to reduce the overall rate of oxidation (Amorati *et al.*, 2013). Mint oils are a mixture of different types of oxidizable terpenoids, and depending on composition and experimental conditions, synergistic or antagonistic interaction can occur. Under experimental procedures used in the present study, results with forced oxidation test provided some preliminary evidence to support the hypothesis that polyunsaturated fatty acids of soybean oil to be protected were co-oxidized with mint oil components.

The effect of mint tisanes on UV absorption at 233 nm of refined soybean oil samples is shown in Figure 1b. Soybean samples treated with mint tisanes had a gain of DV significantly (Tukey test, $P<0.05$) lower than that from control sample for up to 10 days, except for sachet 2 which had a gain of DV significantly ($P<0.05$) higher. In general, the CPS tisanes had less antioxidant power in the course of propagation stage making soya oil treated with CPS tisanes have gained of DV significantly higher ($P<0.05$) than that control. Soybean samples treated with CPS tisanes showed DV significantly ($P<0.05$) higher than those treated with NCP and NCC tisanes. Soybean samples treated with NCP tisanes had gained of DV similar to that from soybean samples treated with NCC tisanes during induction period (up to 5 days). In the propagation period, however, the gain of DV in soybean samples treated with NCP tisanes was significantly ($P<0.05$) lower. A possible explanation for this difference would be that NCC tisanes did not contain rosmarinic acid and presented hesperidin content *circa* 2-fold lower than that from NCP ones (Table 2). Further, hesperidin content in CPS tisanes was by about 2- to 10-fold lower than that from NCP tisanes. Previous reports have described that hesperidin and rosmarinic acid could provide strong antioxidant protection against the harmful effects of

Table 3. Reducing ability of the essential oil and aqueous extract (tisane) from non-commercial peppermint (NCP) and cornmint (NCC) samples and commercial peppermint sachets (CPS).

Assays	*M. piperita*	*M. arvensis*	S.1	S.2	S.3	S.4	S.5
Essential oil							
TPC (mg g^{-1})	0.34 ± 0.18	< 0.10	0.98 ± 0.07	0.98 ± 0.05	1.01 ± 0.11	1.13 ± 0.17	0.81 ± 0.05
DPPH (IC50) (mgmL^{-1})	13.58 ± 0.16	26.72 ± 0.13	36.08 ± 0.67	56.63 ± 0.61	37.07 ± 0.54	38.80 ± 0.48	40.60 ± 0.57
Aqueous extract							
TPC (mg g^{-1})	3.47 ± 0.04	1.18 ± 0.02	2.44 ± 0.22	2.53 ± 0.34	2.80 ± 0.28	2.41 ± 0.27	1.73 ± 0.24
DPPH (IC50) (mgmL^{-1})	12.24 ± 0.23	15.45 ± 0.12	17.43 ± 0.25	23.64 ± 0.30	13.74 ± 0.32	23.56 ± 0.22	20.38 ± 0.19

TPC = total phenolic content assay; DPPH = 2,2-diphenylpicrylhydrazyl reagent; S.1 – S.5 = commercial peppermint sachets (CPS).

Table 4. Minimum inhibitory concentration of the essential oil from non-commercial peppermint (NCP) and cornmint (NCC) samples and commercial peppermint sachets (CPS).

Fungi	*M. piperita*	*M. arvensis*	S.1	S.2	S.3	S.4	S.5
A. fumigatus	2.50 ± 0.0	1.25 ± 0.0	1.25 ± 0.0	N.I.	1.25 ± 0.0	1.25 ± 0.0	2.50 ± 0.0
A. niger	1.25 ± 0.0	1.25 ± 0.0	2.50 ± 0.0	N.I.	2.50 ± 0.0	1.25 ± 0.0	2.50 ± 0.0
F. moniliforme	2.50 ± 0.0	2.50 ± 0.0	2.50 ± 0.0	1.25 ± 0.0	2.50 ± 0.0	1.25 ± 0.0	1.25 ± 0.0

Results expressed as mg mL^{-1}; N.I. = not inhibited; S.1 – S.5 = commercial peppermint sachets.

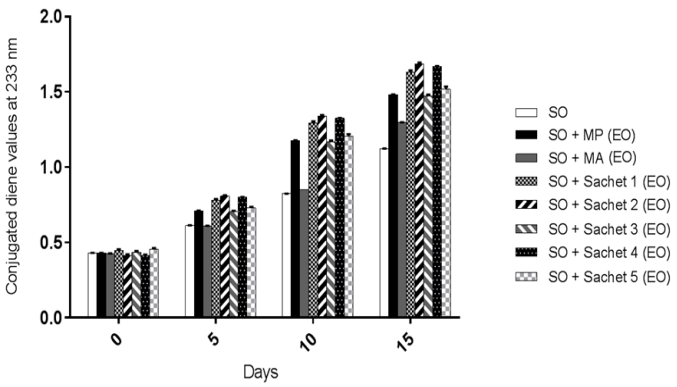

Figure 1a. Effects of non-commercial peppermint and cornmint essential oils and commercial peppermint sachet essential oils on ultraviolet absorption of fresh refined soya oil at 55°C.
SO = soya oil; MP = *M. piperita*; MA = *M. arvensis*; EO = essential oil

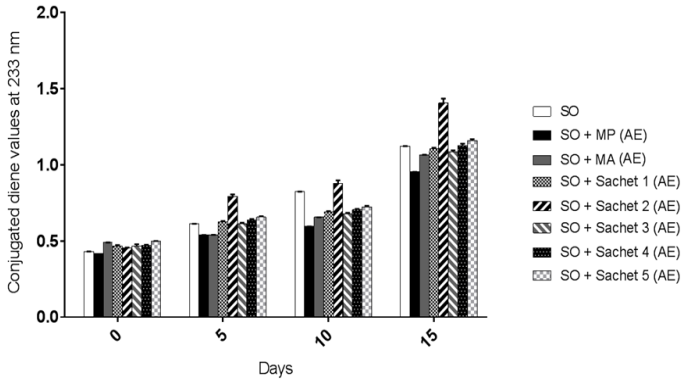

Figure 1b. Effects of non-commercial peppermint and cornmint tisanes and commercial peppermint sachet tisanes on ultraviolet absorption of fresh refined soya oil at 55°C.
SO = soya oil; MP = *M. piperita*; MA = *M. arvensis*; AE = aqueous extract (tisane)

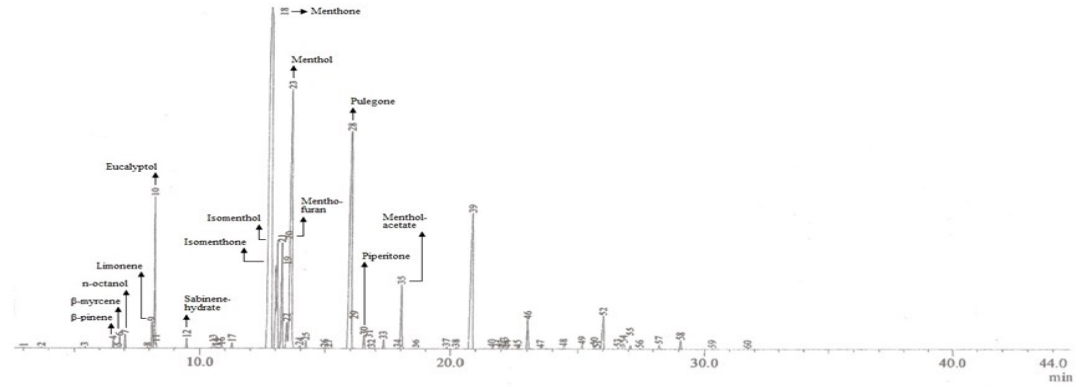

Figure 2. A total ion chromatogram of a non-commercial genuine peppermint (NCP) sample.

reactive oxygen species (Wilmsen *et al.*, 2005; Riachi and De Maria, 2015). It should be taken into account that other minor phenolic compounds which were not analyzed here could contribute to increased antioxidant activity of the NCP tisanes. Peppermint leaves contain more than ten compounds belonging to different groups of phenolics of which 53% are flavonoids, followed by phenolic acids (42%), lignans and stilbenes (2.5%) (Fecka and Turek, 2007; Riachi and De Maria, 2015).

Antifungal potential of mint oils was determined by MIC test which is defined as the lowest concentration of an antimicrobial capable of inhibiting the visible growth of a microorganism. The MIC of mint oils for three pathogenic fungi ranged from 1.25 to 2.50 mg L^{-1} (Table 4). NCC and NCP oils showed a similar tendency in the antifungal activity against *F. moniliforme* and *A. Niger*. It could be attributed to similar inhibition action of major p-menthanes against these two fungi. This finding was in accordance with that described in the previous study which associated the antifungal activity of mint oils with the presence of menthol and menthone (Hussain *et al.*, 2010). NCC oil, on the other hand, demonstrated antifungal activity against *A. fumigatus* at MIC of 1.25 mg mL^{-1} which was half of that from NCP oil. The best antifungal activity of NCC oil against *A. fumigatus* may be due to the high content of menthol isomers. A previous study has reported that chiral compounds with substituents in the equatorial position could induce changes in the chiral organization of biomembranes (Bernardini *et al.*, 2009). In fact, the menthol enantiomers were the most active monoterpenes in the inhibition of fumonisin B1 biosynthesis (Dambolena *et al.*, 2010). The lower water solubility of menthol enantiomers made it easier for the chiral compounds to accumulate inside the membrane and therefore disrupt the membrane integrity, causing its rapid depolarization (Maffei *et al.*, 2001; Dambolena *et al.*, 2010). MIC results from the CPS oils showed a great variation. Three CPS and NCC oils showed similar MIC against *A. fumigatus*. CPS oils had lower antifungal activity against *A. niger* than NCP and NCC oils. In contrast, three CPS oils demonstrated inhibitory activity against *F. moniliforme* at MIC of 1.25 mg mL^{-1} which was half of those from NCP and NCC oils. In general, CPS, NCP and NCC oils used here showed some inhibitory potential on the fungal growth, which paralleled to that reported elsewhere (Hussain *et al.*, 2010; Oh *et al.*, 2013). On the other hand, antifungal activity was not accompanied by a corresponding antioxidant activity. The presence of terpenic prooxidants could positively contribute to antifungal property but not to antioxidant effect. On the other hand, results reported previously in the literature have shown a positive association between antioxidant power and antimicrobial activity (Oh *et al.*, 2013; Sumalan *et al.*, 2013). This divergence could be attributed to the use of indirect methods to monitoring antioxidant behavior. As described in the present study, DPPH values of CPS, NCP and NCC oils indicated some reducing ability. Conversely, mint oils increased the overall rate of lipid oxidation reflecting prooxidant status.

4. Conclusion

Results from the present study allowed to identify differences in the composition and, antioxidant and antifungal activities between CPS, and NCP and NCC samples analyzed here. The low menthofuran content suggested that cornmint plant parts could have been added to CPS, causing ones to have low menthofuran content (<0.4 %). The addition of cornmint could explain the low menthofuran content, but not the low yield of essential oil. This last alteration may be explained by the possible addition of essential oil non-producing plant materials from other genera which may not change the relative distribution of p-menthanes. This issue needs further investigation. Even though DPPH test had shown a modest reducing ability of mint oils, results obtained with forced oxidation of soybean oil test indicated that CPS, NCP and NCC oils are able to have prooxidant behavior. The presence de α-pinene and linalool could contribute for this prooxidant effect. Therefore, use of mint oils as an antioxidant array should be viewed with caution. Different from observed with mint oils, antioxidant potential of mint tisanes was unequivocally demonstrated by both forced oxidation of soybean oil, DPPH, and TPC tests. CPS tisanes showed an antioxidant potential significantly (P<0.05) lower in the soybean oil oxidation test when compared to that found in NCC and NCP tisanes. This last seems to be more efficient in delaying propagation reaction during soya oil oxidation than NCC tisanes. The higher antioxidant efficiency of NCP tisanes could be partially explained by the larger amounts of rosmarinic acid and hesperidin found. It also should be taken into account that distribution of phenolics and terpenes in plants can vary with edaphoclimatic conditions, which influence the plant secondary metabolism. In general, CPS and NCC samples showed lower antioxidant power in comparison with NCP samples. In terms of antifungal activity, CPS, NCP and NCC oils did not show a uniform standard which could be attributed to compositional differences. It was not possible to find any association between the antioxidant potential and the antifungal activity in this

study. Producers and decision-makers must be aware of the necessity to reach stricter quality standards for CPS samples in order to attend consumer expectations.

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