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The chemical composition of the pharmacologically active *Thymus* species, its antibacterial activity against *Streptococcus mutans* and the antiadherent effects of *T. vulgaris* on the bacterial colonization of the *in situ* pellicle

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ABSTRACT

The pharmacological active genus *Thymus* L. comprises over 200 species. Besides its traditional pharmacological use, thyme may reduce the risk of caries disease, however, there is very little respective literature. The pharmacological effects can be attributed to the secondary plant metabolites. The composition of the essential oil and the polyphenols is important for the evaluation of the pharmacological activity. Nevertheless, there are no studies regarding a comparative analysis of the different pharmacological thyme species. In the present study, four different pharmacology *Thymus* species were cultivated under comparable conditions, and the volatile compounds as well as the polyphenols were characterized. In addition, the *in vitro* antibacterial activity against *S. mutans*, one of the primary cariogenic bacterial species, as well as of the essential oil and of the polyphenols were investigated. Furthermore, the bacterial viability and its effect on the initial bacterial adhesion under oral conditions were evaluated *in situ* for the essential oil and the polyphenols. By GC–MS, 69 volatile compounds, and by LC-DAD-MS/MS, 46 polyphenols could be identified. The comprehensive examination of the essential oils and the polyphenols revealed that the main compounds were equal. However, the yield of the essential oil and the polyphenol content differed clearly. The essential oils of the four investigated *Thymus* species exhibited an antibacterial activity against *S. mutans in vitro*, in contrast to the polyphenols of *T. vulgaris*. Rinsing with polyphenol-rich infusions reduced the initial bacterial colonization while the essential oil inhibited the bacterial growth on dental enamel *in situ*.

1. Introduction

The genus *Thymus* L. (thyme) of the Lamiaceae family comprises over 200 different species [1,2]. The *Thymus* species have wide application in medicine as well as in food seasoning and flavoring, but only a few species are economically important, particularly those used as spices such as the leaves of *T. vulgaris* and *T. zygis* [3,4]. Other important thyme species are also those with known pharmacological activity. According to the European Pharmacopoeia [5] these include Thymi herba and Serpylli herba. Thymi herba comprises the two Mediterranean species *T. vulgaris* and *T. zygis* [2,6], whereas Serpylli herba is comprised of *T. pulegioides*, *T. alpestris*, *T. pannonicus*, *T. oenipontanus*, *T. glabrescens*, *T. praecox*, and *T. serpyllum* seven species from Central Europe or Asia [6–8]. However, according to Schmidt [8], not all seven species of the Serpylli herba group are suited for medical usage equally well. Thymi herba and Serpylli herba are used for the treatment of catarrhs of the upper respiratory tract, bronchitis, and pertussis [9]. Furthermore, thyme also has antioxidative properties [10–13] and antimicrobial activities against a wide range of gram-positive and gram-negative bacteria, yeast, and fungi [4,14–19]. These versatile pharmacological effects can be attributed to the secondary plant metabolites, especially to essential oil and polyphenols.

The composition of the essential oil in the genus *Thymus* L. varies widely. In the pharmacological relevant *Thymus* species of Thymi herba and Serpylli herba, monoterpenes and sesquiterpenes dominate with a content of about 80% [15,16,20–22] while non-terpenoid aliphates, benzene derivatives, and phenylpropanoids have been found only sporadically [4]. Despite the numerous compounds known for essential thyme oil, there are only a few substances with a content higher than 10%. In particular, these are for the pharmacologically relevant *Thymus* species monoterpenes such as thymol, carvacrol, p-cymene, γ-terpinene, and β-linalool, whereas sesquiterpenes like β-caryophyllene and

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β-bisabolene are characteristic for Serpylli herba [15–17,19–32]. However, the essential oil of *Thymus* L. possesses an interspecific as well as a high intraspecific variability; therefore, different chemotypes exist for each species. For *T. vulgaris*, *e.g.*, six different chemotypes were described [4,33,34].

The polyphenols have been examined far less than the essential oils. In many thyme species, rosmarinic acid is the main component [35–43]. In addition, derivatives of flavones such as apigenin and luteolin or flavanones such as eriodictyol, naringenin, and hesperetin as well as salvianolic acids were identified [39,42,44,45].

Due to the antibacterial activity of thyme, the possible applications go far beyond traditional pharmacological use. Like many other plants rich in secondary plant substances, *e.g.*, *Cistus* [46], *T. vulgaris* as well as other *Thymus* species have the potential for application in preventive dentistry. Azuma et al. [47], Ohara et al. [48], and Nikolić et al. [49] already examined the positive antibacterial effects of the essential oil against *Streptococcus mutans*, one of the primary cariogenic bacterial species, *in vitro*. However, in these studies, only *T. vulgaris* and *T. serpyllum* were considered. The literature on the *in vitro* antibacterial activity of the polyphenols gained from *T. vulgaris* is insufficient. In contrast to the studies by Babpour et al. [50] and Ohara et al. [48], Hammad et al. [51] described an antibacterial effect against *S. mutans*. Hence, further examinations are needed. Furthermore, caries is caused by pathogenic oral biofilms, based on bacterial adhesion to non-shedding surfaces. Until now, only a few studies have reported on the reduction of bacterial adhesion due to the polyphenols of *T. vulgaris* [51,52], but no studies are available on the essential oil.

Knowledge about the composition of the secondary plant metabolites is important for the evaluation of pharmacological activity. Nevertheless, there are no studies concerning a comparative analysis of the different pharmacological *Thymus* species. The objective of the present study was to examine the essential oil as well as the entire spectrum of the polyphenols in four different pharmacological *Thymus* species. Therefore, *T. vulgaris*, *T. zygis*, *T. serpyllum*, and *T. pulegioides* were chosen and cultivated under comparable conditions. The essential oil was extracted by traditional steam distillation according to the guidelines of the European Pharmacopoeia [5], and the volatile compounds of an ethyl acetate extract were characterized by GC–MS. For the extraction of the polyphenols, an aqueous infusion was prepared, and the extracts were analyzed by LC-DAD-MS/MS. In addition, the *in vitro* antibacterial activity of the essential oil of the four different thyme species against *S. mutans* was examined. For this purpose, three different *in vitro* test systems were adopted, including turbidity measurements, the determination of colony-forming units (CFU) as well as a live/dead staining method (BacLight®). In addition, the minimum inhibitory concentration and the antibacterial activity of individual compounds were determined. Furthermore, the *in vitro* antibacterial activity of the polyphenols of *T. vulgaris* against *S. mutans* was investigated by turbidity measurements. Additionally, the effect on the initial bacterial adhesion under oral conditions as well as the effect on the viability of the adherent bacteria were evaluated for the essential oil and the polyphenols of *T. vulgaris* in an *in situ* experiment.

2. Results and discussion

For the current study, four pharmacological *Thymus* species including *T. vulgaris*, and *T. zygis* (Thymi herba) as well as *T. serpyllum*, and *T. pulegioides* (Serpylli herba) were cultivated and harvested under comparable conditions.

2.1. Characterization of essential oil compounds

The essential oils of the leaves of the four investigated pharmacological thyme species were extracted by steam distillation. The essential oil content in Thymi herba with yields of 23.0 ml/kg for *T. vulgaris* and 21.9 ml/kg for *T. pulegioides* was higher than in Serpylli herba with

Table 1
Chemical composition (%) of *T. vulgaris*, *T. zygis*, *T. serpyllum*, and *T. pulegioides* volatile compounds.

Compounds	*RI*	*T. vulgaris*	*T. zygis*	*T. serpyllum*	*T. pulegioides*
α-Pinene[a]	934	+	+	+	+
Camphene	948	+	+	+	+
Sabinene	976	0.1	0.2	0.2	0.3
β-Pinene[a]	978	0.2	0.2	0.2	0.3
1-Octen-3-ol[a]	981	1.9	0.3	1.7	1.5
3-Octanon	989	0.1	0.3	0.2	1.4
β-Myrcene[a]	992	1.4	0.8	0.8	0.6
3-Octanol	996	0.3	0.2	0.3	1.0
α-Phellandrene	1005	0.3	0.2	0.2	0.2
3-Carene[a]	1011	0.2	0.1	0.2	0.2
α-Terpinene	1018	1.2	0.5	0.4	0.4
p-Cymene[a]	1026	7.5	5.6	6.3	5.6
D-Limonene[a]	1030	0.7	0.6	0.5	0.4
Eucalyptol[a]	1033	0.5	0.3	0.5	0.6
β-*trans*-Ocimene	1040	0.7	0.4	0.5	
β-cis-Ocimene	1050	0.1	0.1	0.2	
γ-Terpinene[a]	1061	10.4	6.9	13.7	4.8
Geranyl-isobutyrate	1068	0.2	0.3		
β-Terpineol[a]	1070	2.0	1.9	1.2	0.8
Terpinyl-acetate	1073	0.2	0.1	0.5	0.2
Linalool oxide	1091	0.2	0.4	0.3	0.3
β-Linalool[a]	1100	4.2	5.5	1.3	0.7
Camphor[a]	1150	0.6	4.2	1.1	0.2
Borneol[a]	1171	0.6	4.2	1.1	1.3
Isoborneol	1177		0.4	0.1	
Terpinen-4-ol[a]	1182	0.5	0.6	0.3	0.3
p-Cymene-8-ol	1191			0.1	0.2
α-Terpineol	1195	0.3	0.4	0.2	0.3
Dihydrocarvone	1201	0.1	0.3	< 0.1	
Guaiol	1222	0.1	0.1		
Bornyl formate	1236				0.3
Thymol methyl ether	1238	2.5	0.6	1.1	4.2
cis-Citral	1246	0.1			
Carvacrol methyl ether	1248	2.8	0.9	3.1	3.6
Thymoquinone[a]	1256	0.3	0.3	0.9	0.7
p-Thymol	1289	0.5	0.5	< 0.1	0.3
Bornyl acetate	1292		0.2	< 0.1	
Thymol[a]	1298	37.7	41.7	13.7	44.5
Carvacrol[a]	1306	5.0	6.2	13.6	3.2
p-Menth-2.4(8)-diene	1315	0.6	0.7	0.8	0.7
γ-Elemene	1358			2.1	
Thymol acetate	1359	0.4	0.4	0.4	0.5
α-Cubebene	1386	0.2	0.1	0.1	0.1
Geraniol acetate[a]	1387	1.2	1.0	0.4	0.3
β-Bourbonene	1396				0.1
γ-Gurjunene	1411		0.2	0.2	
α-Gurjunene	1423		0.2	0.2	
β-Caryophyllene[a]	1433	6.3	3.4	2.7	6.2
Aromadendrene	1441	0.4	0.2	0.2	0.4
Contamination	1453	+	+	+	
α-Caryophyllene[a]	1467	0.5	0.4	2.0	0.7
Alloaromadendrene	1475	0.4	0.4	1.3	
α-Amorphene	1487	0.5	0.3	0.4	0.6
β-Cubebene	1494	0.2	0.1	2.2	1.0
Farnesol	1511	0.3	0.5	3.6	0.1
β-Bisabolene[a]	1517			8.5	7.0
γ-Cadinene	1527	0.8	0.4	0.3	0.5
δ-Cadinene	1535	0.8	0.7	0.8	0.4
Viridiflorene	1550	0.2	0.3	0.3	
Elemol	1559			3.6	
Germacrene-4-ol	1592		0.1	0.1	
Spathulenol	1593		0.4	1.1	0.2
Caryophyllene oxide[a]	1599	0.7	0.5	0.5	1.2
Cubenol	1630	0.2			
γ-Eudesmol	1637	0.3		0.1	
τ-Cadinol	1654	1.0	0.2	0.1	
α-Cadinol	1669	0.2		0.7	
β-Eudesmol	1670			1.0	
Ledol	1711		0.8		
Total identified		98.1	96.7	98.0	97.7
Monoterpene hydrocarbons		23.8	16.1	23.7	13.0
Oxygenated		60.0	70.0	39.9	62.7

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