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MAJOR PHENOLIC ACIDS OF LOCAL AND EXOTIC MINT GERMPLASM GROWN IN ISLAMABAD

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Abstract

Phenolic acids have gained considerable interest during the past few years, owing to their potential health benefits from antiallergenic, anti-inflammatory, antimicrobial and antioxidant properties. During this investigation, selected phenolic acid (caffeic acid, rosmarinic acid, ferulic acid and eugenol) concentrations were determined in fifteen local and exotic mint genotypes grown in the Islamabad area, using reverse phase High Performance Liquid Chromatography (HPLC). Statistically significant ($p>0.01$) differences in concentrations were observed for caffeic, rosmarinic and ferulic acid while eugenol concentrations showed non-significant variation. Rosmarinic and caffeic acid were the major phenolic acids detected in mint germplasm. Ferulic acid was also detected in considerable concentrations in local mint cultivars. The presented data suggest that mints have the potential to be used as dietary sources of caffeic, rosmarinic and ferulic acids.

Introduction

Plants belonging to Lamiaceae (Mint Family) include mint, basil, sage, rosemary, lavender, oregano and have long been used in food preservation, culinary seasoning and perfumery. Their extracts can be used directly or indirectly for the treatment of different ailments. Interest has revived recently in the investigation of medicinal plants to identify novel active phytochemicals that might lead to drug development. Currently more than 50% of drugs in clinical use have a natural-product origin, and about half of the world's 25 best-selling pharmaceutical agents are derivatives of natural products (Aslam, 2006). Mints are inhabitants of six continents and are important sources of traditional medicine. The genus *Mentha* possesses a great degree of morphological and phytochemical diversity (Saric-Kundalic *et al*., 2009). Essential oils isolated from the mints have a long history of use in products that span the range from pharmaceuticals to food (Hayes *et al*., 2007).

Mints are well known for their antioxidant properties (Shan *et al*., 2005; Hossain *et al*., 2008) and recent research has indicated that they are a rich source of polyphenols. Natural antioxidants can protect the human body from free radicals and could retard the progress of many chronic diseases; they can also reduce lipid oxidative rancidity in foods (Regnault-Roger *et al*., 2004; Arts & Hollman, 2005; Williamson & Manach, 2005). Many researchers have determined the concentrations of polyphenols including caffeic acid, rosmarinic acid, ferulic acid, quercetin, myercetin and luteaolin in oregano (Radusiene *et al*., 2009), basil (Kruma *et al*., 2008), thyme (Kulisic *et al*., 2006) and mint (She *et al*., 2010).

Polyphenolic compounds have high antioxidant activity due to the reactivity of the phenol ring (Fialova *et al*., 2008; Tekel'ova *et al*., 2009). Polyphenols are categorized into different classes depending upon the number of phenol rings. The main groups are flavonoids, phenolic acids, phenolic alcohols, stilbenes and lignans (D'Archivio *et al*., 2007). Polyphenols, in their role as antioxidants, may guard cell components against oxidative stress/damage and therefore reduce the risk of various degenerative diseases such as diabetes and cardiovascular disease (Scalbert *et al*., 2005). The antioxidant activity of phenolic acids is due to their ability to scavenge free radicals, donate hydrogen atoms or electrons or chelate metal cations (Amarowicz *et al*., 2004). The structure of phenolic compounds is a key determinant of their radical scavenging and metal chelating activity and this is referred to as a structure activity relationship (SAR). In the case of phenolic acids, the antioxidant activity depends on the numbers and positions of the hydroxyl groups in relation to the carboxyl functional group (Robards *et al*., 1999) and increases with increasing the degree of hydroxylation, as is the case of trihydroxylated gallic acid which shows a high antioxidant activity.

Among the variety of polyphenolic compounds, phenolic acids have attracted considerable interest in the past few years because they exhibit many potential health benefits such as antiallergenic, antiatherogenic, anti-inflammatory, antimicrobial, antioxidant, antithrombotic cardioprotective and vasodilatory effects (Middleton *et al*., 2000; Puupponen-Pimiä *et al*., 2001; Manach and Mazur, 2005). Phenolic acids can be further divided in two types: derivatives of benzoic acid and derivatives of cinnamic acid. The hydroxybenzoic acids such as gallic and protocatechuic acid are found in very few plants eaten by humans. Coumaric, caffeic and ferulic acids are hydroxycinnamic derivatives and rarely found in free form. Mostly they exist as conjugated mono- and polysaccharides, linked to one or more of the phenolic groups, but they may also occur as functional derivatives such as esters and methyl esters. Rosmarinic acid has anti-inflammatory and cancer chemopreventive activity (Scalbert *et al*., 2005). Prevention of isoniazid induced oxidative damage in red blood cells implies a strong antioxidant nature of caffeic acid (Yilmaz *et al*., 2008). Ferulic acid is part of plant cell walls, leaves and seeds and possesses a free radical scavenging nature due to its formation of a resonance-stabilized phenoxy radical (Marimuthu *et al*., 2007). Eugenol is a phenylpropene and acts as an antibacterial agent by disrupting cytoplasmic membranes (Devi *et al*., 2010).

This study determined the phenolic acid profiles in different mint cultivars collected from local and exotic sources. Since no information on determination of

polyphenols in Lamiaceae is available in Pakistan, this investigation provides basic information for further work on antioxidant potential in herbs and other medicinal plants.

Materials and Methods

The mint cultivars under investigation were acquired from different sources and are being preserved and maintained in a medicinal plant clonal repository at the Institute of Agri-Biotechnology & Genetic Resources (IABGR), National Agricultural Research Centre (NARC), Islamabad. The fresh leaves of 15 mint cultivars (Table 1) belonging to the genera *Mentha*, *Melissa* and *Nepeta*, were collected in April, 2010 (spring), washed thoroughly to remove dirt and other contaminants and dried in the glass house (ca. 32-35°C) for three days. Dried leaves were sealed in plastic bags and stored at room temperature for further analysis.

Table 1. Mint species used during the present study.

S. No.	Common name	Scientific name	Origin
1.	Common mint (white flower)	*Mentha arvensis*	Local
2.	Common mint (purple flower)	*Mentha arvensis*	Local
3.	Mint Asavi	*	Saudi Arabia
4.	Peppermint	*Mentha piperita*	Local
5.	Lavender mint	*Mentha* x *piperita* var. "Lavender mint"	Unknown
6.	Lemon mint	*Melissa officinalis*	Local
7.	Aquatic mint	*Mentha aquatica*	Local
8.	Pennyroyal	*Mentha pulegium*	Unknown
9.	Cool mint	*Mentha spicata*	Canada
10.	Chinese mint	*Mentha arvensis*	China
11.	Pahari Pudina	*Mentha royleana*	Local
12.	White Mint	*Mentha longifolia*	Local
13.	Mint Camphor	*Mentha piperita*	AJK**
14.	Catnip mint	*Nepeta cataria*	Local
15.	Field mint	*Mentha arvensis*	Local

*Not known; **Azad Jammu & Kashmir

Phenolic acids were extracted following Javanmardi *et al*. (2002). The dried leaves (500 mg) were ground into powder and suspended in 5 mL methanol and left overnight at 4°C in the dark. Supernatants were decanted and filtered using syringe filters and transferred into HPLC vials. The Ultimate 3000 Dionex was used for reversed phase HPLC analysis with Waters Spherisorb ODS2 column, 5 µm (250 mm x 4.6 mm). Major phenolic acids were determined following Kruma *et al*. (2008). Elution was performed at a flow rate of 1.0 ml/min, using mobile phase, a mixture of 0.05% formic acid (HPLC grade) in water (solvent A) at pH=3.1, acetonitrile (HPLC grade) (solvent B). The solvent gradient was changed according to the following conditions: 1) 0-5 min, B: 10-35%; 2) 5-20 min, B: 35-70%; 3) 20-40 min, B: 70-90%; 4) B: 40-41 min, B: 90-95%; 5) 41-42 min, B: 50-25%; 6) 42-43 min, B: 25-5%; 7) 43-44 min, B: 5-0%; and 8) 44-46 min, B:0-10%. The monitoring wavelength was 280 nm for the phenolic acids. Four standard compounds (caffeic acid, rosmarinic acid, ferulic acid and eugenol) were purchased from Sigma-Aldrich. The identification/quantification of each compound in the samples was based on the comparison of retention time with standards of known concentration. All the chemicals/solvents used in the study were of analytical/HPLC grade.

The experiments were conducted in triplicate. Analyses of variance (ANOVA) were calculated using the statistical package MSTAT-C with least significant differences (LSD) at the 5% probability level. The statistical differences between means were calculated using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Phenolic acid concentrations were determined in 15 cultivars belonging to the genera *Mentha*, *Melissa* and *Nepeta* through reverse phase HPLC. Statistically significant differences for caffeic acid concentration among different mint cultivars ($P \leq 0.01$) were observed. *Nepeta cataria* exhibited the highest concentration of caffeic acid (315.4 mg/100 g) while *Mentha longifolia* and *Mentha pulegium* contained 314.8 and 189.2 mg/100 g caffeic acid (Table 2). The lowest amount of caffeic acid was observed in *Mentha arvensis* from China (4.14 mg/100 g). The range of caffeic acid concentration observed during the present study showed great variation. Proestos *et al*. (2008) analyzed naturally occurring phenolic compounds in *Origanum dicamnus, Origanum vulgare* and *Melissa officinalis* through RP-HPLC. They detected caffeic acid only in *Melissa officinalis* (13.8±0.1 mg/100 g). Lee (2010) reported low levels of caffeic acid in *Melissa officinalis*, peppermint and spearmint (2.68, 5.76 and 4.80 mg/100 g, respectively) available in the US market. Lemon mint and peppermint grown in the Islamabad area showed remarkable differences in caffeic acid concentrations (35.25 and 15.05 mg/100 g, respectively). These differences may be attributed to different geographical origins, growing conditions (Cabrera *et al*., 2006) and different accessions used in the experiments.

Highly significant differences were also observed for rosmarinic acid among all 15 cultivars ($P \leq 0.01$). *Mentha arvensis* (field mint), with 362.2 mg/100 g, had the highest concentration of rosmarinic acid observed among

the tested plants. *Mentha spicata* (cool mint) and *Mentha piperita* (mint camphor from AJK) had 298.7 mg/100 g and 287.4 mg/100 g of rosmarinic acid, respectively. The lowest concentration was detected in *Mentha aquatic* (water mint) (26.45 mg/100 g). *Melissa officinalis* (lemon mint) contained 89.29 mg/100 g of rosmarinic acid. Fialova *et al.* (2008) also reported 13% rosmarinic acid in *Melissa officinalis*. Sanchez-Medina *et al.* (2007) reported a higher range of rosmarinic acid (11-22.5 mg/ml) in dried lemon mint leaves than in fresh leaves while studying the rosmarinic acid contents in *Melissa officinalis* tinctures. The present investigation revealed a wide range of rosmarinic acid among different plants of *Mentha*, *Melissa* and *Nepeta* (Table 2). Feka & Turek (2007) reported a narrower range of variation in rosmarinic acid concentration, up to 182.2 mg/100 g, in plants of Lamiaceae, including peppermint and sage, in Poland. These differences could be attributed to different genotypes, growing and environmental conditions (Cavaliere, 2009).

Table 2. Mean values of major polyphenols in mint germplasm in medicinal plant clonal repository at IABGR, NARC, Islamabad.

S. No.	Variety	Caffeic acid (mg/100g)	Rosmarinic acid (mg/100g)	Ferulic acid (mg/100g)	Eugenol (mg/100g)
1.	Common mint (White flower)	5.85 ± 0.059 ^{d}	188.4 ± 0.036 ^{e}	44.46 ± 0.016 ^{e}	0.02 ± 0.002 ^{b}
2.	Common mint (Purple flower)	9.28 ± 0.032 ^{cd}	130.7 ± 0.0315 ^{f}	51.33 ± 0.034 ^{d}	0.03 ± 0.001 ^{b}
3.	Mint Asavi	11.78 ± 0.434 ^{cd}	215.0 ± 0.01 ^{d}	14.05 ± 0.054 ^{h}	8.44 ± 0.043 ^{b}
4.	Peppermint	15.05 ± 0.028 ^{cd}	32.98 ± 0.043 ^{m}	63.88 ± 0.064 ^{b}	2.24 ± 0.029 ^{b}
5.	Lavender mint	21.12 ± 0.015 ^{cd}	91.26 ± 0.037 ^{g}	0.18 ± 0.029 ^{n}	66.81 ± 0.028 ^{a}
6.	Lemon mint	35.25 ± 0.023 ^{c}	89.29 ± 0.079 ^{h}	0.00 ± 0.000 ^{o}	79.57 ± 0.140 ^{a}
7.	Aquatic mint	19.64 ± 0.023 ^{cd}	26.45 ± 0.026 ^{n}	4.43 ± 0.046 ^{j}	24.81 ± 0.006 ^{b}
8.	Pennyroyal mint	189.2 ± 0.027 ^{b}	65.35 ± 0.036 ^{i}	0.45 ± 0.033 ^{l}	4.56 ± 0.009 ^{b}
9.	Cool mint	8.72 ± 0.027 ^{cd}	298.2 ± 2.307 ^{b}	67.71 ± 0.054 ^{a}	25.42 ± 0.045 ^{b}
10.	Chinese mint	4.14 ± 0.037 ^{d}	56.74 ± 0.033 ^{k}	0.36 ± 0.047 ^{l}	0.74 ± 0.025 ^{b}
11.	Pahari Pudina	179.2 ± 0.015 ^{b}	46.93 ± 0.029 ^{l}	41.76 ± 0.017 ^{f}	0.00 ± 0.000 ^{b}
12.	White mint	314.8 ± 0.055 ^{a}	61.74 ± 0.047 ^{j}	0.94 ± 0.008 ^{k}	16.79 ± 0.012 ^{b}
13.	Mint camphor	10.64 ± 0.039 ^{cd}	287.4 ± 0.034 ^{c}	28.93 ± 0.036^{g}	11.32 ± 0.021 ^{b}
14.	Catnip mint	315.4 ± 0.200 ^{a}	61.20 ± 0.045 ^{j}	12.23 ± 0.025 ^{i}	0.00 ± 0.000 ^{b}
15.	Field mint	4.83 ± 0.010 ^{d}	362.2 ± 0.029 ^{a}	62.50 ± 0.077 ^{c}	9.09 ± 0.030 ^{b}

*Means with same letters are not statistically different

Rosmarinic acid concentrations were higher than caffeic acid concentrations in the different mints studied during this investigation. Kwon *et al*., (2006) also reported higher concentrations of rosmarinic acid (16.56 mg/g dry weight) than caffeic acid (0.10 mg/g dry weight) while evaluating the clonal herbs of Lamiaceae including, oregano, rosemary and lemon balm.

Ferulic acid concentrations showed significant ($p \leq 0.01$) differences among the studied cultivars of mints. There was no ferulic acid detected in *Melissa officinalis*, while only 0.18 mg/100 g of ferulic acid was detected in lavender mint (*Mentha* x *piperita*) (Table 2). The highest ferulic acid concentration was identified in *Mentha spicata* (67.71 mg/100 g), while peppermint and *Mentha arvensis* (field mint) contained 63.88 and 62.50 mg/100 g, respectively. Ferulic acid exhibits a wide range of therapeutic properties that are attributed to its phenolic nucleus and conjugated side chain (Marimuthu *et al*., 2007). Ferulic acid is found in many mint family plants including lemon mint (*Melissa officinalis*) (Hanganu *et al*., 2008). Shan *et al.* (2005) reported the presence of ferulic acid in *Mentha canadensis* in the range of 186.1 mg/100 g of dried leaves. Delazar *et al.* (2004) also detected ferulic acid and its two derivatives (hexacosyl-(*E*)-ferulate and leucosceptoside) in different mint family plants from Iran.

No significant differences in eugenol concentrations were found among the studied mint cultivars ($p > 0.05$). Mean values presented in Table 2 indicate differences in eugenol concentrations, but due to high coefficient of variance percentage, these differences become statistically non-significant. *Melissa officinalis* contained the highest concentration of eugenol (79.57 mg/100 g), followed by lavender mint (66.81 mg/100 g). *Nepeta cataria* contained no eugenol while locally collected *Mentha arvensis* cultivars (white & purple flower) contained traces of eugenol.

Differences in phenolic acid profiles may be due to different agro-climatic (climatic, seasonal and geographical) variations, extraction procedures and physiological conditions of the plants. Ravn *et al*., (1994) reported higher levels of rosmarinic and caffeic acid during spring than summer and winter, and noted a loss of polyphenols during sample preparation. Our data suggest that mints have the potential to be used as dietary sources of caffeic, rosmarinic and ferulic acids. However, further investigations are required to study the antioxidant and antimicrobial potential of mint cultivars grown in Pakistan.

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