Polyphenolic compounds of Mentha longifolia and Lemon Balm (*Melissa officinalis* L.) in Iran

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**ABSTRACT:** Lemon balm, *Melissa officinalis*, is a member of the mint family that is native to Europe. Its use as a medicinal herb dates from the Middle Ages, and it is very well known for its ability to reduce stress and anxiety, promote sleep, improve appetite, and ease pain and discomfort associated with digestion and *Mentha longifolia* (*Lamiaceae*), commonly known as wild mint. Various biological activities have been reported for some species of Mentha, such as antibacterial, antifungal and insecticidal properties. The aerial parts of *Melissa officinalis* and *Mentha longifolia* were collected at the full-flowering stage from Iran during August 2012 and dried by shade drying methods. Polyphenols are the most abundant antioxidants in our diet and are widespread constituents of fruits, vegetables, cereals, olive, dry legumes, chocolate and beverages, such as tea, coffee and wine. Polyphenolic compounds were identified by HPLC-DAD. There were 3 polyphenols detected in *Mentha longifolia*. Gallic acid detected in *Mentha longifolia* and *Melissa officinalis* but in *Mentha longifolia* more than *Melissa officinalis*. Catechin (216.1306mg/g), Caffeic acid (210.3761mg/g) found only in *Mentha longifolia*.

**Keywords:** *Melissa officinalis*, *Mentha longifolia*, polyphenolic compounds; Catechin; Caffeic acid

**INTRODUCTION**

Polyphenols are a group of secondary metabolites involved in the scavenging of plant cells. Interest in plant materials rich in polyphenolic compounds are on the increase due to their high antioxidant potency, which may offer protection against cancer, through the inhibition of oxidative damage, known to be a potential cause of mutation. Free radicals cause oxidative damage to lipids, proteins, and nucleic acids (Shui and Leong, 2004). The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity (Yang et al., 2001; Cotelle, 2001; Facino et al., 1990). They possess ideal structural chemistry for radical scavenging activity and are more effective than tocopherol and ascorbate (Pand- hair and Sekhon, 2006). In the aerobes, due to large generation of reactive oxygen species such as superoxide radical, hydrogen peroxide, and hydroxyl radical leads to severe effects on the cardiovascular system either through lipid peroxidation or vasoconstriction and other ailments such as inflammation, cancer, diabetes mellitus etc (Lachance et al., 2001; Nickavar et al., 2007). Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Wolfe et al., 2003; Naik et al., 2006). Flavonoids are 15-carbon compounds generally distributed through the plant kingdom (Harborne, 1988). Lemon balm (*Melissa officinalis* L.) is one of the oldest and still most popular medicinal plants. It is a representative of the *Lamiaceae* family that is known for many aromatic and medicinal plants commonly used in Europe’s traditional medicine and gastronomy. Originally growing in the Mediterranean area, lemon balm is now spread in the flora of South Slovakia, and Moravia as well (Bertova, 1993). The most commonly known therapeutic properties of lemon balm are sedative, antispasmodic, antibacterial, antiviral, anti-inflammatory and anti oxidative (Wagner and Sprinkmeyer, 1973; May and Willuhn, 1978; Masakova et al., 1979; Koch-Heitzmann and Schultze, 1984; Lamaison et al., 1991; Vogt et al., 1991; Borkowski and Biesiadecka, 1996; Yamaski et al., 1998). The genus Mentha belongs to the family *Lamiaceae* (*Labiatae*), and consists of about 25-30 species, most of which are found in temperate regions of Eurasia, Australia and South Africa (Lange and Croteau, 1999). *Mentha longifolia* (*Lamiaceae*), commonly known as wild mint, is a perennial herb that can grow 1-2 m high. Various biological activities have been reported for some species of Mentha, such as antibacterial (Oyedeji and Afolayan, 2006; Hajlaoui et al, 2008), antifungal (Bouchra et al., 2003), and
insecticidal properties (Franzios et al., 1997; Lamiri et al., 2001; Pavela, 2005; Saljoqi et al., 2006). Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid, a type of organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants (Reynolds and Wilson, 1991). The chemical formula is C₆H₆(OH)₃ COOH.

![3,4,5-trihydroxybenzoic acid](image1)

**Figure 1. Chemical structure of Gallic acid**

Caffeic acid is an organic compound that is classified as hydroxycinnamic acid. This yellow solid consists of both phenolic and acrylic functional groups. It is found in all plants because it is a key intermediate in the biosynthesis of lignin, one of the principal sources of biomass (Boerjan et al., 2003). Catechin is a flavan-3-ol, a type of natural phenol and antioxidant. It is a plant secondary metabolite. It belongs to the group of flavan-3-ols (or simply flavanols). It is often considered to belong to the family of flavonoids.

![Caffeic acid](image2)

**Figure 2. Chemical structure of Caffeic acid**
Sample preparation
The aerial parts of Melissa officinalis were collected at the full-flowering stage from Eram Garden in Fars province (South of Iran) during August 2012. And the aerial parts of Mentha longifolia were collected at the full-flowering stage in Sepidan city in Shiraz, during August 2012. The samples were obtained by cutting the herb manually in a height of about 5 cm above ground. Samples were collected in the course of full flowering period. The herb was dried at air-dried at ambient temperature in the shade and stored in sacks in a dark, cool and dry depository. They were authorized by Ahmad Hatami (Department of biology Fars research center, Shiraz, Iran). A voucher specimen of the plants is deposited in the herbarium of the Research Center for Agriculture and Natural Resources, Shiraz, Iran.

Extraction of Polyphenol
For extraction of polyphenol from plant material we used the procedure that was reported by (Justesen et al., 1998). The procedure summarized in Figure 4. The resulting extract was directly subjected to HPLC analysis.

Powdered material of plant
Extraction with MeOH-CH₃COOH (85:15 v/v) (2ml per 200mg of sample)
Leave at freezer temperature (-18 °C) for 24hr
Sonicate for 15 min
Centrifuge at 10000 rpm for 20 min in 0 °C
Separate the supernatants and add n-Hexane (1:1 v/v) and mix thoroughly
Centrifuge at 10000 rpm for 20 min in 0 °C
Remove polyphenol fraction (lower phase)
Filter through 0.2 μm pore size membrane filter
Store in darkness in freezer at -18 °C until analyse

Figure 4. Procedure For extraction of polyphenols

HPLC analysis
HPLC analysis was carried out on a Agilent 1200 series, equipped with a Zorbax Eclipse XDB-C18 column (10cm × 5 μm i.d.; × 150 mm film thickness, RP), and a photodiode array detector (PDA). Elution was monitored at 280 and 230 nm. Gradient elution was selected to achieve maximum separation and sensitivity. The elution was performed by varying the proportion of solvent A (formic acid 1% in deionized water) to solvent B (Methanol (v/v)) as follows: Methanol: formic acid 1% (10:90), at 0 min; Methanol: formic acid 1% (25:75), at 10 min; Methanol: formic acid 1% (60:40), at 20 min and finally, Methanol: formic acid 1% (70:30), at 30 min. The total running time was 30 min. The column temperature was 30 °C. The injection volume was 20µL and it was done automatically using autosampler.
RESULTS AND DISCUSSION

There were 3 polyphenols detected in Mentha longifolia. They were Gallic acid, Catechin, Caffeic acid (Table 2). Gallic acid detected in Mentha longifolia and Melissa officinalis but in Mentha longifolia more than Melissa officinalis. Both Melissa leaf and herb are used as herbal drugs in Slovak and Czech Republics, western pharmacopoeias prefer the leaf that is the main source of therapeutic principles (BLA-Suchar, 1956; Pharmacopoea, 1987; Codex, 1993; Codex, 1997; Pharmacopoea, 1997-2002; Pharmacopoea, 1997-1999; European, 2002). Phenolic acids, flavonoids and tannins are the most commonly found polyphenolic compounds in plant extracts (Wolfe et al., 2003; Naik et al., 2006). Measurement of the polyphenols and free radical scavenging activity of herbs has become important tools for the understanding of the relative importance of plant species especially from the health point of view (Chang et al., 2007). Gallic acid is found both free and as part of hydrolyzable tannins. Salts and esters of gallic acid are termed 'gallates'. Despite its name, it does not contain gallium. Gallic acid is commonly used in the pharmaceutical industry (Fiuza, 2004). Gallic acid can also be used as a starting material in the synthesis of the psychedelic alkaloid mescaline (Makepeace, 1951). Gallic acid seems to have anti-fungal and anti-viral properties. Gallic acid acts as an antioxidant and helps to protect human cells against oxidative damage. Gallic acid was found to show cytotoxicity against cancer cells, without harming healthy cells. Gallic acid is used as a remote astringent in cases of internal haemorrhage. Gallic acid is also used to treat albuminuria and diabetes. Some ointments to treat psoriasis and external haemorrhoids contain gallic acid (phytochemicals.info). Catechin, Caffeic acid found only in Mentha longifolia (Table 2). Caffeic acid, which is unrelated to caffeine, is biosynthesized by hydroxylation of coumaroyl ester of quinic ester. This hydroxylation produces the caffeic acid ester of shikimic acid, which converts to chlorogenic acid. It is the precursor to ferulic acid, coniferyl alcohol, and sinapyl alcohol, all of which are significant building blocks in lignin (Boerjan et al., 2003).

Table 1. Linear regression equation and correlation coefficient for Caffeic acid, Catechin and Gallic acid in Melissa officinalis and Menthe longifolia.

<table>
<thead>
<tr>
<th>Standard name</th>
<th>Linear regression equation</th>
<th>Correlation coefficient</th>
</tr>
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<tbody>
<tr>
<td>Gallic acid</td>
<td>Y = 40.507x - 33.427</td>
<td>0.9992</td>
</tr>
<tr>
<td>Catechin</td>
<td>Y = 9.2191x - 77.022</td>
<td>0.9966</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>Y = 12.586x + 42.447</td>
<td>0.9987</td>
</tr>
</tbody>
</table>

Table 2. Phenolic compounds of Melissa officinalis and Mentha longifolia.

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Caffeic acid</th>
<th>Catechin</th>
<th>Gallic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentha longifolia</td>
<td>Amount (a)</td>
<td>210.376</td>
<td>216.1306</td>
</tr>
<tr>
<td>Melissa officinalis</td>
<td>Amount (a)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
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\(a\) Calculated mean amount of the polyphenol (mg/g) based on the weight of the ground dry plant.

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