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Overview on *Mentha* and *Thymus* Polyphenols

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Abstract: *Mentha* and *Thymus* are important genera of the Lamiaceae family widely distributed in the entire World and commonly used in traditional medicine. Indeed, many species of the two genera have been credited with a large list of health-benefit effects, including antioxidant, anti-inflammatory, antimicrobial, analgesic, neuroprotective and anticarcinogenic. In turn, these properties have been associated to the polyphenolic composition of the plants. The present review summarizes the phenolic constituents found in *Mentha* and *Thymus* genera, as well as the main methods applied in their extraction, purification and identification. Reported species of *Mentha* and *Thymus* usually comprise derivatives of caffeic acid and distinct glycosidic forms of the flavonoids luteolin, apigenin, eriodictyol and naringenin. At present, the phenolic composition of many relevant plants of *Mentha* and *Thymus* is still unknown and thus, more studies are required for the adequate phenolic characterization of these two genera. In this context, the present implementation of faster and reliable analytical methodologies, as e.g. the chromatographic techniques hyphenated with mass spectrometry, will surely be an enormous tool in the upgrading of the missing information.

Keywords: *Mentha*, *Thymus*, Phenolic compounds, Phenolic acids, Flavonoids, HPLC, Mass spectrometry, NMR.

1. INTRODUCTION

Lamiaceae is one important botanical family with about 230 genera, some of which have been used for centuries in traditional medicine. One important Lamiaceae genus, the *Mentha*, encloses species distributed over the worldwide, with special abundance in temperate regions of Eurasia, Australia and South Africa [1, 2]. In general, the plants of this genus growth in distinct environments, although they are preferentially found in damp or wet places. *Mentha* plants are mainly perennial and grow 10 - 120 cm tall, with erect, branched, four-sided or squared stems. They have white to purple flowers, small fruits containing a few number of seeds (one to four) and have leaves typically arranged in opposite pairs, also showing a great color diversity [3-5].

Several mint species are of a great economic importance and are used in food, flavour, cosmetic and pharmaceutical industries mainly because of peculiar properties of their essential oils. The most used mints are peppermint *(Mentha × piperita)* and *M. spicata*, commonly known as spearmint [6, 7]. Due to the frequent occurrence and to the vast distribution of *Mentha* cultivation, there is a high polymorphism in this genus. More, hybridization in wild and cultivated plants is emerging. These facts lead to the difficult and not consensual systematic classification of *Mentha* species. Despite this, recent studies based in morphological, cytological, and chemical markers, indicated that the *Mentha* genera consists of eighteen species and includes eleven hybrids.

Other important genus of Lamiaceae family is *Thymus*. This genus comprises 300 to 400 endemic species, widely distributed throughout the world and particularly abundant in West Mediterranean region [8]. Many *Thymus* plants appear as perennial and aromatic subshrubs or shrubs with quadrangular stem erect to prostrate. Usually, the plants have big clusters of flowers of diverse color (white, cream, pink or violet) [9]. The leaves are simple, entire or sometimes toothed, frequently revolute, glabrous or hairy. Several species of this genus are often cultivated for culinary purposes and to be used as medicinal plants. The latter usage is due to several pharmacological of these plants, which are mostly described for thyme and its wild form (*Thymus vulgaris* L. and *T. serpyllum*, respectively).

Previous studies on the chemical composition of *Mentha* and *Thymus* have mainly dealt with their essential oils [10-13]. However in recent years, an increasing number of investigations have focused on other secondary metabolites, including the polyphenols. In fact, phenolic compounds of *Mentha* and *Thymus* plants have been associated to their beneficial properties, supporting their ethnopharmacological usage. These effects include the antioxidant capacity, that has been described for *M.* x *piperita, M.* "Native Wilmet", *M. dalmatica* and *M. spicata* [1, 14, 15], the antitumorogenic ability, that has been reported for *M. spicata* and *M. piperita* L. species [16, 17], the neuroprotective action reported for *M. × piperita* and *M. aquatica* species [18-20] and the anti-inflammatory activities showed for *M. aquatica* plant [21]. In the case of *Thymus* genus, *T. vulgaris* L. species has been

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described to possess antitumorogenic [16, 22] and free radical-scavenging [23] activities. This species together with *T. mastichina* and *T. capitata* also have important antioxidant activities [24-26], while *T. broussonetii* and *T. pulegioides* have been shown to exert analgesic [27] and cardioprotective capacities [28], respectively. Note that *Mentha* and *Thymus* genus show many similarities regarding the type of polyphenols in their composition, despite differences in their prevalence. As a result of that, several studies have simultaneously focused species of these two genera [29-31].

The phenolic characterization of plants commonly engage their extraction with water, methanol, ethanol or aqueous mixtures and their further analysis by reversed phase high performance liquid chromatography (HPLC/UV/PDA) [32] combined or, alternatively, hyphenated with electrospray mass spectrometry (LC-MS). These techniques have allowed to perform the identification and quantification of phenolic compounds in many extracts of *Mentha* and *Thymus* genera plants. Nuclear magnetic resonance (NMR) appears as a powerful complementary technique for structural assignment [33], in particular of unknown or complex phenolic compounds. The present manuscript revises the main polyphenols described up to date in extracts of *Mentha* and *Thymus* plants and also examines the analytical methods used in their extraction, isolation and structural identification.

2. POLYPHENOLS OF *MENTHA* AND *THYMUS* GENERA

Several studies have demonstrated that *Mentha* and *Thymus* plants are rich in phenolic compounds, particularly in phenolic acids and flavonoids. As usually, the total polyphenolic content of these plants has been mainly estimated through the use of Folin Ciocalteu method on enriched phenolic extracts, which in turn have been obtained by water or, more frequently, by ethanolic or methanolic aqueous solutions.

The total polyphenolic content of *M. pulegium* and *M. viridis* leaves and that of leaves and branches of *M. canadensis* has been previously established as approximately 8, 17 and 52 mg of gallic acid equivalent (GAE)/g of dry plant, respectively [34]. Moreover, Dorman et al [1] have reported a total phenolic content in the range of 47 – 77 mg of GAE/g of dry plant, for the aerial parts of nine *Mentha* species. Alternatively, values of aproximatly 20 – 46 mg of GAE/g of dry plant have been described in aerial parts for the most common *Thymus* species (*T. vulgaris* L.) [34-36] while a lower amount of total polyphenols (12 mg of GAE/g dry plant) has been reported for *T. serpyllum* [37].

2.1. Phenolic Acids

Phenolic acids occur in nature as hydroxycinnamic (C_6-C_3) or hydroxybenzoic structures (C_6-C_1). Diversity in this group is mainly due to the variation on the number and positions of hydroxyl functions in the phenolic ring. In the plant kingdom, these compounds are often present in their free form, as well as hexoside derivatives or as esters of benzoic or cynnamic acids. From all phenolic acids, *Mentha* and *Thymus* genera are particularly enriched in caffeic acid derivative compounds. These polyphenols, as well as other hydroxycinnamic and hydroxybenzoic acids detected in these genera will be described bellow in more detail. A resume of these phenolic compounds and the respective species where they have been found are shown in Table **1**. Moreover, the structure of these compounds is depicted in Fig. (**1**).

Caffeic Acid and Derivatives

Reported literature on *Mentha* and *Thymus* genera indicates that caffeic acid and its derivative compounds represents 15 - 60% and 60 - 80% of their total phenolic compounds, respectively [34, 38-46]. The free form of this acid is frequently detected, but most representative compounds are those commonly known as dimeric and trimeric forms of the acid, as well as their derivatives.

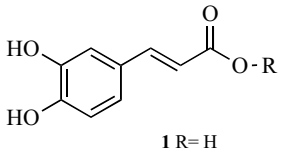
Caffeic acid **(1)** is vastly described in *Mentha* genus [1, 38-41, 47], although its relative abundance greatly varies in between the reported studies. In general, the free form of the acid appears as a minor phenolic component (approximately 1% of total phenolic content), as described by Dorman et al [1] for nine distinct *Mentha* species.

As for *Mentha* species, caffeic acid in *Thymus* plants commonly appears in low relative quantities [43, 46]. Several authors have described caffeic acid in *T. vulgaris* L. [34, 42, 43, 45, 46, 48-50] and reported data indicated a total of 0.1-0.48 mg/g of dry plant in this species with similar results for *T. serpyllum* [46, 49].

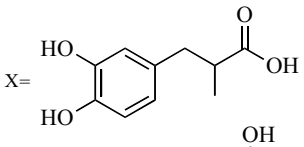
Besides the free form of the acid, simple ester or glucoside derivatives of caffeic acid were also reported to occur as minor phenolic components of *Mentha* and *Thymus*. Namely, two esters of caffeic acid, the (*Z,E*)-[2-(3,5-dihydroxyphenyl)ethenyl] 3-(3,4-dihydroxyphenyl)-2-propenoate **(2)** and the (*Z,E*)-[2-(3,4-dihydroxyphenyl)-ethenyl] 3-(3,4-dihydroxyphenyl)-2-propenoate **(3)**, also named nepetoidin A e B, respectively, have been reported in *M. aquatica, M. longifolia* and *M. x villosa* species [51] while chlorogenic acid **(4)** was reported in *M.* × *piperita* L. [40]. For *Thymus*, caffeic acid ethyl ester **(5)** has been described for *T. serpyllum* [37] and hexoside forms of caffeic acid **(6)** [35, 50], the compound chlorogenic acid [37, 50, 52] and dicaffeoylquinic acid **(7)** [50] have been described for *T. vulgaris* L. Moreover these two latter compounds also have been reported to occur in *T. webbianus* [52].

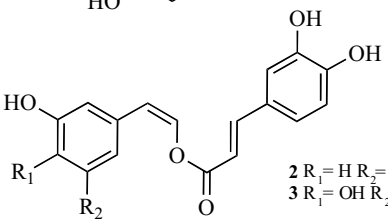
On the other hand, rosmarinic acid **(8)**, the ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid and commonly known as a caffeic acid dimeric form, is the most abundant phenolic in *Mentha* and *Thymus* genera. Its content in *M. x piperita* L. species (the most studied *Mentha* species regarding phenolic compounds) was reported as being approximately 30% of the total phenolics [38-42] and even higher in other *Mentha* species, such as *M. aquatica, M. x dalmatica,* and *M. canadensis* L., where this phenolic was reported to account for 3.1, 3.4 and 19.1 mg/g of dry plant (40, 46 and 90% of the total phenolics, respectively) [1, 34, 41]. Rosmarinic acid has also been reported in *M. spicata* (1.1 mg/g of dry plant) and in *M. longifolia*, although no content has been estimated for the latter species [1, 2, 41, 47, 53].

Rosmarinic acid is even more abundant in *Thymus* genus. In *T. vulgaris* L. species this phenolic acid has been described to account close to 70% of its total polyphenols,

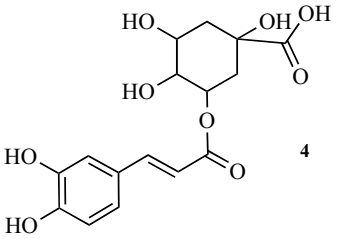


1 R= H
5 R= CH_2CH_3
6 R= Glc





2 R_1= H R_2= OH
3 R_1= OH R_2= H



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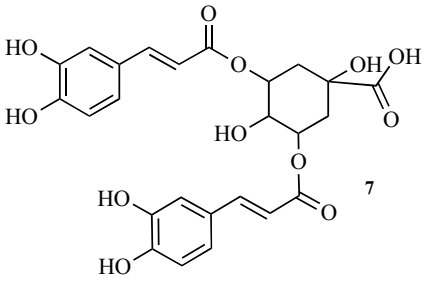

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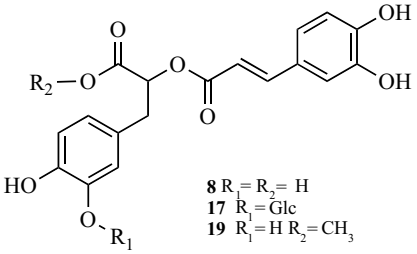
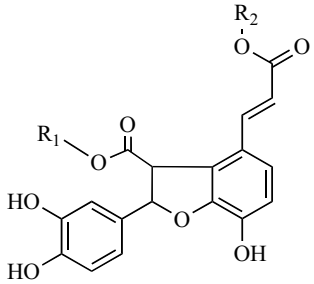
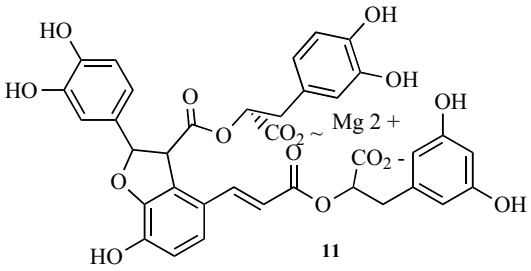


8 R_1= R_2= H
17 R_1= Glc
19 R_1= H R_2= CH_3



9 R_1= H R_2= X
10 R = R_1= X_2



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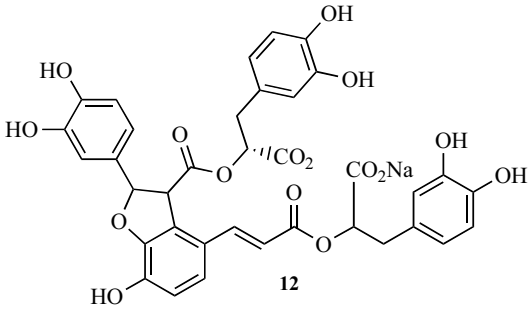

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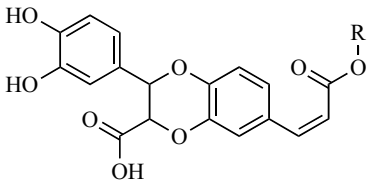
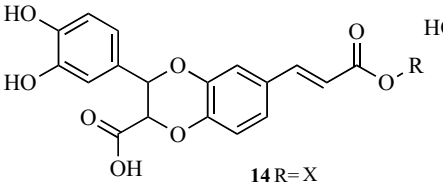
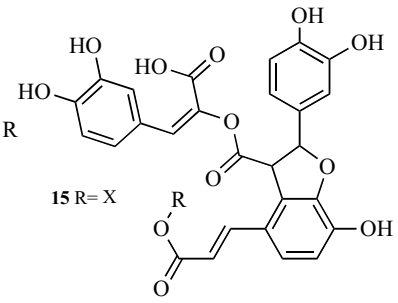


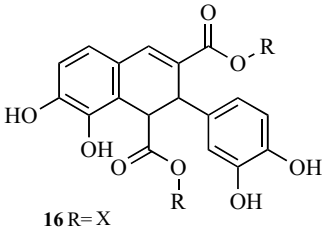
13 R= X



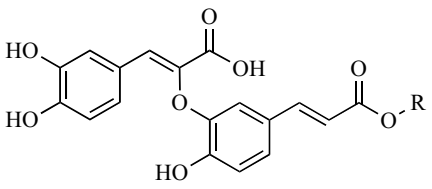
14 R= X



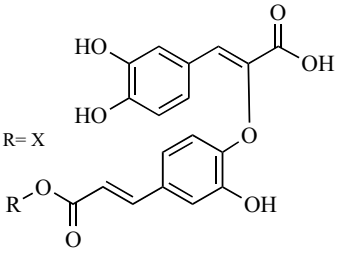
15 R= X



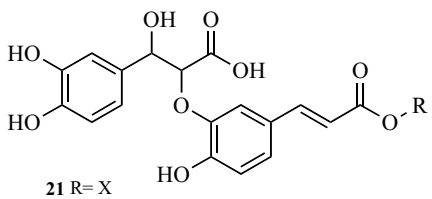
16 R= X



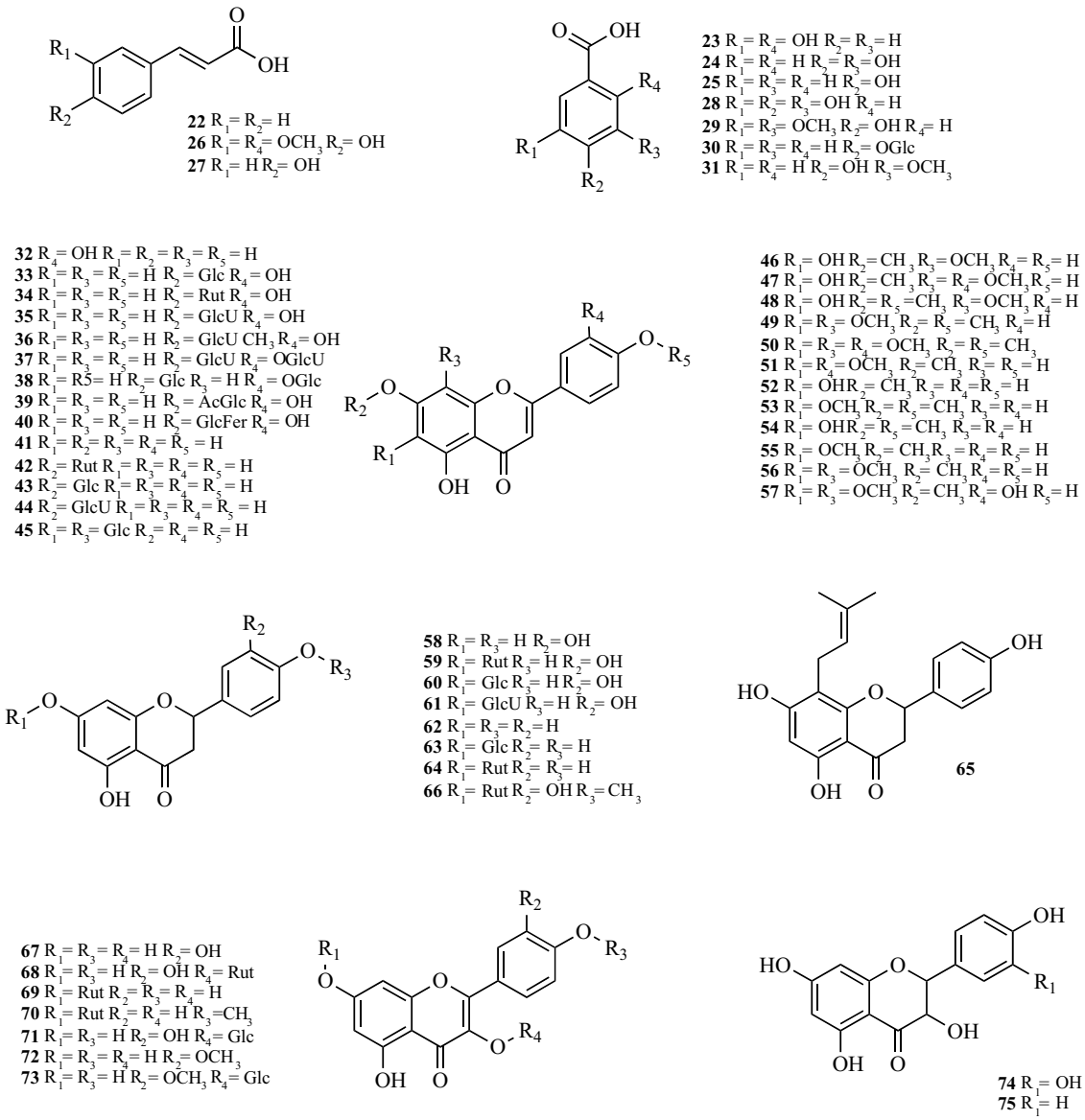
18 R= X



20 R= X



21 R= X



GlcU- Glucuronide unit; Glc- Glucoside unit; Rut- Rutinoside unit; Ac- Acetyl unit; Fer- Feruloyl unit

Fig. (1). Chemical structures of polyphenols described in *Mentha* and *Thymus* plants. The reference numbers for the compound structures are used throughout this paper.

Table 1. Phenolic Acids of *Mentha* and *Thymus* Genera

Compound	*Mentha* species		*Thymus* species	
Caffeic acid derivatives				
Caffeic acid **(1)**	*M. x piperita L.*	[1, 38-42, 58]	*T. vulgaris L.*	[34, 42, 43, 46, 48-50, 53]
	M. aquatica	[41]	*T. serpyllum*	[37, 46, 47, 49]
	M. spicata	[1, 47, 53]	*T. quinquecostatus*	[54]
	M. canadensis L.	[34]		
	M. x dalmatica	[1]		
	M. "Morocco"	[41]		
	M. "Native Wilmet"	[41]		
	M. arvensis	[1, 41]		

Table 1. contd…

Compound	***Mentha* species**		***Thymus* species**	
Nepetoidin A **(2)**	*M. aquatica* *M. longifolia* *M. x villosa*	[51]		
Nepetoidin B **(3)**				
Chlorogenic acid **(4)**	*M. x piperita L.*	[40]	*T. vulgaris L.*	[50]
			T. serpyllum	[37]
			T. webbianus	[52]
Caffeic acid ethyl ester **(5)**			*T. serpyllum*	[37]
Caffeic acid glucoside **(6)**			*T. vulgaris L.*	[35, 50]
Dicaffeoylquinic acid **(7)**			*T. vulgaris L.*	[50]
			T.webbianus	[52]
Rosmarinic acid **(8)**	*M. x piperita L.*	[1, 2, 38-42, 58, 61-63]	*T. vulgaris L.*	[34, 35, 42-46, 49, 53, 57, 58, 64]
	M. aquatica	[41]	*T. serpyllum*	[37, 46, 47, 49]
	M. spicata	[1, 41, 47, 53]	*T. sipyleus*	[55]
	M. canadensis L.	[34]	*T. quinquecostatus*	[54]
	M. x dalmatica	[1]		
	M. haplocalyx	[1, 56]		
	M. "Morocco"	[41]		
	M. "Native Wilmet"	[41]		
	M. x verticillata	[41]		
	M. arvensis	[1, 41]		
	M. longifolia	[2]		
Lithospermic acid **(9)**	*M. x piperita L.*	[39]	*T. serpyllum*	[49]
	M. haplocalyx	[56]		
Lithospermic acid B **(10)**	*M. haplocalyx*	[56]		
Magnesium lithospermate B **(11)**	*M. haplocalyx*	[56]		
Sodium lithospermate B **(12)**	*M. haplocalyx*	[56]		
Cis salvianolic acid J **(13)**	*M. haplocalyx*	[56]		
Salvianolic acid J **(14)**	*M. haplocalyx*	[56]		
Didehydro-salvianolic acid **(15)**	*M. x piperita L.*	[2]		
	M. longifolia	[2]		
Salvianolic acid L **(16)**	*M. x piperita L.*	[2]		
	M. longifolia	[2]		
Rosmarinic acid glucoside **(17)**			*T. vulgaris L.*	[35]
3'-*O*-(8''-*Z*-Caffeoyl)rosmarinic acid **(18)**			*T. vulgaris L.*	[57]
Methyl rosmarinate **(19)**			*T. vulgaris L.*	[49]
Salvianolic acid I **(20)**			*T. vulgaris L.*	[35]
Salvianolic acid K **(21)**			*T. vulgaris L.*	[35]

Table 1. contd…

Compound	*Mentha* species		*Thymus* species	
Other phenolic acids				
Cinnamic acid **(22)**	*M. x piperita L.*	[6]		
Gentisic acid **(23)**	*M. x piperita L.*	[42]	*T. vulgaris L.*	[42]
Protocatechuic acid **(24)**	*M. x piperita L.*	[42]	*T. vulgaris L.*	[42, 50]
Hydroxybenzoic acid **(25)**	*M. x piperita L.*	[42]	*T. vulgaris L.*	[42, 46, 50]
			T. serpyllum	[37]
Ferulic acid **(26)**			*T. vulgaris L.*	[48, 50]
p-Coumaric acid **(27)**			*T. vulgaris L.*	[34, 42, 50]
			T. webbianus	[52]
			T. serpyllum	[37]
Gallic acid **(28)**			*T. vulgaris L.*	[34, 43, 50]
			T. webbianus	[52]
Syringic acid **(29)**			*T. vulgaris L.*	[42]
Hydroxybenzoic acid-*O*-hexoside **(30)**			*T. vulgaris L.*	[50]
Vanillic acid **(31)**	*M. x piperita L.*	[42]	*T. vulgaris L.*	[42, 50]
			T. serpyllum	[37]

representing approximately 3.4 to 22 mg/g of dry plant [34, 43, 49]. Besides this species, rosmarinic acid was also detected as a major phenolic compound in other *Thymus* species, such as *T. serpyllum*, *T. sipyleus* and *T. quinquecostatus* var. japonica [37, 46, 49, 54, 55].

In addition to rosmarinic acid, other caffeic acid derivative compounds enclosed in the class of depsides (esters formed by the condensation of two or more molecules of phenolic carboxylic acids compounds), are relevant in *Mentha* and *Thymus* genera. Lithospermic acid **(9)**, usually classified as a caffeic acid trimer, is a phenolic constituent of *M. x piperita L.* and *M. haplocalyx* and its content in the first species has been described to be approximately 3 mg/g of dry plant [39, 56]. Other caffeic acid derivatives, namely the lithospermic acid B **(10)**, magnesium lithospermate B **(11)**, lithospermate B **(12)** and two salvianolic acid derivatives (*cis* salvianolic acid J **(13)**, salvianolic acid J **(14)**) were only detected in *M. haplocalyx*. Alternatively, the first species contained didehydro-salvianolic acid **(15)** and one tetrameric form of caffeic acid (salvianolic acid L **(16)**) [2, 56].

In respect to *Thymus* genus, five derivatives of rosmarinic acid namely, its glucoside **(17)**, the 3'-*O*-(8''-*Z*-Caffeoyl)rosmarinic acid **(18)**, methyl rosmarinate (0.6 mg/g of dry plant) **(19)** and the two trimers of caffeic acid (salvianolic acid I **(20)** and salvianolic acid K **(21)**) [35, 49, 57] have been described to occur in *T. vulgaris* L.. Alternatively, lithospermic acid has been described as accounting for approximately 12 mg/g of dry plant in plant extract of *T. serpyllum* [49].

Other Phenolic Acids

Besides caffeic acid and their derivatives, other phenolic acids have also been described in *Mentha* and *Thymus* plants [1, 55] and, according to literature data, these compounds are much frequently detected in the latter genus. In more detail, *M. piperita* L. has been described to contain minor amounts of cinnamic acid **(22)** [6], gentisic acid **(23)**, protocatechuic acid **(24)** and *p*-hydroxybenzoic acid **(25)** [42]. Several hydroxycinnamic and hydroxybenzoic acids were reported for *Thymus* genus. Besides the caffeic acid, hydroxycinnamic acids previously detected in *Thymus* include ferulic acid **(26)** [48, 50] and *p*-coumaric acid **(27)** [34, 37, 42, 50, 52]. More, the hydroxybenzoic acids gallic acid **(28**) [34, 43, 50], gentisic acid [42], protocatechuic acid [42, 50, 54], syringic acid **(29)** [37, 42, 50], *p*- hydroxybenzoic acid [37, 42, 46], hydroxybenzoic acid-*O*-hexoside [50] **(30)** and vanillic acid **(31)** [37, 42, 50] have been detected in *T. vulgaris* L. while some of those have also been described to occur in *T. serpyllum*, *T. quinquecostatus* and *T. webbianus*.

2.2. Flavonoids

Mentha and *Thymus* species are rich in flavonoids. *Mentha* species are particularly abundant in flavanones (10-70% of their total phenolics), although their flavones content is also relevant. The latter class of flavonoids is the most common in *Thymus* genus. On the other hand, flavonols and dihydroflavonols are only found as minor components in these two plant genera. Table **2** resumes the main flavonoids found in *Mentha* and *Thymus* genera and their respective structure is shown in Fig. (**1**).

Flavones

Luteolin and its derivatives are the main flavones described in *Mentha* and *Thymus* genera [1, 39-41, 49, 50, 58, 59]. Luteolin **(32)** has been detected in *M. piperita* L., *M. aquatica, M. longifolia, M. pulegium, M. arvensis*, *M. haplocalyx* and *M. spicata* [1, 40, 58-60] and, as reported by Dorman *et al.* [1] for the latter three species, its content can

Table 2. Main Flavonoids in *Mentha* and *Thymus* Genera

Compound	Mentha Species		Thymus Species	
Flavones				
Luteolin (**32**)	*M. pulegium*	[60]	*T. vulgaris L.*	[44-46, 49, 50, 58]
	M. x piperita L.	[39, 40, 58, 60]	*T. serpyllum*	[37, 46, 49]
	M. longifolia	[59]	*T. sipyleus*	[55]
	M. spicata	[1]	*T. herba-barona*	[68]
	M. haplocalyx	[1]	*T. striatus*	[67]
	M. arvensis	[1, 41]	*T. webbianus*	[52]
	M. aquatica	[60]		
Luteolin-*O*-glucoside (**33**)	*M. x piperita L.*	[1, 41, 61]	*T. vulgaris L.*	[44, 46, 50, 65, 58, 64]
	M. longifolia	[59]	*T. serpyllum*	[37, 46]
	M. aquatica	[41]	*T. sipyleus*	[55]
	M. spicata	[1]	*T. webbianus*	[52]
	M. x dalmatica	[1]		
	M. haplocalyx	[1]		
	M. "Morocco"	[41]		
	M. "Native Wilmet"	[41]		
	M. x verticillata	[41]		
	M. arvensis	[1, 41]		
Luteolin-*O*-rutinoside (**34**)	*M. x piperita L.*	[2, 39, 58, 61-63]	*T. vulgaris L.*	[49, 50]
			T. serpyllum	[49]
Luteolin-*O*-glucuronide (**35**)	*M. x piperita L.*	[2, 39, 58]	*T. vulgaris L.*	[35, 49, 50, 57, 64]
	M. longifolia	[2]	*T. serpyllum*	[37, 49]
			T. sipyleus	[55]
Luteolin-*O*-glucuronide-methyl (**36**)	*M. x piperita L.*	[2]		
	M. longifolia	[2]		
Luteolin-*O*-diglucuronide (**37**)	*M. x piperita L*	[2]		
	M. longifolia	[2]		
Luteolin-*O*-diglucoside (**38**)			*T. vulgaris L.*	[64]
Luteolin-acetyl-*O*-glycoside (**39**)			*T. vulgaris L.*	[64]
Luteolin-7-*O*-(6"-feruloyl)-ß-glucopyranoside (**40**)			*T. sipyleus*	[55]
Apigenin (**41**)	*M. x piperita L.*	[40]	*T. vulgaris L.*	[44, 46, 50]
	M. spicata	[1]	*T. serpyllum*	[37, 46]
	M. pulegium	[60]	*T. herba-barona*	[68]
	M. arvensis	[1, 41]	*T. striatus*	[67]
	M. aquatica	[60]	*T.webbianus*	[52]
	M. x piperita L.	[60]		

Table 2. contd…

Compound	Mentha Species		Thymus Species	
Apigenin-7-*O*-rutinoside (**42**)	*M. x piperita L.*	[1, 39, 41, 58, 61-63]	*T. vulgaris L.*	[50]
	M. aquatica	[41]		
	M. spicata	[1]		
	M. x dalmatica	[1]		
	M. haplocalyx	[1]		
	M. "Morocco"	[41]		
	M. "Native Wilmet"	[41]		
	M. x verticillata	[41]		
	M. arvensis	[1, 41]		
Apigenin-7-*O*-glucoside (**43**)			*T. vulgaris L.*	[43, 46, 50]
			T. serpyllum	[46]
			T. webbianus	[52]
Apigenin-7-*O*-glucuronide (**44**)			*T. vulgaris L.*	[35, 58, 64]
			T. serpyllum	[37]
Apigenin-6,8-di-*C*-glucoside (**45**)			*T. vulgaris L.*	[58]
			T. webbianus	[52]
Thymusin (**46**)	*M. spicata*	[66]	*T. herba-barona*	[68]
	M. x piperita L.	[60, 66]	*T. striatus*	[67]
Thymonin (**47**)	*M. spicata*	[60, 66]	*T. striatus*	[67]
	M. x piperita L.	[60, 66]		
	M. suaveolens	[60]		
	M. pulegium	[60]		
	M. longifolia	[60]		
Pebrellin (**48**)	*M. citrata*	[66]	*T. striatus*	[67]
	M. aquatica	[66]		
	M. x piperita L.	[60, 61, 66]		
Gardenin B (**49**)	*M. citrata*	[66]	*T. striatus*	[67]
	M. aquatica	[66]		
	M. x piperita L.	[60, 61, 66]		
Desmethylnobiletin (**50**)	*M. spicata*	[60, 66]	*T. striatus*	[66, 67]
	M. x piperita L.			
Cirsilineol (**51**)	*M. spicata*	[66]	*T. vulgaris L.*	[71]
			T. herba-barona	[68]
Sorbifolin (**52**)	*M. x piperita L.*	[60]	*T. herba-barona*	[68]
	M. pulegium	[60]		
Salvigenin (**53**)	*M. citrata*	[60, 66]	*T. striatus*	[67]
	M. aquatica	[66]		
	M. x piperita L.	[60, 66]		

Table 2. contd…

Compound	Mentha Species		Thymus Species	
Ladanein (**54**)	*M. x piperita L.*	[60, 66]	*T. striatus*	[67]
	M. pulegium	[60]		
Cirsimaritin (**55**)			*T. serpyllum*	[37]
			T. herba-barona	[68]
			T. vulgaris L.	[50]
Xanthomicrol (**56**)	*M. x piperita L.*	[60]	*T. striatus*	[67]
			T. herba-barona	[68]
Sideritoflavone (**57**)	*M. spicata*	[66]	*T. herba-barona*	[68]
Flavanones				
Eridioctyol (**58**)	*M. x piperita L.*	[1, 39]	*T. vulgaris L.*	[44, 49, 57]
	M. "Morocco"	[41]	*T. herba-barona*	[68]
	M. "Native Wilmet"	[41]	*T. serpyllum*	[37, 49]
			T. webbianus	[52]
Eriocitrin (**59**)	*M. x piperita L.*	[1, 2, 38, 39, 58, 61, 62, 72]	*T. vulgaris L.*	[46, 49, 65]
	M. aquatica	[41]	*T. serpyllum*	[46, 49]
	M. spicata	[1]		
	M. x dalmatica	[1]		
	M. haplocalyx	[1]		
	M. "Morocco"	[41]		
	M. "Native Wilmet"	[1, 41]		
	M. x verticillata	[41]		
	M. arvensis var. japanensis	[1, 41]		
Eridioctyol-*O*-glucoside (**60**)	*M. x piperita L.*	[39, 61, 63]	*T. vulgaris L.*	[35, 49]
Eridioctyol-*O*-glucuronide (**61**)			*T. vulgaris L.*	[35, 64]
			T. serpyllum	[37]
Naringenin (**62**)	*M. x piperita L.*	[39, 40]	*T. vulgaris L.*	[49]
	M. aquatica	[19]	*T. herba-barona*	[68]
			T. webbianus	[52]
Naringenin-7-*O*-glucoside (**63**)	*M. x piperita L.*	[38, 39]	*T. vulgaris L.*	[49]
	M. haplocalyx	[1]		
	M. x verticillata	[41]		
	M. arvensis	[1, 41]		
Naringenin-7-*O*-rutinoside (**64**)	*M. x piperita L.*	[39, 62, 63, 72]	*T. vulgaris L.*	[49]
Prenylnaringenin (**65**)			*T. serpyllum*	[37]
Hesperidin (**66**)	*M. x piperita L.*	[39, 58, 61-63, 72]	*T. vulgaris L.*	[49]
	M. longifolia	[59]		

Table 2. contd…

Compound	Mentha Species		Thymus Species	
Flavonols and dihydroflavonols				
Quercetin (**67**)	*M. x piperita L.*	[40]	*T. vulgaris L.*	[46, 50]
			T. serpyllum	[46]
Rutin (**68**)	*M. x piperita L.*	[40]	*T. vulgaris L.*	[50]
Kaempferol-7-*O*-rutinoside (**69**)	*M. x piperita L.*	[72]		
4'-methoxykaempferol-7-*O*-rutinoside (**70**)	*M. x piperita L.*	[72]		
Quercetin-3-*O*-hexoside (**71**)			*T. vulgaris L.*	[50]
Isorhamnetin (**72**)			*T. vulgaris L.*	[50]
Isorhamnetin-*O*-glucoside (**73**)			*T. vulgaris L.*	[50]
Taxifolin (**74**)			*T. vulgaris L.*	[57]
			T. quinquecostatus	[54]
Aromadendrin (**75**)			*T. quinquecostatus*	[54]

account for up to 0.12 mg/g of dry plant. Moreover, glycosidic derivatives of luteolin are often described as major phenolic compounds in *Mentha*. In this sense, Dorman *et al.* [1] reported the occurrence of approximately 0.1 to 3 mg of luteolin-*O*-glucoside **(33)**/g of dry plant [1, 41, 61], while luteolin-*O*-rutinoside **(34)** has been shown to account close to 8 mg/g of dry *M. x piperita* L. [2, 39, 58, 61-63]. Besides those compounds, glucuronide luteolin derivatives, namely the luteolin-*O*-glucuronide **(35)**, luteolin-*O*-glucuronide-methyl **(36)** and luteolin-*O*-diglucuronide **(37)** have been detected in *M. piperita* L. and in *M. longifolia* [2, 39, 58]. In a similar way, luteolin has been reported to occur in several *Thymus* species, including *T. vulgaris* L. [44-46, 49, 50, 58], *T. serpyllum* [37, 49] and *T. webbianus* [52] and its amount was established as 0.6 mg/g of dry plant for *T. vulgaris* L. or even higher for *T. serpyllum* (1.5 mg/g of dry plant) [49]. Additionally, luteolin-*O*-glucoside, luteolin-*O*-diglucoside **(38)** or luteolin-acetyl-*O*-glycoside **(39)** have been detected in *T. vulgaris* L. [43, 44, 46, 50, 52, 55, 58, 64, 65] and the first compound also in wild thyme (*T. serpyllum*) [37, 46]. Besides these species, *T. sipyleus* has been described to contain luteolin-*O*-glucoside and luteolin-7-*O*-(6"-feruloyl)-ß-glucopyranoside **(40)** [55]. Moreover, luteolin-*O*-rutinoside is a luteolin glycosidic derivative commonly found in *Thymus*. Its content in *T. serpyllum* and *T. vulgaris* L. has been estimated to be approximately 1.5 and 1.3 mg/g of dry plant, respectively [49, 50]. On the other hand, glucuronide derivatives of luteolin seem to be much abundant in *Thymus* than in *Mentha* species [35, 37, 49, 50, 55, 57]. In particular, a high concentration of luteolin-*O*-glucuronide has been found in *T. vulgaris* L. and *T. serpyllum* (close to 8 and 14 mg/g of dry plant, respectively) [49].

Apigenin and its derivatives can also be found in *Mentha* and *Thymus* genera. In particular, variable concentrations of apigenin **(41)** has been described in plant extracts of *M. spicata* (0.01 mg/g of dry plant) and *M. arvensis* (0.03 mg/g of dry plant) [1, 41]. Moreover, its diglycosidic derivative, the apigenin-7-*O*-rutinoside **(42)**, was detected in several *Mentha* species [1, 39, 41, 58, 61-63] with a concentration of approximately 0.8 mg/g of dry plant in *M. x piperita* L. [39, 41]. Apigenin has also been reported in *Thymus*, including *T. vulgaris* L. [44, 46, 50], *T. serpyllum* [37, 46] and *T. webbianus* [52]. These three *Thymus* species were also described to contain mono-*O* glycosidic forms of apigenin, namely apigenin-7-*O*-glucoside **(43)** [43, 46, 50, 52] and apigenin-7-*O*-glucuronide **(44)** [35, 37, 58, 64]. Moreover, apigenin 7-*O*-rutinoside has been described to occur in *T. vulgaris* L. [50], while a di-*C*-glycosidic derivative, the apigenin-6,8-di-*C*-glucoside **(45)**, has been detected in the latter species and in *T. webbianus* [52], although no quantification data has been delivered.

Besides the above compounds, *Mentha* and *Thymus* plants were shown to contain several OMe flavones. Thymusin **(46)**, thymonin **(47)**, pebrellin **(48)** and gardenin B **(49)** are the most cited for *Mentha* species. They have been detected in *M. spicata, M. x piperita* L.*, M. aquatica, M. citrata, M. longifolia, M. suaveolens* and *M. pulegium* [59-61, 66]. Some of these OMe flavones and others (compounds **(46)** to **(57)** in Table **2**) have been found in *T. striatus* [67], *T. herba-barona* [68] and also in other *Thymus* species [37, 45, 50, 69-71].

Flavanones

As previously mentioned, *Mentha* species are rich in flavanones and, according to the reported data in this genus, compounds of this class enclose mainly derivatives of eriodictyol, naringenin and hesperitin, which frequently appear as *O*-glucoside derivatives. In this context, eridioctyol **(58)** was reported in *M. x piperita* L. at concentrations in the range of 0.1 - 0.5 mg/g of dry plant and in *M.* "Native Wilmet" with a concentration of 0.05 mg/g dry plant [1, 39]. On the other hand, eriodictyol-7-*O*-rutinoside (eriocitrin) **(59)**, which is the most abundant flavavone in *Mentha* plants, was detected by distinct authors in *M.x piperita* L. [1, 2, 38, 39, 58, 61, 62, 72] and its content in that species was established

as approximately 13 - 19 mg/g of dry plant [1, 39]. Other studies focusing *M.* "Native Wilmet", *M. x dalmatica* and *M. spicata* concluded that these species also have considerable amounts of eriocitrin, although its levels were much lower than that reported for *M. x piperita* L. (7.4 mg/g dry plant in *M.* "Native Wilmet" and 2.5 mg/g dry plant in *M. x dalmatica* and *M. spicata* [1]).

According to literature data, flavanones in *Thymus* represent approximately 3-9% of their total polyphenolics [44, 46, 49], which mostly appear as eriodictyol and as its derivatives. In fact, eriodictyol has been described to occur in *T. vulgaris* L. (1.5 mg/ g of dry plant) [44, 49, 57], in *T. serpyllum* [37, 49], in *T. webbianus* [52] and in *T. herba-barona* [68], and eriodictyol-*O*-glucoside **(60)** and eriodictyol-7-*O*-rutinoside were both found in *T. vulgaris* L. (0.6 mg/g and 1.2 mg/ g of dry plant, respectively). The latter has also been described for *T. serpyllum* (1.2 mg/ g of dry plant) [46, 49]. Note that eriodictyol-*O*-glucuronide **(61)** has been found in thyme and wild thyme (Table **2**), but no quantitative information has been delivered on that compound [35, 37, 64].

Naringenin **(62)** has been detected as a minor phenolic component in *M. x pipperita* L. [40] and in *M. aquatica* [19] while its 7-*O*-glucoside **(63)** was found in *M. x piperita* L. (1.0 mg/g of dry plant) [38, 39] and in *M. arvensis* (0.1 mg/g of dry plant), among others [1, 41]. The major naringenin derivative described in this genus is narirutin (naringenin-7-*O*-rutinoside) **(64)** [39, 62, 63, 72].

Despite less frequently described, naringenin derivatives have also been reported in *Thymus*. Naringenin and prenylnaringenin **(65)** were the only naringenin derivatives detected in *T. webbianus* [52] and in *T. serpyllum* [37] respectively, while significant amounts of this aglycone, of naringenin-7-*O*-glucoside and of naringenin-7-*O*-rutinoside have been reported for *T. vulgaris* L. (approximately 0.4, 0.6 and 0.3 mg/ g of dry plant, respectively [49]).

To our knowledge, hesperitin aglycone has not been detected so far in *Mentha*, but its 7-*O*-rutinoside derivative, the hesperidin (**66**), has been found in significant amounts (1.7 mg/g dry plant) in *M. x piperita* L. and also detected in *M. longifolia* [58, 59, 61, 63, 72]. In *Thymus* plants, hesperidin has been described in *T. vulgaris* L., accounting for 1 mg/g of dry plant [49].

Flavonols and Dihydroflavonols

Despite less mentioned, flavonols have been reported to be minor phenolic components of some *Mentha* and *Thymus* species. In particular, *M. x pipperita* L. has been described to contain quercetin **(67)**, rutin **(68)**, kaempferol-7-*O*-rutinoside **(69)** and its 4'-methoxy derivative **(70)** [40, 72]. Quercetin has also been described to occur in several *Thymus* species, including the *T. vulgaris* L. [46, 50]. This latter species also contains two quercetin glycosides (quercetin-3-*O*-hexoside **(71)** and rutin), the methylated flavonol isorhamnetin **(72)**, its glucoside **(73)** [50] and the dihydroflavonol taxifolin **(74)** [57]. The latter phenolic has been also detected in *T. quinquecostatus*, together with aromadendrin (dihydrokaempferol) **(75)** [50, 54] and, besides these two dihydroflavonols, others have been described to occur in several species of *Thymus* [70].

3. METHODS OF EXTRACTION AND PURIFICATION

3.1. Sample Preparation

As for the majority of reported work on natural products, those focusing on *Mentha* and *Thymus* phenolics have entail regular practices to allow the improvement of the resulting analytical data. Indeed, in the sample preparation step, the material has been commonly dried [34, 42, 43, 47, 48, 57, 58], lyophilized [34, 64] or frozen ideally at -80ºC [45], thus minoring the instability of polyphenols and the action of several degradative enzymes. In addition, grounding is a well establish procedure before the extraction step [2, 34, 42, 48, 50, 53, 61, 63], as the particle size reducement of the plants increases the yield of extraction. Note that authors occasionally defatted the plant material with apolar solvents (e.g. *n*-hexane) [44, 62, 71], for prevention of high levels of lipophilic compounds in the extracts which can interfere in the polyphenols analysis. Some authors have also performed acidic or enzymatic hydrolysis. These procedures have been done before or simultaneously to the extraction procedure, when only aglycones were intended to characterize [36, 40, 44, 48].

3.2. Extraction

Polyphenols have been mainly obtained by solvent extraction. Aqueous mixtures of methanol or ethanol are the most used ones, though pure water is also often applied. In fact, as the majority of polyphenolics in *Mentha* and *Thymus* occur in glycosidic forms, aqueous mixtures are preferred. Distinct species of these two genera have been extracted with hydromethanolic solutions of 60 - 80% (*v/v*) to obtain phenolic acids [34, 42, 53], as well as this group combined with flavones, flavanones and flavonols [2, 35-37, 40, 43, 50, 53, 54, 58, 61, 64, 72, 73]. In a similar way, hydroethanolic solutions of 80% (*v/v*) have been preferentially used for extracting phenolic acids or flavonoids in *Mentha* [40, 53, 58, 61, 72] or *Thymus* [53, 57] species. According to the study of Reichling *et al*. [58], this mixture was much efficient than that of water:ethanol (80:20) in recovering rosmarinic acid from four *Mentha* species, including *M. x pipperita* L. and *T. vulgaris* L.. Besides the above solvents, water [39, 46, 62], acetone [45, 56, 71], aqueous acetone mixtures [56] and diethyl ether [68-70] have been used.

Authors have applied different techniques in the extraction process of *Mentha* or *Thymus* polyphenols. Stirring [35, 61], homogenization using a tissue homogenizer [43, 45, 50], maceration [40], sonication [47, 53, 72, 73] were the most frequently applied. Commonly, these techniques have been performed at room temperature [43, 50, 56, 58], to minimize degradation of phenolic compounds [74]. Water extraction is the main exception, since authors frequently have applied boiling or refluxing [39, 46, 62].

3.3. Clean-up and Fractionation

The main extraction process can be followed by additional purification of the enriched phenolic extracts. This practice allows obtaining a cleaner sample for characterization or to be used in biological assays. Reports on *Mentha* and *Thymus* genera have applied liquid-liquid extraction [55]

and, most commonly, solid phase extraction on C_{18} cartridges or column chromatography on Sephadex LH-20. The two latter usually enclose sequential solubilisation with distinct solvents, according to the nature of compounds that are intended to separate [39, 42, 44, 56, 57, 62, 68, 75, 76].

4. ANALYTICAL TECNHIQUES

As known, HPLC is the main technique applied in the analysis of plant phenolics, since it allows a rapid qualitative and quantitative screening [76]. In fact, the HPLC analysis of the *Mentha* and *Thymus* plant extracts have been essentially carried out in C_{18} reversed-phase columns. Additionally, in order to control the reproducibility of the method, the temperature of the column is usually maintained constant (20-30ºC).

Other important feature for achieving a good separation of phenolic constituents and consequently, high accuracy in the method, is the choice of the mobile phase. Distinct combinations of mobile and stationary phases provide diferent separation, since this is based on polarity differences among polyphenols [77]. For e.g., studies on *Mentha* and *Thymus* species applied acetonitrile/water or methanol/water combinations in an isocratic mode for fractionation of phenolic acids [42]. However, in general, *Mentha* and *Thymus* polyphenols have been preferentially analysed in a binary system of solvents, such as acetonitrile/water [37, 39, 43, 45, 48, 49, 58, 61, 63], methanol/water [34, 35, 40, 42, 61, 73] or water/water-acetonitrile [2]. Note that acidified water (0.1% to 5% of formic acid or other acids such as phosphoric acid or acetic acid) is preferentially used, as this procedure impairs analytes ionization and thus allows a better resolution and superior reproducibility of the retention times, as well as the minimization of peak tailing [76-78].

As commonly, the HPLC separation of *Mentha* and *Thymus* polyphenols has been achieved at constant flow rates of approximately 1mL/min and their identification and quantification has been frequently done by comparison of the retention times and integrated peak areas of the separated compounds, to those of the corresponding reference compound [34, 38-40, 43, 45, 46]. This information has also been combined to spectral information gathered by photodiode array detector (PDA) [1, 2, 34, 37, 40, 43, 52, 69, 70]. Spectral data in those studies has been obtained in the range of 200 to 450 nm, while the chromatograms have been plotted according to their maximum absorbance peaks at 280 nm for flavanones and hydroxybenzoic acids, at 320-330 nm for hydroxycinnamic acids and flavones and at 350-370 nm for flavonols [34, 40, 45, 48]. Alternatively, in case of exclusive usage of UV-Vis detector, the polyphenolic profiles were only recorded at a wavelength of 280 nm [36, 39, 42, 49, 54, 63].

However, due to commercial unavailability of many phenolic plant constituents, fine analytical techniques have also been implemented in order to improve the phenolic characterization. In this field, mass spectrometry has been playing a crucial role, as its coupling to chromatographic analysis allowed an increment on the sensitivity and selectivity of the method. HPLC fractionation combined with electrospray ionization-MS/MS analyses have been used e.g. by Krzyzanowska and colleagues [2] for structural determination of phenolic acids, flavones and flavanones in two species of *Mentha* [2, 73]. Since these procedures entails a long time of analysis, the present implementation of faster and reliable analytical methodologies, as e.g. the chromatographic techniques hyphenated with mass spectrometry appears as a good alternative. On-line LC–MS/MS analysis has been used in the identification of phenolic acids and distinct classes of flavonoids in *M. pipperita* L. [72], as well as in several *Thymus* species [35, 37, 50, 70], between others. In the majority of these studies, mass spectrometry analysis was performed using electrospray ionization (ESI), a soft mode of ionization that is suitable for structural characterization of a high number of polar biomolecules, in particular the phenolic compounds [35, 43, 72]. Moreover, the mass spectrometry analysis has been mainly carried out in the negative ion mode, due to its high sensitivity in detecting distinct classes of phenolic compounds [79].

Besides mass spectrometry, NMR spectroscopy has played an important role in structural analysis of polyphenols in *Mentha* [39, 56, 60, 62, 66] and *Thymus* [54, 55, 57, 68, 71] plants, coupled with other techniques as LC or MS. The main drawback in NMR is its low sensibility when compared to MS and thus, there is the need of getting higher amounts of sample for analysis [80]. In this sense, when using NMR technique, samples need to be obtained by preparative chromatography, as done for *Mentha* [51, 62] and *Thymus* [57]. A good alternative is the coupling of HPLC with RMN techniques (LC-NMR) that actually appears as the most powerful method for the separation and structural determination of organic compounds. Regardless of its efficiency for identification of on the nature of the polyphenol skeletons and on their substitution patterns, the method is not widely used at present due the high entailed costs [77].

5. CONCLUDING REMARKS

Mentha and *Thymus* species have been mostly studied for their oil composition. However, the close association of some of their beneficial properties with their content in phenolic compounds has encouraged the search on these latter metabolites. So far, the reported data allows us to conclude that plants of *Mentha* and *Thymus* genera are mostly rich in caffeic acid derivatives. *Thymus* plants are also particularly rich in glycosidic forms of the flavones luteolin and apigenin while glycosidic derivatives of eriodictyol and naringenin are particularly abundant in *Mentha* species. On the contrary, flavonols and dihydroflavonol are less described in these genera. Despite the considerable number of studies focusing on *Mentha* and *Thymus* polyphenols, it must be noted that to date, only one third of *Mentha* species have been studied and a diminutive number of *Thymus* (less than 5% of total known species) have been investigated. Thus besides summarizing the existing information on this theme, we hope that the present manuscript also encourages the search of the missing data on these genera.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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