Horsemint as a potential raw material for the food industry: survey on the chemistry of a less studied mint species

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Abstract  Horsemint (Mentha longifolia L), is wild-growing species, widespread in Eurasia and Africa. The review focuses on its potential utilization as a preservative and flavoring in the food industry based on the polyphenolic and terpenoid composition. Several phenolic antioxidants were detected in horsemint, among which rosmarinic acid may have a key role. Nineteen other acids, and fifty-five flavonoids (six which are de novo) were also identified. The antiradical efficacy in horsemint extract has not yet been adequately justified. Similarly, systematic screening of the flavonoid composition of the species is lacking. Horsemint essential oils possess an outstandingly wide variability in composition which may serve as basis of special flavoring or antimicrobial agents. The efficacy of horsemint volatiles have been demonstrated against more than twenty microbes. As current literature of horsemint lacks comparable results, the present review provides the broadest and therefore, a critical overview, on its most important secondary compounds and the factors influencing their accumulation.

Keywords  Mentha longifolia · Polyphenols · Antioxidants · Volatiles · Antimicrobial · Food

Abbreviations
AAPH  2,2'-Azobis(2-amidinopropane) dihydrochloride
AO  Antioxidant
DCM  Dichloromethane
dp  Dry plant material
DPPH  2,2'-Diphenyl pycrylhydrazyl
EC50  Effective concentration-50
EO  Essential oil
FRAP  Ferrous reducing activity
IC%  Inhibitory concentration in percentage
MIC  Minimal inhibitory concentration
RA  Rosmarinic acid
TAC  Total antioxidant capacity
TF  Total flavonoid content
THD  Total hydroxycinnamic acid content

Introduction

Mentha longifolia L, horsemint, wild or biblical mint is a perennial herb belonging to the Mentha genus in the Nepetoideae subfamily of Lamiaceae. According
to the monography of the genus (Tucker and Naczi 2007), its natural distribution area is the largest among wild-growing Mentha species, covering temperate and mediterranean regions of Eurasia and Africa. (Tucker and Naczi 2007; Sevindik et al. 2017; Sevindik 2018).

This may be evaluated as a sign of adaptivity. The large number of taxa included by the species indicates its genetic diversity. The monography lists 22 subspecies of Mentha longifolia described from different regions of the world (Table 1).

In Turkey, Iraq, Iran, Pakistan and Arabic countries, leaves or flowering shoots are used as a spice, i.e. for dairy specialties (Tunc¸turk et al. 2011; Mahmoudi et al. 2012; Ehsani and Mahmoudi 2012), as leafy vegetables, herbal tea and an ethnomedicinal remedy (Ghoulami et al. 2001; Başer et al. 2012; Iqbal et al. 2013; Mikaili et al. 2013; Murad et al. 2016; Sevindik et al. 2017). A recent review of Farzaei et al. (2017) provides ethnopharmacological data in the aforementioned regions, with a wide variety of traditional indications. Beside collection, Mentha longifolia is reported to be cultivated in Tunisia (Hajlaoui et al. 2009) and its intraspecific taxon Mentha schimperi var. schimperi syn. ssp. schimperi in Sudan (Younis and Beshir 2011).

In Europe, Mentha longifolia is far less known and used, contrary to its abundance in wet meadows, forests and ruderal areas. A couple of works have however, been published on analyzing constituents and/or preparations of Mentha longifolia due to potential industry-related uses (Dudai et al. 2006; Güllüce et al. 2007; Krzyzanowska et al. 2011; Bertoli et al. 2011; Orhan et al. 2012). Beside them, some works are also available on Mentha longifolia as a medicinal plant, primarily of antiinflammatory and chemoprotective effects (Mimica-Dukić et al. 1996, 1999; Shen et al. 2011; Baris et al. 2011; Vladimir-Knežević et al. 2014). Nevertheless, Mentha longifolia is less studied as other Mentha species, partially concerning its non-volatile constituents. Until now it has not been used either industrially or pharmaceutically on a large-scale. However, it seems to be a cheap and prosperous

| Subspecies            | Country or region                              |
|-----------------------|------------------------------------------------|
| ssp. calliantha       | Southwestern Iran, Eastern Anatolia            |
| ssp. capensis         | Cape Colony, Zimbabwe, Lesotho, Namibia        |
| ssp. caucasica        | Caucasus                                       |
| ssp. cyprica          | Cyprus, mountainous regions                    |
| ssp. diabolina        | Eastern Europe; Asia                           |
| ssp. dumortieri       | Belgium                                        |
| ssp. erminea          | Crete, Southern and Eastern Greece, Turkey     |
| ssp. grisella         | Asia Minor, and Greece, Macedonia, Romania, Hungary. |
| ssp. hymalaiensis     | Himalaya. Afghanistan                          |
| ssp. lavandulaceae    | Spain                                          |
| ssp. longifolia       | Whole Europe                                   |
| ssp. minutiflora      | Hungary, Macedonia and Crete                   |
| ssp. modesta          | Asia Minor, Iran, Tibet                        |
| ssp. mollis           | Romania, Former Yugoslavia                     |
| ssp. noeana           | Southeastern Anatolia, Western Iran, Iraq.     |
| ssp. pellita          | Syria and Ethiopia                             |
| ssp. polyadenica      | South Africa; Lesotho                          |
| ssp. royleana         | Siberia, Asia Minor, Iran, Afghanistan, Tibet  |
| ssp. schimperi        | Ethiopia, Yemen, Sinai peninsula               |
| ssp. syriaca          | Syria and Ethiopia                             |
| ssp. typiooides       | Aegean region, Northern Iran, Northern Iraq, Egypt, Lebanon and Israel |
| ssp. wissii           | South Africa, Namibia                          |
additive in numerous products. A promising application may be the usage of *M. longifolia* polyphenols in the food industry as antioxidants (AOs) to increase shelf life. This potential use may be considered with regard to the high demand of plant-originated antioxidants (AOs) and in parallel, health concerns due to some synthetic phenolic AOs (Shahidi and Ambigaipalan 2015). Another alternative may be utilization of the volatiles of selected *M. longifolia* chemotypes against foodborne microbes, or as flavoring agents. In the present review, these two potential ways of utilization of this adaptive species, having a large tolerance for various habitats, in different preparations are in focus. Therefore, a detailed survey was carried out on the respective secondary compounds and a thorough evaluation is presented.

**Materials and search strategy**

Beside the comprehensive review *Labiatae* flavonoids and their bioactivity (Ulubelen et al. 2005) as a starting point, studies on *M. longifolia* and related species evaluated here were primarily obtained from electronic databases, namely SpringerLink, ScienceDirect, Journal of Agricultural and Food Chemistry, JEOR, Wiley Online Library, Taylor and Francis and MDPI. PubMed, Google Scholar and ResearchGate were used to search reliable but less known sources like the study of Jahan et al. (2001) on a novel flavone detected from ML. To check the background of journals providing some of the latter, Scimago was used. References in available publications were also screened in further sources like dissertations or less cited articles. One example was the study on phenolics of *M. x piperita* (Guédon and Pasquier 1994) referring to the earliest available work on *M. longifolia* flavonoids (Bourwieg and Pohl 1973) as a nowadays less known source to exploit. Further references considered to be necessary (e.g. studies dealing with structure—AO activity relationships of flavonoids), were searched also at the above mentioned databases. In the present review the cited data on concentrations of phenolics in *M. longifolia* will be given both in the original measuring units as they were published and also, in the majority of cases, in mg/kg dry plant material (mg/kg dp) for the sake of better comparability.

**Cinnamic acid derivatives in ML**

**Rosmarinic acid**

Rosmarinic acid or ‘Labiatae tannin’ (further: RA) is the caffeoyl ester of caffeic acid and 3',4'-dihydroxyphenyllactic acid. Accumulation of RA is characteristic in the *Nepetoideae* subfamily of the *Lamiaceae* family. Petersen and Simmonds (2003) summarize RA as adstringent, AO, antiviral, antimutagen and anti-inflammatory agent. Investigations on *M. longifolia* phenolics have predominantly been focusing on RA as a potent AO (Dudai et al. 2006; Fialová et al. 2008; Krzyzanowska et al. 2011; Patonay et al. 2017) antiinflammatory (Shen et al. 2011) and anti-cholinesterase (Vladimir-Knežević et al. 2014) molecule. The available quantitative data dealing with RA content of *M. longifolia* is summarized in Table 2. However, as large differences are observable in the investigated drug types and plant developmental stages (if defined), comparison of data can not be totally adequate. The most thorough publication on RA and caffeic acid content of *M. longifolia* is the work of Dudai et al. (2006) being the only one analyzing large sample numbers of *M. longifolia* for any phenolics. Results represent the highest RA content available in the literature of *M. longifolia*, covering 20–80 mg/g dp. (20,000–80,000 mg/kg dp). On the other hand, a recent work (Park et al. 2019) gives unconventionally low RA concentration (18.68 μg/g dp. viz. 18.68 mg/kg dp) from a single *M. longifolia* sample of undefined phenophase. In general, there is a relatively large variability in RA concentrations of *M. longifolia* mentioned by different authors, and they seem to be determined not only genetically but might be the result of differences in the plant developmental stage, harvest time (Fialova et al. 2008), cultivation technics, drug types (Krzyzanowska et al. 2011), extraction methods or other factors (Table 2).

Other phenolic acids, esters and phenylpropanoid volatiles

Beside RA, further phenolics have been detected in *M. longifolia* samples. Table 3 summarizes their concentrations. Nepetoidin A and B are reported to be present in *M. longifolia* (Grayer et al. 2003) as a chemotaxonomical marker of *Nepetoideae* plants. Salvianolic
acid L and dedihydro-salvianolic acid was detected by Krzyzanowska and co-workers (2011). It may be important to note that m/z data and UV maxima (283.3, 344.4 nm) of dedihydro-salvianolic acid were provided in this study, but molecular structure of a compound with this name was found neither in PubChem, PhenolExplorer, Human Metabolome Database or NIST Webbook, nor in literature. Hexacosyl ferulate and bis-2-ethylhexil-benzene 1,2-dicarboxylate were reported from Mentha longifolia L ssp. noéâna sampled in Turkey (Ertas¸ et al. 2015). This is the first report of them from M. longifolia, thus, based on this single reference it is impossible to evaluate the frequency and level of their concentration in horsemint. In general, data of minor phenolic acids in this species outline a rather wide variability, but unfortunately there is hardly any data about the influencing factors of the accumulation of them until now.

Table 2 Rosmarinic acid content obtained from different Mentha longifolia samples

| References            | Quantitative data | Plant part investigated | Phenophase     |
|-----------------------|-------------------|-------------------------|----------------|
|                       | Concentration measured | Concentration in mg/kg dp. |                  |
| Dudai et al. (2006)   | 20–80 mg/g dp       | 20,000–80,000            | Leaves and stem | Not defined   |
| Fialová et al. (2008) | 1.19 m/m % in dp    | 11,900                   | Not defined     | Not defined   |
| Fialová et al. (2008) | 0.88 m/m % in dp    | 8800                     | Not defined     | Not defined   |
| Krzyzanowska et al. (2011) | 1.933 mg/g dp           | 1933                     | Not defined     | Not defined   |
| Krzyzanowska et al. (2011) | 4.445 mg/g dp           | 4455                     | Not defined (in vitro plant) | Not defined |
| Krzyzanowska et al. (2011) | 12.765 mg/g dp           | 12,765                   | Cell suspension | (-)           |
| Krzyzanowska et al. (2011) | 21.576 mg/g dp           | 21,576                   | Callus culture  | (-)           |
| Tahira et al. (2011)  | 61.47 mg/100 g       | 614.7                    | Leaves          | Spring vegetative |
| Shen et al. (2011)    | 6.99 mg/g dp         | 6990                     | Aerial p. without flowers | Flowering |
| Shekarchi et al. (2012)| 26.6 mg/g dp          | 26,600                   | Aerial parts    | Flowering     |
| Vladimir-Knežević et al. (2014) | 22.33 mg/g extract | 1639                     | Aerial parts    | Flowering     |
| Elansary and Mahmoud (2015) | 40.91 mg/g extract | NA^a                   | Leaves          | Not defined   |
| Bahadori et al. (2018) | 2225–6260 µg/g       | NA^a                     | Leaves, flowers, juvenile stems | Flowering |
| Patonay et al. (2017) | 6418–11,366 mg/kg     | 6418–11,366^b            | Leaves, stem, inflorescence | Flowering |
| Park et al. (2019)    | 18.68 µg/g dp        | 18.68                    | Leaves and stem | Not defined   |

^aConcentration given in extracts without providing yields, thus in mg/kg dp is not possible to calculate
^bConcentration depending on the extraction solvent
^cHarvested in July
^dHarvested in September
^eHarvested in June

Flavonoids in horsemint

Table 4 summarizes structural information of flavonoids reported in Mentha longifolia.

Flavanones

Flavanones (‘citrus flavonoids’) are usually a dominant or a major flavonoid subclass in mints, together with flavones (Pereira and Cardoso 2013). Regarding M. longifolia, data about them is sporadic and a majority of the references report only the presence of these compounds (eriocitrin, hesperidin and narirutin) without quantitative data. The spectrum of flavanones include relatively widespread compounds (Table 4), but a special flavanone, 4′-methoxy-naringenin-7-O-fucopyranosil-1 → 6-glucoside or longitin, reported
| Subclass                      | Name                        | References                          | Quantitative data                                                                 | Plant part investigated                      | Plant phenophase                  |
|------------------------------|-----------------------------|-------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------|-----------------------------------|
| Cinnamic acid derivative      | *trans*-Cinnamic acid       | Park et al. (2019)                  | Concentration measured 6.5 ug/g dp                                                   | Leaves and stem                            | Not defined                       |
|                              | *trans*-Cinnamic acid       | Bahadori et al. (2018)              | Concentration, mg/kg dp. 6.5                                                        | Flowers, leaves, juvenile stems             | Flowering                         |
|                              | *para*-Coumaric acid (bound)| Dudai et al. (2006)                 | Concentration measured ca. 25–250 mg/g dp                                          | Leaves and stem                            | Not defined                       |
|                              | *para*-Coumaric acid        | Bahadori et al. (2018)              | Concentration, mg/kg dp. 5–15                                                         | Flowers, leaves, juvenile stems             | Flowering                         |
|                              | *para*-Coumaric acid        | Park et al. (2019)                  | Concentration measured 6.15 ug/g dp                                                  | Leaves and stem                            | Not defined                       |
|                              | *ortho*-Coumaric acid       | Bahadori et al. (2018)              | Concentration, mg/kg dp. 134–328                                                       | Flowers, leaves, juvenile stems             | Flowering                         |
|                              | Caffeic acid (free)         | Dudai et al. (2006)                 | Concentration measured ca. 10–24                                                       | Leaves and stem                            | Not defined                       |
|                              | Caffeic acid (bound)        | Dudai et al. (2006)                 | Concentration measured ca. 6–16                                                       | Leaves and stem                            | Not defined                       |
|                              | Caffeic acid                | Tahira et al. (2011)                | Concentration measured 314.8 mg/100 g dp                                              | Leaves                                     | Spring vegetative                 |
|                              | Caffeic acid                | Benedec et al. (2013)               | Concentration measured < 0.2 mg/dp                                                    | Aerial parts                               | Flowering                         |
|                              | Caffeic acid                | Vladimír-Knězević (2014)            | Concentration measured 1.18 mg/g dry extract                                           | Aerial parts                               | Flowering                         |
|                              | Caffeic acid                | Patonay et al. (2017)               | Concentration measured 0–273.3                                                        | Leaves, stem, inflorescence                | Flowering                         |
|                              | Caffeic acid                | Bahadori et al. (2018)              | Concentration measured 86–119                                                         | Flowers, leaves, juvenile stems             | Flowering                         |
|                              | Caffeic acid                | Park et al. (2019)                  | Concentration measured 58.50                                                           | Leaves and stem                            | Not defined                       |
|                              | Ferulic acid                | Tahira et al. (2011)                | Concentration measured 0.94 mg/100 g dp                                               | Leaves                                     | Spring vegetative                 |
|                              | Ferulic acid                | Benedec et al. (2013)               | Concentration measured < 0.2 mg/dp                                                    | Aerial parts                               | Flowering                         |
|                              | Ferulic acid                | Patonay et al. (2017)               | Concentration measured 0–30.59                                                        | Leaves, stem, inflorescence                | Flowering                         |
|                              | Ferulic acid                | Park et al. (2019)                  | Concentration measured 38.70                                                           | Leaves and stem                            | Not defined                       |
|                              | Hexacosyl ferulate          | Ertas et al. (2015)                 | Concentration measured 6 mg/1350 g dp                                                 | Aerial parts                               | Not defined                       |
|                              | Sinapic acid                | Bahadori et al. (2018)              | Concentration measured 4604–7132                                                       | Flowers, leaves, juvenile stems             | Flowering                         |
|                              | Caftaric acid               | Benedec et al. (2013)               | Concentration measured < 0.2 mg/100 g dp                                              | Aerial parts                               | Flowering                         |
|                              | Chlorogenic acid            | Benedec et al. (2013)               | Concentration measured < 0.2 mg/100 g dp                                              | Aerial parts                               | Flowering                         |
Table 3 continued

| Subclass                         | Name                     | References                           | Quantitative data | Plant part investigated | Plant phenophase |
|----------------------------------|--------------------------|--------------------------------------|-------------------|-------------------------|------------------|
|                                  |                          |                                      | Concentration measured | Concentration, mg/kg dp. |                  |
| Chlorogenic acid                 | Vladimír-Knezević et al. (2014) | 1.50 mg/g dry extract              | 110.1             | Aerial parts            | Flowering        |
| Chlorogenic acid                 | Bahadori et al. 2018    | 27–64 ug/g extract \(^b\)            | NA\(^a\)          | Flowers, leaves, juvenile stems | Flowering        |
| Chlorogenic acid                 | Park et al. (2019)      | 170.90 ug/g                        | 170.9             | Leaves and stem         | Not defined\(^c\) |
| Salvianolic acid L               | Krzyzanowska et al. (2011) | 0.285 mg/g dp                     | 285.0             | Not defined             | Not defined      |
| Dedihydro-salvianolic acid       | Krzyzanowska et al. (2011) | 0.084 mg/g dp                     | 84.0              | Not defined             | Not defined      |
| Nepetoidin A                     | Gray et al. (2003)      | Quantity not given                  | NA                | Leaves                  | Flowering        |
| Nepetoidin B                     | Gray et al. (2003)      | Quantity not given                  | NA                | Leaves                  | Flowering        |
| Benzoic acid derivative/other    | Vanillic acid            | 0–62.17 mg/ kg dp                  | ND-62.17\(^b\)    | Leaves, stem, inflorescence | Flowering        |
|                                  | Gallic acid              | 0–2583 mg/ kg dp                   | ND-2583           | Leaves, stem, inflorescence | Flowering        |
|                                  | Bahadori et al. 2018    | 2–72 ug/g extract \(^c\)            | NA\(^a\)          | Flowers, leaves, juvenile stems | Flowering        |
|                                  | Patonay et al. 2017     | 0–56.75 mg/ kg dp                  | ND-56.75          | Leaves, stem, inflorescence | Flowering        |
|                                  | Bahadori et al. 2018    | 9–33 ug/g extract \(^c\)            | NA\(^a\)          | Flowers, leaves, juvenile stems | Flowering        |
|                                  | Patonay et al. 2017     | 0–56.75 mg/ kg dp                  | ND-56.75          | Leaves, stem, inflorescence | Flowering        |
|                                  | Bahadori et al. 2018    | 6–7 ug/g dry extract \(^b\)         | NA\(^a\)          | Flowers, leaves, juvenile stems | Flowering        |
|                                  | Ertas et al. (2015)     | 4 mg/1350 g dp                     | 2.9               | Aerial parts             | Not defined      |
|                                  | 0.6 w/w % of EO         |                                     | 0.3               | Aerial parts             | Not defined      |
|                                  | Vanillin                 | 6–31 ug/g dry extract \(^b\)        | NA\(^a\)          | Flowers, leaves, juvenile stems | Flowering        |
|                                  | Tahira et al. (2011)    | 16.79 mg/ 100 g dp                 | 168               | Leaves                  | Spring vegetative |

\(^a\) Concentration given without extraction yields, thus in mg/kg dp is not possible to calculate

\(^b\) Concentration depending on the extraction solvent

\(^c\) Harvested in June
| Subclass       | Substitution pattern | Name(s)                                      | Ref (*) |
|---------------|---------------------|---------------------------------------------|---------|
| Flavanones    |                     | Naringenin                                  | 18      |
|               | H OH H OH           | Naringenin-7-O-rutinoside, narirutin         | 18      |
|               | H OH H O-rut        | 4'-methoxy-naringenin-7-O-fucopyranosil-1 → 6-glucoside, longitin | 6       |
|               | H OH H O-fuc-glu    | Eriodyctiol                                 | 20      |
|               | H OH H OH           | Eriodyctiol-7-O-rutinoside, eriocitrin       | 1;18    |
|               | H OH? H OH?         | Eriodyctiol-7-O-glucoside-rhamnoside        | 7       |
|               | H OH H O-rut        | Hesperetin-7-O-rutinoside, hesperidin        | 1;5     |
| Flavones      | H OH H OH           | 5,7,4′-trihydroxy-flavone, apigenin          | 10;15;16;18;20 |
|               | H OH H O-glu        | Apigenin-7-O-glucoside, cosmosiin, apigetrin| 11      |
|               | H OH H O-glc        | Apigenin-7-O-glucuronide                    | 1;11    |
|               | H OH H O-rut        | Apigenin-7-O-rutinoside, isorhoifolin       | 11      |
|               | H OH H OH           | Apigenin-4′-O-glucoside                     | 14      |
|               | H O-glu H OH        | Apigenin-5-O-glucoside                      | 14      |
|               | H OH C-glu OH       | Apigenin-6,8-C-diglucoside, vicenin-2       | 8       |
|               | H OH H OH           | 4′-methoxyapigenin-7-O-rutinoside, acacetin-7-O-rutinoside | 1       |
|               | H O-glu H OMe       | 7′-methoxyapigenin-5-O-glucoside, genkwanin-5-O-glucoside | 14      |
|               | H O-6′-mal-glu OMe  | Genkwanin-5-O-[6′-O-malonyl]-glucoside       | 14      |
|               | H OH H OMe          | Genkwanin-4′-O-glucoside, fegopolin          | 14      |
|               | H OH H OH           | 5,7,3′,4′-tetrahydroxy-flavone, luteolin, luteolol | 5; 15; 16;18;20 |
|               | H OH H O-glu        | Luteolin-7-O-glucoside, cynaroside           | 1;5;12;16;10 |
|               | H OH H O-glc        | Luteolin-7-O-glucuronide                    | 1;12    |
|               | H OH H O-rut        | Luteolin-7-O-rutinoside, lonicerin, veronicastroside | 1;12    |
|               | H OH H O-neothes    | Luteolin-7-O-neohesperoside                 | 8       |
Table 4 continued

| Subclass | Substitution pattern | Name(s)                                                                 | Ref (*) |
|----------|----------------------|------------------------------------------------------------------------|---------|
|          | C3 C5 C6 C7 C8 C2' C3' C4' C5' |                                                                        |         |
| H        | OH H O-neohes H H H H O-soph H | Luteolin-7-O-neohesperoside-4'-O-sophoroside                          | 4       |
| H        | O-glu H OH H H OH OH OH H | Luteolin-5-O-glucoside, galuteolin                                     | 14      |
| H        | OH? H OH? H H OH? OH? OH? H | Luteolin-glucuronide, an other isomer                                  | 7       |
| H        | OH? H OH? H H OH? OH? OH? H | Luteolin-glucuronide, an other isomer                                  | 7       |
| H        | OH? H OH? H H OH? OH? OH? H | Methylated luteolin-glucuronide                                        | 7       |
| H        | OH? H OH? H H OH? OH? OH? H | Luteolin-diglucuronide                                                 | 7       |
| H        | OH H O-rut H H H OMe H | Diosmetin-7-O-rutinoside, diosmin                                      | 1       |
| H        | OH H OH H H H OH H OH H | 5,7,8,4'-tetrahydroxy-flavone, 8-OH-luteolin, Hypolaetin              | 8       |
| H        | OH C-glu OH C-glu H H H OH H | Hypolaetin-6,8-C-diglucoside, lucenin-1                                | 8       |
| H        | OH H OH H H H OMe H | Hypolaetin-4'-methyl ether                                              | 4       |
| H        | OH H OH H H H O-glu OH O-rha | Tricetin-3'-O-glucoside-5'-O-rhamnoside                                | 3       |
| H        | OH H OH H H H O-rha-rha OH OH | Tricetin-3'-O-dirhamnoside                                             | 3       |
| H        | OH H OMe H H H O-glu OH O-rha | 7-methoxy-tricetin-3'-O-glucoside-5'-O-rhamnoside                         | 3       |
| H        | OH OH OMe OMe OMe H OMe OH H | 5,6, 4'-tri hydroxy-7,8,3'-trimethoxy-flavone, thymonin                 | 5       |
| H        | OH OMe OMe OMe OH H OMe OH H | 5,8, 4'- trihydroxy-6,7,3'-trimethoxy-flavone                           | 4       |
| H        | OH OH OMe OMe OMe H OMe OMe OMe H | 5,6,-dihydroxy-7,8,3'4'-tetramethoxy-flavone, pebrellin               | 2;5     |
| H        | OH H OMe OMe OMe OMe OMe H H H | 5-hydroxy-7,8,2',3'-tetramethoxy-flavone                                | 4       |
| H        | OH OMe OH H OMe OMe OMe OH H | 5,7,4'- trihydroxy-6,2',3'-trimethoxy-flavone                           | 5       |
| H        | OH OMe OMe OMe H H OMe OMe OMe H | 5-hydroxy-6,7,3',4'-tetramethoxy-flavone, belamcanidine                 | 17      |
| H        | OH OH OMe OMe H H OMe H H H | 5,6,4'- tri hydroxy-7,3'-dimethoxy-flavone                             | 8       |
| Flavonols| OH OH H OH H H H OH H | Kaempferol                                                             | 19;21   |
|          | O-glu OH H OH H H H OH H | Kaempferol-3-O-glucoside, astragalin                                  | 10;14   |
from a Pakistani sample may be mentioned as a novelty (Ali et al. 2002).

Flavones

Table 5 shows the available quantitative data of flavones in *M. longifolia*. This flavonoid subclass shows a very wide variability in ML samples. Among them, there are some compounds which have not been known before and detected especially in ML for the first time. A novel aglycone with unconventional substitution pattern, 5,7,4\(^\prime\)-trihydroxy-6,2\(^\prime\),3\(^\prime\)-trimethoxy-flavone, was detected by Ghoulami et al. (2001) from Morocco. Besides, a low concentration of another new aglycone, 5,8,4\(^\prime\)-trihydroxy-6,7,3\(^\prime\)-trimethoxy-flavone was found by Jahan et al. (2001), from Pakistan. Exploration of three previously unknown tricetin derivatives in a *M. longifolia* sample from Saudi Arabia is reported by Sharaf et al. (1999). According to the authors, it is the first report on flavones bearing trisubstituted B ring in the whole *Lamiaceae* family. However, the occurrence of the mentioned special flavones in horsemint seems to be supported only by the cited single reference.

Table 4 continued

| Subclass | Substitution pattern | Name(s) | Ref (*) |
|----------|----------------------|---------|---------|
|          | C3 C5 C6 C7 C8 C9 C10 C11 |         |         |
| O-soph   | OH H OH H H H OH H | Kaempferol-3-O-sophoroside, sophoravonoside | 14 |
| O-rha    | OH H OH H H H OH H | Kaempferol-3-O-rhamnoside, afzelin | 14 |
| OH       | OH H O-rha H H H OH H | Kaempferol-7-O-rhamnoside | 14 |
| O-6\(^\prime\)-mal-glu | OH H O-rha H H H OH H | Kaempferol-3-O-[6\(^\prime\)-O-malonyl]-glucoside-7-O-rhamnoside | 14 |
| OH       | OH H OH H H OH H OH H | Quercetin | 16;19;21 |
| O-glu    | OH H OH H OH OH H | Quercetin-3-O-glucoside, isoquercitrin | 10;15 |
| O-rut    | OH H OH H H OH OH H | Quercetin-3-O-rutinoside, rutin | 15;18;19;20;21 |
| O-glu    | OH H O-glu H H OH OH H | Quercetin-3,7-O-diglucoside | 10;15 |
| OH       | OH H OH H O-rha H OH OMe OH | 4\(^\prime\)-methoxymyricetin-3-O-rhamnoside | 4 |

*fuc:* Fucose, *glu:* Glucose, *glc:* Glucuronic acid, *neohes:* Neohesperidose, *rut:* Rutinose, *rha:* Rhamnose, *soph:* Sophorose, *mal:* Malonyl, *OMe:* Methoxyl."?

*For the sake of transparency, References in this table are numbered: (1) Bourwieg and Pohl (1973); (2) Tomás-Barberán et al. (1988); (3) Sharaf et al. (1999); (4) Jahan et al. (2001), (5) Ghoulami et al. (2001); (6) Ali et al. (2002); (7) Krzyzanowska et al. (2011); (8) Ulubelen et al. (2005); (9) Fialová et al. (2008); (10) Akroum et al. (2009); (11) Baris et al. (2011); (12) Orhan et al. (2012); (13) Pereira and Cardoso (2013); (14) Stanislavžič et al. (2012); (15) Benedek et al. (2013); (16) Elansary and Mahmoud (2015); (17) Ertas et al. (2015); (18) Hawryl et al. (2016); (19) Patonay et al. (2017); (20) Bahadori et al. (2018) (21) Park et al. (2019) Among flavone glycosides, cynaroside has been detected repeatedly, (Table 4) although its concentration is low (or not provided) (Table 5). In some cases, the sites of the glycosidic bonds are not designated, thus the exact glycoside molecule remains questionable, e.g. luteolin-glucorhamnoside and luteolin-glucuronides in study of Krzyzanowska et al. (2011). It can be established, that the sporadic data about flavone-7-O-glycosides as summarized in Tables 4 and 5 do not seem to represent strong support for the universal and frequent accumulation of them in horsemint, although these ingredients have been frequently described in other mint species (Guédon and Pasquier 1994; Areias et al. 2001; Damien-Dorman et al. 2003a, b; Koşar et al. 2004).

Flavonols

Although the previous reviews (Pereira and Cardoso 2013; Mikaili et al. 2013; Farzaei et al. 2017) do not deal with this subclass in detail when discussing the flavonoids of *M. longifolia*, the available literature shows that flavonols may frequently be present in this species. Quercetin and kaempferol together with their
Table 5 Available quantitative data of flavones in *Mentha longifolia* L samples

| Name | References | Quantitative data | Plant part investigated | Phenophase |
|------|------------|-------------------|-------------------------|------------|
| 5,7,4′-trihydroxy-flavone, apigenin | Elansary and Mahmoud (2015) | 3.86 mg/g dry extract | Leaves | Not defined |
| 5,7,4′-trihydroxy-flavone, apigenin | Patonay et al. (2017) | 19.7–144.2 mg/kg dp | Leaves, stem, inflorescence | Flowering |
| 5,7,4′-trihydroxy-flavone, apigenin | Bahadori et al. (2018) | 94–124 ug/g dry extractc | Flowers, leaves, juvenile stems | Flowering |
| Apigenin-7-O-glucoside, cosmosin, apigetrin | Baris et al. (2011) | 3.6 mg isolated from 1 kg sample | Leaves, stem | Flowering |
| Apigenin-7-O-glucuronide | Baris et al. (2011) | 5.2 mg isolated from 1 kg sample | Leaves, stem | Flowering |
| Apigenin-7-O-rutinoside, isorhoifolin | Baris et al. (2011) | 6.3 mg isolated from 1 kg sample | Leaves, stem | Flowering |
| Apigenin-4′-O-glucoside | Stanislavljević et al. (2012) | 0.81 mg/g extract | Above-ground parts | Flowering |
| Apigenin-5-O-glucoside | Stanislavljević et al. (2012) | 7.53 mg/g extract | Above-ground parts | Flowering |
| 5,7,3′,4′-tetrahydroxy-flavone, luteolin | Benedec et al. (2013) | 1.764 mg/g dp | Aerial parts | Flowering |
| 5,7,3′,4′-tetrahydroxy-flavone, luteolin | Elansary and Mahmoud (2015) | 3.21 mg/g dry extract | Leaves | Not defined |
| 5,7,3′,4′-tetrahydroxy-flavone, luteolin | Bahadori et al. (2018) | 84–162 ug/g dry extractc | Flowers, leaves, juvenile stems | Flowering |
| Luteolin-7-O-glucoside, cynaroside | Elansary and Mahmoud (2015) | 3.91 mg/g dry extract | Leaves | Not defined |
| Luteolin-7-O-glucoside, cynaroside | Orhan et al. (2012) | 7.0 mg isolated from 1 kg sample | Leaves, stem, inflorescence | Flowering |
| Luteolin-7-O-glucuronide | Orhan et al. (2012) | 4.0 mg isolated from 1 kg sample | Leaves, stem, inflorescence | Flowering |
| Luteolin-7-O-rutinoside, lonicerin, veronicastroside | Orhan et al. (2012) | 18.3 mg isolated from 1 kg sample | Leaves, stem, inflorescence | Flowering |
| Luteolin-5-O-glucoside, galuteolin | Stanislavljević et al. (2012) | 1.69 mg/g extract.d | Above-ground parts | Flowering |
| Luteolin-glucuronide | Krzyzanowska et al. (2011) | 2.237 mg/g dp | Not defined (field plant) | Not defined |
| Luteolin-glucuronide | Krzyzanowska et al. (2011) | 0.007 mg/g dp | Not defined (in vitro plant) | Not defined |
| Luteolin-glucuronide | Krzyzanowska et al. (2011) | traces mg/g dp | Cell suspension | (–) |
| Luteolin-glucuronide, an other isomer | Krzyzanowska et al. (2011) | 0.285 mg/g dp | Not defined (field plant) | Not defined |
| Luteolin-glucuronide, an other isomer | Krzyzanowska et al. (2011) | 0.005 mg/g dp | Not defined (in vitro plant) | Not defined |
| Luteolin-glucuronide, an other isomer | Krzyzanowska et al. (2011) | 0.074 mg/g dp | Callus culture | (–) |
| Luteolin-glucoside-rhamnoside | Krzyzanowska et al. (2011) | 3.576 mg/g dp | Not defined (field plant) | Not defined |
| Name                          | References                          | Quantitative data | Plant part investigated | Phenophase |
|------------------------------|-------------------------------------|-------------------|-------------------------|------------|
|                              |                                     | Concentration measured | Concentration (mg/kg dp) |            |
| Luteolin-glucoside-rhamnoside| Krzyzanowska et al. (2011)          | 1.134 mg/g dp      | 1134                    | Not defined (in vitro plant) |
|                              |                                     | 0.018 mg/g dp      | 13.0                    | Cell suspension (–) |
|                              |                                     | 0.013 mg/g dp      | 18.0                    | Callus culture (–) |
| Methylated luteolin glucuronide| Krzyzanowska et al. (2011)          | 0.007 mg/g dp      | 7.0                     | Not defined (field plant) |
|                              |                                     | 0.013 mg/g dp      | 13.0                    | Not defined (in vitro plant) |
| Luteolin-diglucuronide       | Krzyzanowska et al. (2011)          | 1.432 mg/g dp      | 1432                    | Not defined |
| Tricetin-3'-O-glucoside-5' -O-| Sharaf et al. (1999)                | 28 mg isolated from | 140                     | Aerial parts |
| rhamnoside                   |                                     | 200 g dp           |                         | Not defined |
| Tricetin-3'-O-di-rhamnoside  | Sharaf et al. (1999)                | 31 mg isolated from | 155                     | Aerial parts |
|                              |                                     | 200 g dp           |                         | Not defined |
| 7-methoxy-tricetin-3'-O-    | Sharaf et al. (1999)                | 21 mg isolated from | 105                     | Aerial parts |
| glucoside-5'-O-rhamnoside   |                                     | 200 g dp           |                         | Not defined |
| Genkwanin-5-O-glucoside      | Stanislavljević et al. (2012)       | 0.56 mg/g extract  | 64.9                    | Above-ground parts |
|                              |                                     | 0.52-0.57 mg/g     | 58.8–60.3               | Flowering |
| Genkwanin-5-O-[6'-O-        | Stanislavljević et al. (2012)       | 1.96 mg/g extract  | 183.                    | Above-ground parts |
| malonyl]-glucoside           |                                     | 0.56 mg/g extract  | 183.                    | Flowering |
| Genkwanin-4'-O-glucoside,    | Stanislavljević et al. (2012)       | 0.52-0.57 mg/g     | 58.8–60.3               | Flowering |
| fegopolin                    |                                     | extractd           |                         |            |
| 5,6,-dihydroxy-7,8,3'4'-    | Ghoualami et al. (2001)             | 0.015 w/w % dp     | 150                     | Aerial parts |
| tetrametoxi-flavone, pebrellin|                                     |                   |                         | End of vegetative cycle |
| 5,7,4'-trihydroxy-6,2',3'-  | Ghoualami et al. (2001)             | 0.010 w/w % dp     | 100                     | Aerial parts |
| trimetoxi-flavone            |                                     |                   |                         | End of vegetative cycle |
| 5-hydroxy-6,7,3',4'-         | Ertas et al. (2015)                 | 5 mg isolated from | 3.7                     | Not defined |
| tetrametoxi-flavone, belamcanidine|                                     | 1350 g dp         |                         | Not defined |
| 5-hydroxy-7,8,2',3'-         | Jahan et al. (2001)                 | 25 mg isolated from | 1.25 × 10^-3           | Not defined |
| tetramethoxy-flavone         |                                     | 20 kg dp           |                         | Not definedd |
| Hypolaetin-4' methyl ether   | Jahan et al. (2001)                 | 30 mg isolated from | 1.5 × 10^-3            | Not defined |
|                              |                                     | 20 kg dp           |                         | Not definedd |
| 5,8,4'-trihydroxy-6,7,3'    | Jahan et al. (2001)                 | 45 mg from 20 kg   | 2.25 × 10^-3           | Not defined |
| trimethoxy-flavone           |                                     | dp                 |                         | Not definedd |

*aConcentration given in mg/g extracts without providing yields, thus in mg/kg dp. is not possible to calculate

bConcentrations depending on the extraction solvent
cConcentrations depending on drying method
dHarvested in March
glycosides are most often reported from *M. longifolia* (Table 4.). The concentration ranges are variable, like in the case of rutin: 0.822 mg/100 g dp (Benedec et al. 2013) or 11.66 mg/100 g dp (Park et al. 2019). Flavonol-rich samples were reported from Serbia (Stanislavljević et al. 2012), Hungary (Patonay et al. 2017) and Korea (Park et al. 2019). Interestingly, Stanislavljević et al. (2012) reported astragalin to be the dominant flavonol constituent of a *M. longifolia* charge (61.36 mg/g extract calculated with yield: 7118 mg/kg dp).

It seems, that the actual amount of flavonoid compounds in the drug may be influenced by drying method (Stanislavljević et al. 2012) or other postharvest treatments like heating the fresh plant material (Stocker and Pohl 1976). These questions may need a further study.

**Antiradical and antimicrobial properties of phenolics occurring in horsemint**

Rosmarinic acid plays an important role in the antioxidant properties of *M. longifolia* extracts. Dudai and co-workers established a tight correlation ($R^2 = 0.38$) between rosmarinic acid content and results of DPPH assay. However, Fialová and co-workers (2008) suggest, that other constituent(s) than this may play a role in the radical scavenging activity of *M. longifolia* as the maxima of THD, TF and antiradical activity do not coincide with the maxima of RA content. Interestingly, the concentration of caffeic acid does not seem to correlate with results of DPPH assay ($R^2 = 0.0119$) contrary to its known AO efficiency (Košar et al. 2004; Csepregi et al. 2016). Grayer et al. (2003) observed nepetoidin B to be a stronger AO than gallic acid in DPPH assay.

As Table 4 shows, a significant proportion of the flavonoids detected in ML are the 7-O-glycosides. Although they are frequent in antioxidant-rich species of plant families e.g. *Lamiaceae*, *Apiaceae*, *Asteraceae*, their AO properties are less known in comparison with 3-O-glycosides (Csepregi et al. 2016). Therefore, the antiradical abilities of 7-O-glycosides may principally be outlined using studies of structure–activity relationship. Bors and co-workers (1990) studied the kinetics of various flavonoids against OH, N$_3$ and tert-butoxyl radicals demonstrating that the key of the AO activity of flavonoids towards radicals is the ability to form a longlife secondary aroxyl radical which could take part in recombinations. In this consideration, authors outlined the necessary structural traits providing better delocalization of the unpaired electron and in consequence, stability of aroxyl radicals. These are the followings (1) free ortho-dihydroxy group at B ring (catechol moiety) (2) the free --OH group at C3 (3) double bond at C2-C3 and carbonyl on C4, because of conjugation (4) additionally, presence of free –OH groups at C5 and C7. Later, studies ranking flavonoids on TEAC (Rice-Evans et al. 1996; Csepregi et al. 2016) and DPPH assays (Burda and Ołeszek 2001; Csepregi et al. 2016) modified this idea. Catechol moiety was repeatedly observed to play a key role in AO properties, followed by free C3–OH. The latter was recently observed to be tightly and significantly correlated with activity in TEAC and FRAP assays but loosely coupled to the activity in DPPH and Folin-Ciocalteu’s assay (Csepregi et al. 2016). The C2–C3–C4 system was reinforced to function only in combination with free catechol moiety and/or C3–OH (Wen et al. 2014; Csepregi et al. 2016). Based on these considerations, some flavonoid-7-O-glycosides detected in ML may deserve attention. Thus, luteolin-7-O-glycosides may be predicted as active against some radicals as rutin as they have catechol moiety and C2-C3-C4 conjugation but free C3–OH is absent. A ranking of flavonoids by activity against DPPH (Burda and Oleszek 2001) supports this idea. Here, rutin showed 90.9 IC% and cynaroside 87.6 IC%. Luteolin itself was also observed to show antiradical activity stronger than of BHT on DPPH assay but weaker efficacy on ORAC (Wen et al. 2014), suggesting that the lack of C3–OH might decrease this kind of AO activity. On the other hand, eriodictiol and 7-O-glycosides may be considered as stronger anti-radical agents than other flavanones of ML because only they have a free catechol group. Damien-Dorman and co-workers (2003a, b) declared, that mints richest in eriocitrin and rich in RA showed the highest activity against DPPH and OH. Their further study (Košar et al. 2004) demonstrated a high correlation between DPPH antiradical activity of *Mentha* extracts and concentration of caffeic acid, rosmarinic acid, lonicerin, eriocitrin and an undefined luteolin-7-O-glycoside. Antiradical activity of luteolin-5-O-glycosides like galuteolin may be supposed to be similar to 7-O-analuges because of the presence of free catechol.
moiety and C2–C3–C4 conjugation. Naringenin and apigenin derivatives however, as it may be expected based on their structure, did not show this response. It must be emphasized, that synergistic effects between some flavonoids and/or flavonoids and caffeic acid derivatives may occur, depending on the ratio of concentration and their redox potential and the presence of catechol moiety in the case of flavonoids (Freeman et al. 2010; Reber et al. 2011; Ołszowy-Tomczyk 2020). A very recent long-needed review of Olszowy-Tomczyk (2020) called attention to the mutual effects of plant phenolics in binary mixtures. The detailed data collected by the author shows that there are some cases when synergistic or additive effects were reported between polyphenols e.g. between rosmarinic acid and quercetin in the case of AAPH induced oxidation; between chlorogenic acid and hesperidin, also between p-coumaric acid and quercetin in ORAC assay. On the other side, no antagonistic effect was reported to rosmarinic acid and flavonoids except an observation on FRAP assay of rutin and rosmarinic acid (Hajimehdipoor et al. 2014). Although an extract is much more complex than a binary mixture, synergistic or antagonistic effects may be considered when the background of antioxidant properties of a ML extract is studied.

Beside the plant material itself, studies rarely focused on other factors which might influence the AO properties of ML extracts. Fialová and co-workers (2008) proved that ML show higher AO activity, THD and TF in July than in September (DPPH EC$_{50}$ in July 24.60 µg/mL, in September 45.20 µg/mL). Further studies are needed in this respect.

Focusing on the food preservative utilisation of _M. longifolia_, beyond the AO activity of phenolic compounds, the activity against bacteria or fungi causing food spoilage and/or foodborne diseases may be taken into account. Akroum and co-workers (2009) established that isouercitrin in _M. longifolia_ showed the strongest growth inhibitory effect against _B. cereus_, _B. subtilis_, _S. aureus_, _E. coli_ and _P. aeruginosa_ (MIC = 0.03–0.09 µg/mL). Synergism among these molecules was observed. Other polyphenols of ML may also be potential antimicrobial agents, as documented in in vitro studies in the case of other species, like apigenin (Basile et al. 1999; Metsäümäen and Sirén 2019), luteolin (Wen et al. 2014) and nepetoidins (Grayer et al. 2003).

**The volatile composition of horsemint**

Essential oil (EO) content of horsemint and classification of its constituents

According to recent data, volatile components accumulate in _M. longifolia_ in a range of 0.5–1% dry weight (Hajlaoui et al. 2009; Sharopov et al. 2012; Iqbal et al. 2013; Llorens-Molina et al. 2015; Kapp 2015). However, earlier studies report significantly higher EO contents, up to 1.6–2.8% from Eastern Crete (Karousou et al. 1998) and 3.8% from Sinai (Fleisher and Fleisher 1991). This wide interval of EO contents may be in part coupled to sampling methods and the varying phenological phase or organic composition of the plants (EO yield of the plant is recently observed by Llorens-Molina et al. (2020) to reach its maximum in advanced flowering stage). Anyhow, the different experimental conditions make a proper evaluation difficult. Illustrating this, the analysed sample types include flowering shoots (Karousou et al. 1998), shoots at the end of flowering, or seed ripening stage (Baser et al. 1999) or even leaves separated from the stems (Orav et al. 2013).

Volatiles of ML show extraordinary wide variability, involving multiple metabolic pathways. Based on works of Başer and co-authors (1999; 2012), volatile terpenes of _M. longifolia_ could be perspicuously grouped by structure (Figs. 1, 2). These groups and their important representants are presented below.

**Open-chain monoterpenes**

Linalool (Mimica-Dukic and Bozin 2008) and linalyl acetate may be present in concentrations above 10% of ML EO (Al-Okbi et al. 2015), although they do not appear in all _M. longifolia_ samples. Thus, they may not be considered as universal constitutent of the species. Myrcene was also reported in concentration around 10% in samples from Lithuania (Venskutonis 1996).

**Limonene and its 2-oxo derivatives**

Carvone, dihydrocarvone, _cis_- and _trans_-carvyl acetate, _cis_- and _trans_-dihydrocarveol frequently appear in EOs of _M. longifolia_ (Başer et al. 1999; Sharopov et al. 2012; Mimica-Dukic and Bozin 2008). 55–66% carvone was present in the EO of the samples from Crete (Karousou et al. 1998) while 50–65% carvone
was reported from Iran, former Yugoslavia, France, Estonia and Tajikistan (Sharopov et al. 2012; Kapp 2015).

Limonene 3-oxo derivatives

Piperitone, the two piperitone oxide isomers, piperitenone and piperitenone epoxide are typical in the EO (Başer et al. 1999; Aksit et al. 2013). Pulegone appears frequently, too. This volatile, a major component also of the pennyroyal (M. pulegium), bears an unpleasant aroma and is considered to be toxic. Target human organs are suggested to be the liver and kidney which may be damaged via reactive metabolites in the case of long-term consumption (EPA/HMPC/138386/2005 Rev 1) (European Medicines Agency, Committee on Herbal Medicinal Products (HMPC) 2016). The EU directive EC1334/2008 (EEC 2008) declares that pulegone and menthofurane are limited to max. 20 mg/kg in general foodstuff, 200 mg/kg in mint/
Fig. 2  a Limonene-3-oxo-derivatives  b miscellaneous cyclic monoterpenes,  c sesquiterpenes detected in *M. longifolia* EOs
peppermint flavoured confectionery, and 100 mg/kg in chewing gums. Proportion of pulegone in a ML EO varies between 20 and 85% (Fleisher and Fleisher 1991; Baser et al. 1999; Ghoulami et al. 2001; Gülüce et al. 2007; Sharopov et al. 2012; Kapp 2015). Further representatives of limonene 3-oxo derivatives in M. longifolia are menthone, isomenthone, menthofurane (Mimica-Dukic and Bozin 2008; Kapp 2015) and an accession rich in menthol is also reported (Llorens-Molina et al. 2017). Besides, Ali and co-workers (2002) report from the Pakistani sample mentioned above, a novel chlorinated limonene-3-oxo ketone. It is 1-hydroxy-2-chloromenthone or longifone.

Other cyclic monoterpenes

This group includes terpinen-4-ol, α-terpineol, α-terpinylacetate, eucalyptol, borneol, trans-sabinene hydrate, thymol etc., which were detected as major ingredients of ML EO in just a few cases. Alpha-terpinyl acetate as a main compound in an EO was described independently from Northern Turkey (Baser et al. 1999), Jiloca basin in Spain (Llorens-Molina et al. 2015), and (Kapp 2015). In the Turkish sample the terpinyl ester was present in 42% of the EO, and in the Estonian oil in 48%. The samples from Spain (18 individuals) showed somewhat lower proportion (39%) of the ester. From Serbia, a unique EO composition was described with presence of thymol (13%) together with its precursors γ-terpinene (5%) and p-cymene (14%) accompanied by eucalyptol (7%), however without the typical limonene-derived ketones (Mimica-Dukić et al. 1993).

Sesquiterpenes

The majority of sesquiterpenes has been detected in EOs of horsemint as minor component except β-caryophyllene, caryophyllene oxide and germacrene D which are regularly demonstrated as major ingredients (Mimica-Dukić et al. 1993; Başer et al. 1999; Sharopov et al. 2012; Iqbal et al. 2013; Llorens-Molina et al. 2015; Kapp 2015). Their proportions in the EO make up 2-10%, however, in a Turkish sample of M. longifolia L ssp. typhoides var. typhoides 29% caryophyllene oxide and 12% β-caryophyllene were determined (Başer et al. 1999). Besides, a major unknown was also detected by Başer and co-workers (2012) in samples from Marmara region (Turkey).

This compound, probably a sesquiterpene is characterized by a GC retention index RI = 2209. It was present in the EO of a single M. longifolia L ssp. longifolia oil (35% of EO) and in six M. longifolia L ssp. typhoides var. typhoides oils (between 6-35%).

Chemotypes of horsemint: open questions

Based on the mentioned varying main compounds of the EO, references declare the presence of different chemotypes. According to Mimica-Dukic and Bozin (2008) the wide diversity of EOs of wild-growing mints is observable in contrary to the relative stability of the composition of cultivated spices. Others authors conclude that EO composition of ML is highly variable even among the wild growing mints (Németh-Zámboriné 2015a) In spite of this, according to our knowledge, no summarizing survey or review of this partial area of the phytochemistry of M. longifolia has been published until now. Here, three larger typologies are considered. Başer and his group (1999) provided EO compositions and typology of Turkish ( Aegean region) samples of two ML taxa. From M. longifolia ssp. longifolia (18 samples) five chemotypes were determined: 1) rich in piperitone oxides (2) linalool-rich or linalool-eucalyptol type (3) type based on carvone or carvone and β-caryophyllene (4) type rich in isomenthone (5) other compositions: one α-terpinyl acetate based sample and another rich in terpinen-4-ol and trans-sabinene hydrate. M. longifolia ssp. typhoides var. typhoides (19 samples) have been classified into six chemotypes (1) rich in piperitone oxides (2) linalool-rich (3) carvone-rich (4) rich in trans-sabinene hydrate (5) type based on menthone or menthone/ trans-piperiton-oxide (6) EOs based on trans-piperitone oxide/β-caryophyllene or trans-piperitone oxide/β-caryophyllene oxide. Another typology is provided by Mimica-Dukic and Bozin (2008) who distinguish nine chemotypes (signed as types I to IX) of the genus based on surveying both cultivated and wild-growing mint species. M. longifolia s.l. is present in five of these chemotypes: II, rich in linalool and/or linalyl acetate; III, based on carvone or dihydrocarvone; IV, dominated by piperitone or piperitenone; V, piperitone oxides or pipertitenone epoxide; IX, menthone, isomenthone or menthol (isomers) as main constituents. In this classification, the chemotype V group contains only M. longifolia and no other Mentha taxa were

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placed here. Interestingly, no thymol—para-cymene chemotype of *M. longifolia* is mentioned, although it was reported by the same authors earlier (Mimica-Dukić et al. 1993). Finally, Sharopov and co-authors (2012) list fourteen chemotypes of *M. longifolia* as the most important ones. This classification is supported by experimental data of *M. longifolia* samples collected from at least one, but usually 3-8 habitats. The mentioned chemotypes, marked by their key component are as follows: piperitenone epoxide; piperitone oxides; piperitone; isopiperitenone; piperitenone; carvone; trans-dihidrocarvone; pulegone; menthone; isomenthone; menthofurane; menthol; eucalyptol; borneol. Authors note that both EO composition and morphological traits of *M. longifolia* are highly diverse without mentioning any correlation between chemical and morphological traits.

Comparing the above mentioned three typology, carvone, piperitone, piperitone with its oxides can be established as the basis and the most widespread monoterpenes of ML chemotypes. Other studies mentioning different chemotypes of this species are scarce but recent works report new chemotypes too. A menthofurane rich accession of *M. longifolia* L ssp. *polyadena* from South-Africa is described by Viljoen et al. (2006). Three novel types in Teruel region, Spain have been explored via careful sampling of chemotaxonomically heterogeneous populations. These were a cis-sabinene hydrate/terpinen-4-ol, a α-terpinyl acetate/carvyl acetate (Llorens-Molina et al. 2015) and an α-terpineol acetate/8-acetoxy carvotanacetone type (Llorens-Molina et al. 2020) respectively.

**Antimicrobial properties of horsemint volatiles**

Beside flavour and aroma, the EO might contribute to the preservation of food products. The most comprehensive study (Güllüce et al. 2007) on *M. longifolia* EO rich in limonene-3-oxo compounds provide the antimicrobial activity against 15 species of molds and 14 strains of bacteria, and also against *C. albicans*. This data is highly valuable because most of the studies work with a far lower number of microorganisms and/or do not provide strain numbers. The tested EO contained cis-piperitone oxide (18.4%), pulegone (15.5%), piperitenone oxide (14.7%), menthone (7.9%), isomenthone (6.6%), trans-piperitone oxide (4.1%) and in lower (1-5%) proportions limonene-2-oxo volatiles, accompanied by 6.6% thymol. MIC of this EO was lower or equal with the values of control antibiotics against the majority of the tested bacteria (except *Streptococcus*, *Pseudomonas*, *Enterobacter* and *Brucella* spp). Good anticandidic activity and efficacy against *Fusarium* spp. was also observed. Based on this finding, authors propose utilization of *M. longifolia* essential oil as a preservative.

Other constituents of the ML oils, like limonene and its 2-oxo derivatives were also demonstrated to show moderate antibacterial activity on a wide range of pathogens, including foodborne ones (e.g. *E. coli*, *P. aeruginosa*, *Enterobacter* sp.- strain numbers not provided), (Oumzil et al. 2002). The study of Aggarwal et al. (2002) demonstrated the activity of S(-)-carvone, being present frequently in *M. longifolia*, was as effective against *K. pneumoniae* and *Candida*. The antimicrobial activity of limonene-3-oxo-ketones and their epoxides, together with the mint oils characterized by them are rather frequently studied in some cases due to their potential preservative properties. Studies on pulegone, piperitenone, piperitone and epoxides isolated from *Mentha* (Oumzil et al. 2002) or *Satureja* species (Tolossa et al. 2007) show that pulegone possesses strong antimicrobial activity. However, as the use of pulegone is limited, its direct utilisation in the food industry does not have much potential. The EO of the thymol-paracymene chemotype from Serbia (Mimica-Dukić et al. 1993) was observed to show considerable activity against *B. subtilis*, *S. aureus*, *C. albicans* and *A. niger*. A review of the antimicrobial activity of EO is given by Mikaili et al. (2013), with data on decreasing antibiotic resistance of food-borne bacteria together with remarkable effects against moulds, pathogen fungi and protozoas. Ehsani, Mahmoudi and co-workers (2012) demonstrated the preservative effects of *M. longifolia* EO (with main components pulegone, eucalyptol, menthofurane and isopulegone) in Iranian white-brined cow cheese. The combination of 150 ppm EO and a probiotic bacterium (*L. casei*) showed a significantly better preservative effect against the dairy-borne pathogens *L. monocytogenes* and *S. aureus*, than any of the treatment alone. According to authors, the limiting factor of the EO concentration in the cheese may be its influence to organoleptic properties. In our opinion, the high proportion of pulegone isomers and menthofurane in the EO might also be dangerous.
Concluding remarks

Among the phenolic compounds of *M. longifolia*, the AO value of rosmaricin has been declared frequently. Nevertheless, a part of the available data brings up the question if it is really the only or maybe the most important constituent of strong antiradical properties of the ML extracts. It was demonstrated, that the plant contains a couple of caffetannins and 55 various flavonoids, primarily flavanones. Considering the relations of structure and antiradical activity, three groups of flavonoids may deserve attention, i) luteolin-7-O-glycosides like lonicerin and cynaroside, ii) eriodyiocytol derivatives (eriocitrin) and iii) derivatives of quercetin, among which rutin is as frequently reported from *M. longifolia* samples as cynaroside. Rutin content was found to be in significant correlation with the FRAP activity of *M. longifolia* (Patonay et al. 2017). Park et al. (2019) also observed strong antioxidant activity to their rutin-dominated *M. longifolia* sample. Further studies are suggested to determine if higher concentrations of quercetin derivatives are really universal characteristics to this species as it was described in some references above.

Unfortunately, well-established conclusions on the available literature data are facing difficulties arising primarily because of methodological problems. Analysis, partially on nonvolatile compounds, are often made on a single batch of questionable representativeness of *M. longifolia*. Bulk samples are unable to represent the real chemical variability of any population and repetability of the results is also hardly possible. Comparison of published results is aggravated through the missing definition of plant part and phenophase at collection, too. Thus, in lack of representativeness and detailed description of sampling methods, separate references are unable to confirm either the universal appearance of any components or the background of the detected differences. It may be proposed to screen flavonoids of *M. longifolia* on a wider range of samples instead of single charges. It seems to be necessary to detect also both the biotic and environmental factors which might influence accumulation of these compounds.

During optimization of industrial uses, the effective solvent of polyphenols of *M. longifolia* should also be determined. In general, polar extraction results in high AO activity (Mikaili et al. 2013), nonpolar solvents such as hexane or DCM are not effective (Iqbal et al. 2013). A recent study of extractability of *M. longifolia*, performed by our team on 36 samples, proposes to use water–ethanol with a 3:7 mixture which makes it possible to avoid the toxic methanol in food products (Patonay et al. 2019). The utilization of aqueous waste of EO hydrodistillation was also described as a potential useful way to gain polyphenolic antioxidants of *Mentha* spp. (Damien-Dorman et al. 2003a, b; Koşar et al. 2004; Shen et al. 2011).

On preservative efficacy of *M. longifolia* drug or nonvolatile extracts in the food matrix, no published data was found. In case of other mint species, some results are available. The drug of *M. spicata* in a dairy dessert under thermal treatment, inhibited lipid peroxidation (Bandopadhyay et al. 2008) with similar efficacy as tert-butyl-hydroquinone. In a highly different matrix, namely a whole raw fish, a *M. arvensis* ethanolic extract was able to increase shelf life by inhibiting lipid peroxidation and release of biogenic amines (Viji et al. 2015). Although these studies do not determine the constituents in the background of the preservative effect, it could be supposed, that phenolics were effective in inhibiting peroxidation based on their radical scavenging properties detailed above. These results allow us to anticipate that a standardized, deodorized *M. longifolia* extract rich in polyphenols may be a cheap and effective inhibitor of lipid peroxidation and coupled oxidative deteriorations in some sensible types of foodstuff, i.e. in dairy, meat or fish products.

Volatile of *M. longifolia* show an extraordinarily wide variability. Because of the complexity of data and eventual contradictions, it can be established, that the chemotaxonomic investigation on *M. longifolia* needs further thoroughl study. It seems, that the geographical habitat is not closely connected to the abundance of any chemotype and the populations may be heterogenous in contrary to the primarily vegetative propagation behaviour of the plant (Llorens-Molina et al. 2015). To the contrary of some comprehensive studies, a well established definition of *M. longifolia* chemotypes is still lacking as chemical variability may have several backgrounds (Németh-Zámboriné 2015b). A more adequate knowledge on the occurrence and stability of chemotypes and those of the EO composition may encourage the utilization of the desired types, primarily those free of pulegone as being a potentially cheap source of flavorings. The rich spectrum of volatiles also enables the selection of
strains or clones with different aroma characters (e.g. carvone-rich: spearmint like, linalool-rich: reminiscent to lavender, etc.). Beside the phenolics, EO of *M. longifolia* might contribute to the preservation of food products, too. Evaluation and comparison of data dealing with antimicrobial activity of *M. longifolia* volatiles is, however aggravating due to the wide and varying spectrum of the investigated microbiota strains and extraction methods as well as the missing details on the enantiomers of the investigated volatiles (Oumzil et al. 2002). Chemotypes rich in piperitone, piperitenone and correspondent oxides, might have a great value as antimicrobial agents against numerous food-borne pathogens. Usage of pulegone-rich EOs should be avoided because of the toxicity of this component.

**Acknowledgements** Authors gratefully acknowledge the support of the Grant EFOP-3.6.1-16-2016-00001 ‘Complex improvement of research capacities and services at Eszterhazy Karoly University’ by the European Social Fund, and by the Ministry for Innovation and Technology (Hungary) within the framework of the Higher Education Institutional Excellence Program (NKFIH-1159-6/2019) in the scope of plant breeding and plant protection researches of Szent István University.

**Funding** Open access funding provided by Eszterhazy Karoly University.

**Compliance with ethical standards**

**Conflict of interest** Authors have no conflict of interest or competing interest to declare.

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