

Identification and semi-quantification of sesquiterpenes and sesquiterpenoids from Chamomile, Hop, Lavender, Basil and Lemon balm

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Abstract

Sesquiterpenes are naturally occurring compounds with 15 carbon atoms and are formally composed of three isoprene units. Together with their derivatives, the sesquiterpenoids represent one of the major substance classes of essential oils. As essential oils are often attributed with positive effects on human health, these compounds are interesting targets for physiological and biochemical investigations. The here investigated chamomile (*Matricaria chamomilla* L.), hop (*Humulus lupulus*), lavender (*Lavandula angustifolia*), basil (*Ocimum basilicum*) and lemon balm (*Melissa officinalis*) are famous members of plants with highly acknowledged beneficial health effects. To study these effects, sesquiterpenes and sesquiterpenoids (SQTs) as major constituents need to be studied more intensely. The here described tentative identification and quantification complement the existing literature by adding a concrete quantitative dimension to these valuable substances in the five plants, and help to further elucidate the contribution of SQTs to the overall properties of essential oils.

Keywords: GC-MS, solvent assisted flavour evaporation, internal standard, isolation, essential oils

Introduction

Essential oil-bearing plants are popular remedies used in traditional medicine. As they often have fewer complications compared to modern pharmaceutical drugs, they are very commonly applied. Many psycho- and physiological effects have been discovered, and the quest for new naturally occurring bioactive substances is still ongoing. Furthermore, we could recently show that sesquiterpenes and sesquiterpenoids (SQTs) can have a high influence on the overall aroma of natural materials [1], revealing the ability of SQTs to have a huge impact even at trace levels. For biochemical and physiological investigations, often the whole essential oil is administered. This may, however, obscure the effects of single compounds. Additionally, as naturally occurring substances like the SQTs are structurally very demanding, only few are synthetically accessible, and are therefore not investigated so far. In this study, we want to complement the research on SQTs and on their identification and semi-quantification. Most investigations only consider a comparison of the peak areas determined by gas chromatography – mass spectrometry (GC-MS), and utilise these for an approximated quantification. Here we report the tentative identification together with semi-quantification based on internal standards.

Experimental

Plant materials and chemicals

Dried chamomile flower heads, dried hop cones and dried lavender flower heads were obtained from a local store (Erlangen, Germany). Fresh samples of basil and lemon balm were purchased from Bioland Kräuter Gut Dworschak-Fleischmann (Nürnberg, Germany). Dichloromethane (DCM, purified by distillation before usage) and sodium sulphate were purchased from VWR (VWR International Radnor, PA, USA). α -Bisabolol, α -humulene, (+)-nootkatone and the series of *n*-alkanes were purchased from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO, USA).

Extraction and distillation

The dried plant materials were finely chopped (Kenwood mini chopper CH180, 300 W, Kenwood Limited, Havant, United Kingdom) until a powder or fine needles, respectively, were obtained. The leaves of the fresh samples were cut into small pieces. Subsequently, dependent on the plant material, different amounts (ranging from 0.5 g to 5.0 g) of the samples were extracted with DCM (50 mL, 30 min) and were dried over sodium sulphate. The resulting extracts were applied for solvent assisted flavour evaporation (SAFE) [2] at 60 °C under vacuum. Finally, the distillates were reduced by Vigreux and micro distillation to 100 μ L (250 μ L for lavender and 200 μ L for hop) [3].

Analysis by GC-MS

The analyses for the identification and quantification were performed on a GC 6890 coupled with a 5973 MSD (Agilent Technologies, Santa Clara, CA, USA). The system was equipped with a Multipurpose Sampler 2 and a CIS 3 injection system (GERSTEL GmbH & Co. KG, Mülheim an der Ruhr, Germany). A DB-5 capillary column was used (30 x 0.25 mm, film thickness 0.25 μ m), connected to an uncoated fused silica capillary pre-column (3 m x 0.53 mm; Agilent J&W Scientific, Santa Clara, CA, USA). The following oven temperature program was used following the instructions by Adams [4]: 60 °C until 246 °C with a temperature ramp of 3 °C/min. A split of 20:1 was used to apply 2.0 μ L of sample (for the dried samples a 1:10 dilution of the original distillate was prepared for the measurements). The helium carrier gas stream was set to 1 mL/min. EI mass spectra were recorded at 70 eV in TIC mode (40-400 amu).

Tentative identification of SQTs

The tentative identification was based on the following criteria: The comparison with the linear retention index (RI) on a DB-5 capillary column and the matching with literature mass spectra. For both, the literature [4] or the NIST mass spectral library (version 2.0, National Institute of Standards and Technologies, Gaithersburg, MD, USA) were consulted. For the RI a difference lower than 15 (value compared to literature values) was required for the tentative identification. For the matches with the NIST a value of >800 was taken as criterion. The following SQTs could not be identified and remain unknown: Chamomile RIs: 1388, 1410, 1479, 1506, 1554, 1580, 1583, 1587, 1590, 1605, 1608, 1614, 1630, 1646, 1659, 1674 and 1718; hop RIs: 1382, 1507 and 1629; lavender RIs: 1443, 1498, 1542, 1589, 1620, 1668 and 1692; basil RIs: 1418 and 1482.

Semi-quantification of SQTs

For the semi-quantification, an internal standard (ISTD, (+)-nootkatone) was used to cover the loss during the workups and measurements. Additionally, two calibration standards (CSTD) were used for the calculation of the concentration of sesquiterpenes (α -humulene) and sesquiterpenoids (α -bisabolol). A 5-point calibration curve was prepared for both CSTDs by plotting the peak area CSTD/ISTD against the concentration of the respective CSTD, while varying the concentration of the CSTD and keeping constant the concentration of the ISTD (α -humulene: $y = 0.0422x + 0.0097$, $R^2 = 0.9992$, α -bisabolol: $y = 0.0297x - 0.0935$, $R^2 = 0.9985$). Each sample was worked up twice and the final values were obtained by calculating the mean value.

Results and discussion

In Table 1, the results of the tentative identification and semi-quantification are summarised. In total 52 SQTs could be tentatively identified in the investigated plant materials. Our findings show that the volatile fractions, especially for the dried samples, reveal high concentrations of SQTs. In contrast, the fresh samples contained a lower amount, which can be explained by the higher content of water. Our findings are in line with earlier reported values from the literature. Interestingly, within the individual plant materials many SQTs are related in terms of their structure. For instance, chamomile contains several SQTs which are derived from α -bisabolol, e.g., α -bisabolol oxide B, α -bisabolone oxide A or α -bisabolol oxide A. In contrast, there are also SQTs which could be tentatively identified in several of the investigated plant materials, e.g., β -caryophyllene, α -humulene or γ -muurolene, which are well researched members of the SQTs family.

Table 1: Compounds tentatively identified (RI: retention index on DB-5) and semi-quantified in the investigated plants; values of the semi-quantification are given in ppm; tr = traces.

N°	RI (DB-5)	Compound	Chamomile	Hop	Lavender	Basil	Lemon balm
1	1345	α -Cubebene		6.3			
2	1348	α -Longipinene	tr				
3	1368	α -Ylangene		28.3			
4	1374	Cedrene	tr				
5	1374	α -Copaene		99.0		0.1	
6	1380	Modhephene	0.2				
7	1405	α -Gurjunene	tr				
8	1416-18	β -Caryophyllene	0.5	1786.1	190.5		3.7
9	1427	β -Gurjunene		72.3			
10	1428	β -Copaene	tr				

Table 1 (cont.)

11	1431	α -trans-Bergamotene				6.9	
12	1436	Aromadendrene	tr				
13	1452	(E)- β -Farnesene	260.9		47.7	6.6	
14	1453-54	α -Humulene		4950.0		tr	0.1
15	1457-58	Alloaromadendrene	0.6				tr
16	1470	(E)-Cadina-1(6),4-diene		tr			
17	1473-79	γ -Muurolene	tr	176.2	26.1	2.5	
18	1477	α -Amorphene		18.4			
19	1488-1506	Valencene	0.3			0.3	
20	1493	Bicyclogermacrene	13.6			0.5	
21	1494	α -Selinene		41.6			
22	1496	α -Muurolene		36.7			
23	1499	α -Bulnesene				0.2	
24	1504	(E,E)- α -Farnesene	tr				
25	1510-11	γ -Cadinene	tr	223.3	134.2	0.2	
26	1515-17	δ -Cadinene	tr	321.4			
27	1520	Zonarene		48.6			
28	1521-23	β -Sesqui-phellandrene	tr			0.1	
29	1530	(E)-Cadina-1,4-diene		34.2			
30	1534	α -Cadinene		39.3			
31	1539	α -Calacorene		18.3			
32	1556	Germacrene B		tr			
33	1574	Spathulenol	140.1				
34	1579	Caryophyllene oxide		151.8	573.3		
35	1585	Gleenol		10.8			
36	1602	Globulol	7.0				
37	1606	Humulene epoxide II		570.5			
38	1612	1,10-di-epi-Cubenol		24.9			
39	1613	Cubenol			23.2		
40	1625	1-epi-Cubenol		32.3			
41	1632	(Z)-Cadin-4-en-7-ol		87.2			
42	1639-41	epi- α -Cadinol	8.2	83.8	145.2	1.1	
43	1645	α -Muurolol		12.8			
44	1653	α -Eudesmol				0.8	
45	1653	α -Bisabolol oxide B	464.7				
46	1654	α -Cadinol		55.8			
47	1679	α -Bisabolone oxide A	317.0				
48	1682	cis-14-nor-Muuro-5-en-4-one			26.9		
49	1684	α -Bisabolol	29.3				
50	1688	Eudesma-4(15),7-dien-1 β -ol	10.5				
51	1709	(Z)- β -Santalol			40.6		
52	1746	α -Bisabolol oxide A	622.5				

Conclusion

SQTs are important compounds present in foods, plants and essential oils and thereby contribute to their respective properties. Chamomile, hop, lavender, basil and lemon balm were chopped and extracted with DCM. After solvent assisted flavour evaporation, the distillates were investigated by means of GC-MS. For the semi-quantification, an ISTD was used and CSTDs enabled the calculation of the respective amounts. The highest amounts of SQTs were detected for the dried samples; especially, hop was found to be rich in α -humulene (4950.0 ppm) and β -caryophyllene (1786.1 ppm). Both compounds were recently characterised to undergo structural changes during digestion in the human body [5]. For chamomile and lavender, the highest amounts were found for α -bisabolol oxide A (622.5 ppm) and caryophyllene oxide (573.3 ppm) respectively. The here reported values complement existing literature and may help to promote research dealing with single administered SQTs in biochemical and physiological assays. We aim to test several of the here investigated SQTs in terms of their neurotropic interaction on the human GABA_A Receptor [6].

References

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