



Modulatory effect of various essential oils on different GABA_A receptor subtypes present in the central nervous system

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ABSTRACT

Introduction: Essential oils (EOs) are complex mixtures with different application areas in human medicine and health. Some harbor calming and anxiolytic effects, which has been attributed to GABA_A receptor (GABA_AR) modulation. Although GABA_AR modulation has been demonstrated, most studies do not discriminate between different GABA_AR subtypes present in the central nervous system.

Objectives: We determined the modulatory effect of five EOs (lavender (*Lavandula angustifolia* MILL.), basil (*Ocimum basilicum* L.), lemon balm (*Melissa officinalis* L.), hops (*Humulus lupulus* L.) and chamomile (*Matricaria chamomilla* L.)) on recombinant $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ GABA_AR and characterized the chemical composition of the EOs.

Methods: The chemical composition of the EOs was investigated by gas chromatography coupled with mass spectrometry. EOs were functionally investigated on transfected HEK293 cells with GABA_AR by patch-clamp whole-cell recordings.

Results: The chemical analysis revealed mono- and sesquiterpene substances being main constituents, yet no specific common compound being present at relevant levels in all five oils. All EOs investigated, displayed negative modulation of GABA_AR lacking the $\gamma 2$ subunit. For GABA_AR containing the $\gamma 2$ subunit, three oils (lavender, basil and chamomile) exhibited positive modulation of up to 98 % increased GABAergic responses while lemon balm and hops showed no significant effect.

Conclusion: EOs which have not been investigated yet for their total modulatory potential on GABA_AR exhibit specific receptor subtype dependent allosteric modulation. This modulatory potential of EOs together with its chemical identification offers a tool for the design of future studies with individual compounds or defined ratios of compounds.

Introduction

Nature produces an amazingly rich and diverse range of natural products, many of which have a positive effect on humans. Compared to the total number of natural products, EOs represent only a small fraction

of these, yet being appreciated since ancient times (Mertens et al., 2009). However, EOs are complex mixtures of compounds with a variety from only a few up to far more than hundreds of substances (de Groot and Schmidt, 2016b). The most commonly used inexpensive, easy to perform and highly reproducible method for the extraction of EOs is

Abbreviations: BZDs, benzodiazepines; CNS, central nervous system; EOs, essential oils; GABA, γ -aminobutyric acid; GC-MS TIC, gas chromatography-mass spectrometry total ion current; MOTs, monoterpenes and monoterpenoids; SQTs, sesquiterpenes and sesquiterpenoids; TM, transmembrane domain.

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steam distillation (Zhou et al., 2023). The components of the oils generally have molecular weights below 300 Da and are usually lipophilic (de Groot and Schmidt, 2016a). EOs share a high therapeutic potential due to their anti-inflammatory (α -pinene), anti-nociceptive (thyme), anti-bacterial (neral), anti-depressive and anxiolytic effects (coriander, lemon) (Abuhamdah et al., 2008; Chioca et al., 2013; Costa et al., 2011; Dhifi et al., 2016; Seol et al., 2010; Wang and Heinbockel, 2018). The underlying molecular pathways altered by EOs are not understood, yet. EOs have been demonstrated to modulate several classes of ion channels and their subsequent signaling pathways in the central nervous system (Wang and Heinbockel, 2018). Alterations of signal transduction pathways in the central nervous system have been delineated for EOs of e.g. chamomile, lavender, rose, clary sage, lemon.

The most important substance classes of EOs are terpenes, monoterpenes and monoterpenoids (MOTs), sesquiterpenes and sesquiterpenoids (SQTs) as well as the phenylpropanoids (Moghaddam and Mehdizadeh, 2017). In general, there are two biosynthetic pathways for dimethylallyl pyrophosphate and isopentenyl pyrophosphate, the two precursors of terpenes and terpenoids. The methylerythritol phosphate pathway thereby takes place in the chloroplasts whereas the mevalonate pathway occurs in the cytoplasm. Both routes are inter-linked since there is exchange and/or cooperation between these pathways (Maffei et al., 2011; Nagegowda, 2010). Phenylpropanoids are synthesized via the Shikimate pathway (Hüsni Can Baser and Buchbauer, 2020). The phytochemical profile of EOs, however, is influenced by numerous factors e.g. plant cultivation, harvest, production condition, storage (de Groot and Schmidt, 2016b).

A crucial aspect regarding potential pharmacological effects of EOs is the interaction of the individual components. The biological effect of a certain amount of a pure substance is commonly divergent if other accompanying substances are present, potentially resulting in antagonistic, additive or synergistic effects (Hemaiswarya et al., 2008). For example, the MOTs linalyl acetate, terpineol and camphor showed little or no activity against two human colon cancer cell lines as individual substances whereas in combinations moderate activity was observed: 33–45 % reduction in proliferation when combining linalyl acetate and terpineol and even 50–64 % for combination of all three substances (Itani et al., 2008). These examples illustrate that compound mixtures such as EOs may be more effective as pharmaceuticals than single substances. This aspect needs to be considered in view of the fact that calming and anxiolytic effects have been shown for specific EOs by the modulation of GABA_ARs in the central nervous system (CNS). Moreover, the presence of a hydroxyl-group at single investigated constituents of EOs seems to be beneficial for GABA_AR modulation (Wang and Heinbockel, 2018).

γ -aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the CNS. It acts through the ligand-gated GABA_ARs and G-protein-coupled GABA_BRs. GABA_ARs are chloride ion permeable channels which consist of five subunits (Lavery et al., 2019; Olsen and Sieghart, 2009; Tretter et al., 1997). Every subunit has a large amino- and a small C-terminus domain (both are extracellular), four transmembrane domains (TMs), and a large intracellular domain between TM3 and TM4. Most GABA_ARs are formed by two α , two β and one γ or δ subunits (Fritschy et al., 1992; Miller and Aricescu, 2014; Pirker et al., 2000). The most common synaptic GABA_AR composition harbors $\alpha 1\beta 2\gamma 2$ subunits enabling fast synaptic neurotransmission, while extrasynaptic receptors which are important for tonic inhibition have been described as $\alpha 4/6\beta 3\delta$ or $\alpha 1\beta 2$ configurations (Kasaragod et al., 2022; Mortensen et al., 2011). Two orthosteric binding sites for the neurotransmitter GABA are located in the extracellular amino domains at the interface of β - α subunits (Masiulis et al., 2019). GABA_ARs share a high potential of modulation and are thus interesting as clinical targets. Different positive and negative allosteric modulators have been described which range from sedatives, sleep aids and anticonvulsants to anesthetic agents. Negative allosteric modulators of GABA_ARs are able to act anxiogenic or as convulsant (Olsen, 2018). The most well-known

modulation of GABA_ARs is positive allosteric modulation by benzodiazepines (BZDs) (Walters et al., 2000; Zhu et al., 2022). One classical binding site of BZDs is located at the junction of the α - γ subunits. However, recent data from cryo-electron microscopy structures also identified an alternative binding site for BZDs localized within the transmembrane segment of the receptors (Masiulis et al., 2019; Zhu et al., 2022). Similarly, modulation of GABA_ARs by neurosteroids has been characterized within the transmembrane segment changing the orientation of the TMs to each other and thus influencing ion channel opening and closing (Sun et al., 2023). Many efforts have been made to identify further specific GABA_AR modulators similar in potency to BZDs, yet without sedative properties. Single substances sharing structural properties of BZDs or neurosteroids, but also plant extracts, have been shown to significantly alter GABA_AR function (Aoshima and Hamamoto, 1999). Substances present in many plant extracts which bear high modulatory potential on GABA_ARs are mono-, sesqui- and diterpenes. Single sesquiterpenes have been shown to either positively or negatively modulate GABA_AR function depending on the subunit composition (Janzen et al., 2021). In the current study, we investigate the modulatory activity of EOs harboring distinct ratios of mono-, sesqui- and diterpenes acutely applied on two recombinant GABA_AR subtypes. Investigation of those GABA_AR subtypes will help to unravel mechanisms underlying functional specificity and physiological relevance of EOs.

Material and methods

EOs and chemicals

Lavender (*Lavandula angustifolia* MILL.) and basil (*Ocimum basilicum* L.) EO (100 %) were obtained from Bergland-Pharma (Heimertingen, Germany). Hops (*Humulus lupulus* L.) and lemon balm (*Melissa officinalis* L.) EO (100 %) were purchased from Ayus (Bühl, Germany). Chamomile (*Matricaria chamomilla* L.) EO (100 %) was obtained from Caesar & Loretz (Hilden, Germany). Dichloromethane (purified by distillation before usage) was from VWR International (Radnor, PA, USA). Reference substances for identification (purity in brackets in %): α -pinene (97), β -pinene (≥ 97), 3-octanone (≥ 98), 6-methyl-5-hepten-2-one (99), myrcene (technical), limonene (96), 1,8-cineole (99), *trans*- β -ocimene (≥ 90), linalool (97), citronellal (≥ 95), terpinen-4-ol (≥ 95), α -terpineol (96), nerol (≥ 97), neral (96), geraniol (98), linalyl acetate (≥ 97), geranial (96), bornyl acetate (≥ 99), neryl acetate (≥ 98), geranyl acetate (98), caryophyllene (≥ 80), α -humulene (≥ 95), (*E*)- β -farnesene (≥ 90), caryophyllene oxide (≥ 95), (*S,S*)- α -bisabolol (≥ 93), α -bisabolol oxide A (≥ 95) were from Sigma Aldrich (Darmstadt, Germany), *p*-cymene (99.5), camphor (95), borneol (≥ 99), estragole (98.5), eugenol (99) from Fluka (Charlotte, NC, USA), sabinene (98.5) from Carl Roth (Karlruhe, Germany), and germacrene D from Chemos (Altdorf, Germany). α -Bisabolone oxide A (94) was isolated from *Matricaria chamomilla* L. by B. Slavik (Slavik et al., 2021).

Preparation of the EOs

The pure EOs were diluted in CH₂Cl₂ until a concentration of 0.2mg/mL was reached.

GC-MS analysis

The analyses for tentative identification of the constituents of the EOs were performed on a GC 6890 (Agilent Technologies, Santa Clara, CA, USA) connected with a 5973 MSD (Hewlett Packard, Palo Alto, CA, USA). The system was equipped with a multipurpose sampler 2 and a Cooled Injection System 3 (GERSTEL, Mülheim an der Ruhr, Germany). A HP-5 ms capillary column was used (30 $\times$ 0.25 mm, film thickness 0.25 μ m), connected to an uncoated fused silica capillary column (3 $\times$ 0.53 mm; Agilent J&W Scientific, Santa Clara, CA, USA). The

temperature program was used following the instructions by Adams (Adams, 2017): 60 °C until 246 °C with a temperature ramp of 3 °C/min. 2.0 µL of sample were injected and a split ratio of 2:1 was set. The helium carrier gas stream was set to 1 mL/min. EI mass spectra were recorded at 70 eV in total ion current (TIC) mode (40–400 amu). A series of homologous alkanes (C₆–C₂₆) was measured to calculate the linear retention indices (RIs).

Tentative identification of the constituents of the EOs

The measured data were analyzed with the Thermo Xcalibur Qual Browser (version 4.2.47, Thermo Fisher Scientific Waltham, MA, USA). The peak detection algorithm ICIS was used to detect peaks. The algorithm was run with the default values, except that the manual noise region was used and set from 45 to 50 min, and the multiplet resolution was set to 5. Peaks with an area greater 100000 were further analyzed. Final peak identification was done by comparing literature mass spectra from the NIST mass spectral library (version 2.4, National Institute of Standards and Technologies, Gaithersburg, MD, USA) and RIs on a DB-5 capillary column measured and calculated by Adams (Adams, 2017). For matches with NIST a value greater 800 was required and for the RIs a difference lower than 10 (compared to the Adams (Adams, 2017)) was necessary for the tentative identification. If available, mass spectra and RIs from reference compounds using an in-house database built with the software AMDIS (Version 2.73, National Institute of Standards and Technology, Gaithersburg, MD, USA) were used for identification. Here, a net value greater than or equal 80 and a RI-RI(lib) value between –10 and 10 was required. The identification steps carried out for each peak are depicted in Table 1 (identification method column). The reference substances used with purity information are listed under EOs and chemicals.

Cell line

HEK293 cells (Human Embryonic Kidney cells; CRL-1573; ATCC – Global Bioresource Center, Manassas, VA, USA) were grown in minimum essential medium (Life Technologies, CA, USA) supplemented with 10 % fetal bovine serum, 200 mM L-glutamine and 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C and 5 % CO₂. 200.000 HEK293 cells were seeded on glass cover slips in 35 mm dishes and transfected after 24 h.

Transfection

HEK293 cells were transfected using a modified calcium phosphate precipitation method. Briefly, 1 µg/µL plasmid DNA (GABA_AR α1:β2 in a ratio of 1:1 or GABA_AR α1:β2:γ2S (γ2S = short variant) in a ratio of 2:2:1, both together with the green fluorescent protein, 0.1xTE buffer (Tris/EDTA), 2.5 M CaCl₂ and 2xHBS (50 mM HEPES, 12 mM glucose, 10 mM KCl, 280 mM NaCl, 1.5 mM Na₂HPO₄) were mixed and incubated for 20 min at room temperature before applying to the cells. Cells were washed after 4–6 h and used for electrophysiological measurements after 48 h.

Electrophysiological measurements

Patch-clamp whole-cell recordings were performed on transfected HEK293 at room temperature. Recording pipettes were pulled with a Sutter P97 horizontal puller (Sutter Instrument, Emmendingen, Germany) from borosilicate capillaries with open resistances of 3–6 MΩ and filled with internal buffer (in mM: 120 CsCl, 20 N(Et)₄Cl, 1 CaCl₂, 2 MgCl₂, 11 EGTA, 10 HEPES, pH 7.2, adjusted with CsOH). 10 µM GABA with or without 0.06 % EO was applied in external buffer (in mM: 137 NaCl, 5.4 KCl, 1.8 CaCl₂, 1 MgCl₂, 5 HEPES, pH 7.35, adjusted with NaOH) for 100 ms by the Octaflow II system (ALA Scientific Instruments, NY, USA). Peak current responses were obtained with a 500 ms delay after application. Current responses were amplified with an EPC-10

amplifier and measured at a holding potential of –60 mV using the Patchmaster Next software (HEKA Elektronik, Lambrecht, Germany). To verify expression of recombinant GABA_AR containing the γ2 subunit, 10 µM GABA with 10 µM ZnCl₂ were co-applied. Cells that showed inhibition of GABA currents with 10 µM ZnCl₂ were sorted into the pool of GABA_AR α1β2 lacking the γ2 subunit. The EOs were solved and diluted using ethanol. Electrophysiological recordings were performed at a GABA concentration of 10 µM in extracellular buffer containing 0.06 % of EOs and 0.6 % of ethanol or only 0.6 % ethanol as control condition. 10 µM GABA was again applied after the application of the different EOs. As representative of the EOs, lavender was also applied alone to exclude an agonistic effect by the EO itself. Moreover, lavender was also co-applied at saturating concentration of GABA (100 µM) followed by 10 µM of GABA and application of 10 µM GABA + 10 µM propofol as a known positive allosteric modulator.

Data analysis

All experiments were performed at least three times (biological replicates, *N* = 3) or as otherwise stated. Statistical analysis was done using the software GraphPad Prism10. Data are presented as individual values and means. Normality of the data was reviewed by Shapiro-Wilk normality test (α =0.05). Statistical significance was calculated using a paired *t*-test. The 0-hypothesis was rejected at a level of *p* < 0.05. Number of measured cells (*n*) and *p*-values are shown in the results part.

Data availability

Data that support the findings of this study are available from the corresponding author, upon reasonable request.

Results

Chemical composition of the EOs

The gas chromatography-mass spectrometry total ion current (GC–MS TIC) chromatograms of the five EOs lavender (*Lavandula angustifolia* MILL.), basil (*Ocimum basilicum* L.), lemon balm (*Melissa officinalis* L.), hops (*Humulus lupulus* L.) and chamomile (*Matricaria chamomilla* L.), all of which are supposed to be rich in MOTs and SQTs, are shown in Fig. 1. The results of the tentative identification of components are summarized in Table 1. The detailed identification procedure and the defined criteria are provided in the materials and methods chapter under tentative identification of the constituents of the EOs. A total of 83 compounds was detected, with 73 being tentatively identified; thereby, 21 of the 29 substances detected in the EO of hops, all 27 substances in basil and 22 substances in lavender, and 16 out of 18 substances from lemon balm were identified. Chamomile exhibited the lowest number of substances with 14 substances being successfully identified out of 14 detected compounds.

Indeed, the majority of detected compounds belonged to the classes of MOTs (27 substances) or SQTs (38 substances). A few other single compounds were from the diterpene family (*m*- and *p*-camphorene), phenylpropanoids (estragole and eugenol) and four were ketones or esters (3-octanone, 6-methyl-5-hepten-2-one, 1-octen-3-yl acetate and hexyl tiglate). Ten constituents could not be revealed due to an equivocal mass spectrum, but these were not among the substances with the highest peak areas. MOTs eluted in the range between 4 and 20 min, corresponding to RIs of 933 to 1385, followed by SQTs eluting between 18 and 38 min; RIs: from 1347 to 1739. The EO of lavender was dominated by MOTs, and hops by SQTs, whereas chamomile solely yielded SQTs. Lemon balm and basil contained equal shares of representatives from both classes, MOTs and SQTs.

Single MOTs and SQTs were generally detected in more than one oil, while substances from other classes were commonly only present in one of the oils investigated. For example, the two phenylpropanoids were

Table 1

Results of the GS-MS measurements and identification methods.

No.	RI (HP-5 ms)	Compound	class/group ^a	EO					Identification method ^b
				Lavender	Basil	Lemon balm	Hops	Chamomile	
1	933	α -Pinene	M		x				1,2,3
2	973	Sabinene	M		x				1,2,3
3	977	β -Pinene	M		x				1,2,3
4	986	3-Octanone	K	x					1,2,3
5	987	6-Methyl-5-hepten-2-one	K			x			1,2,3
6	992/991	Myrcene	M		x		x		1,2,3
7	1005	Pseudolimonene	M				x		1,2
8	1023	Cymene	M		x				1,2,3
9	1027	Limonene	M	x	x				1,2,3
10	1029/1030	1,8-Cineole	M	x	x				1,2,3
11	1035	(Z)- β -Ocimene	M	x					1,2
12	1046	(E)- β -Ocimene	M	x					1,2,3
13	1101	Linalool	M	x	x	x			1,2,3
14	1112	1-Octen-3-yl acetate	E	x					1,2
15	1141/1142	Camphor	M	x	x				1,2,3
16	1147	Unknown	–			x			–
17	1151	Citronellal	M			x			1,2,3
18	1163	Borneol	M	x					1,2,3
19	1166	Lavandulol	M	x					1,2
20	1175	Terpinen-4-ol	M	x	x				1,2,3
21	1189	α -Terpineol	M	x	x				1,2,3
22	1197	Estragole	P		x				1,2,3
23	1227	Nerol	M			x			1,2,3
24	1239	Neral	M			x			1,2,3
25	1254	Geraniol	M			x			1,2,3
26	1256	Linalyl acetate	M	x					1,2,3
27	1270	Geranial	M			x			1,2,3
28	1284	Bornyl acetate	M		x				1,2,3
29	1291	Lavandulyl acetate	M	x					1,2
30	1324	Methyl geranate	M			x			1,2
31	1331	Hexyl tiglate	E	x					1,2
32	1347	α -Cubebene	S		x				1,2
33	1355	Eugenol	P		x				1,2,3
34	1365	Neryl acetate	M	x					1,2,3
35	1371/1372	α -Copaene	S		x	x	x		1,2
36	1384/1385	Geranyl acetate	M	x		x			1,2,3
37	1386	β -Cubebene	S		x				1,2
38	1388	7- <i>epi</i> -Sesquithujene	S	x					1,2
39	1389	β -Elemene	S		x				1,2
40	1402	Isocaryophyllene	S				x		1,2
41	1414	Caryophyllene	S	x	x	x	x		1,2,3
42	1416	α -Santalene	S	x					1,2
43	1423	Unknown	–				x		–
44	1433	(E)- α -Bergamotene	S		x				1,2
45	1433	Aromadendrene	S				x		1,2
46	1445	Unknown	–				x		–
47	1447/1448	α -Humulene	S		x	x	x		1,2,3
48	1454	Alloaromadendrene	S				x		1,2
49	1457	(E)- β -Farnesene	S	x				x	1,2,3
50	1458	Unknown	–				x		–
51	1472	γ -Murolene	S				x		1,2
52	1476/1475	Germacrene D	S		x	x		x	1,2,3
53	1475	α -Amorphene	S				x		1,2
54	1480	β -Selinene	S				x		1,2
55	1489	α -Selinene	S				x		1,2
56	1490	<i>epi</i> -Cubebol	S		x				1,2
57	1491	Bicyclogermacrene	S					x	1,2
58	1496	α -Murolene	S			x	x		1,2
59	1501	α -Bulnesene	S		x				1,2
60	1508	(E,E)- α -Farnesene	S					x	1,2
61	1509	γ -Cadinene	S		x	x	x		1,2
62	1514	Unknown	–				x		–
63	1519	δ -Cadinene	S		x		x	x	1,2
64	1520	Unknown	–			x			–
65	1528	Unknown	–				x		–
66	1532	α -Cadinene	S				x		1,2
67	1535	Selina-3,7(11)-diene	S				x		1,2
68	1546	Unknown	–				x		–
69	1571	Spathulenol	S					x	1,2
70	1575	Caryophyllene oxide	S	x		x	x		1,2,3
71	1592	Unknown	–				x		–
72	1601	Humulene epoxide II	S				x		1,2
73	1621	Unknown	–				x		–

(continued on next page)

Table 1 (continued)

No.	RI (HP-5 ms)	Compound	class/group ^a	EO					Identification method ^b
				Lavender	Basil	Lemon balm	Hops	Chamomile	
74	1636/1635	<i>epi</i> - α -Cadinol	S		x			x	1,2
75	1649	α -Bisabolol oxide B	S					x	1,2
76	1676	α -Bisabolone oxide A	S					x	1,2,3
77	1680	α -Bisabolol	S					x	1,2,3
78	1719	Chamazulene	S					x	1,2
79	1739	α -Bisabolol oxide A	S					x	1,2,3
80	1873	(<i>Z</i>)-En-yn-dicycloether	S					x	1,2
81	1889	(<i>E</i>)-En-yn-dicycloether	S					x	1,2
82	1950	<i>m</i> -Camphorene	D				x		1
83	1983	<i>p</i> -Camphorene	D				x		1
84				22	27	18	29	14	

^a : M: monoterpene or monoterpenoid, K: ketone, E: ester, P: phenylpropanoid, S: sesquiterpene or sesquiterpenoid, D: diterpene.
^b : 1: Comparison with literature mass spectra (NIST mass spectral library), 2: Comparison with RIs from Adams (Adams, 2017), 3: Comparison with mass spectra and RIs from reference compounds via AMDIS (Reference substances used with purity see material and methods: EOs and chemicals).

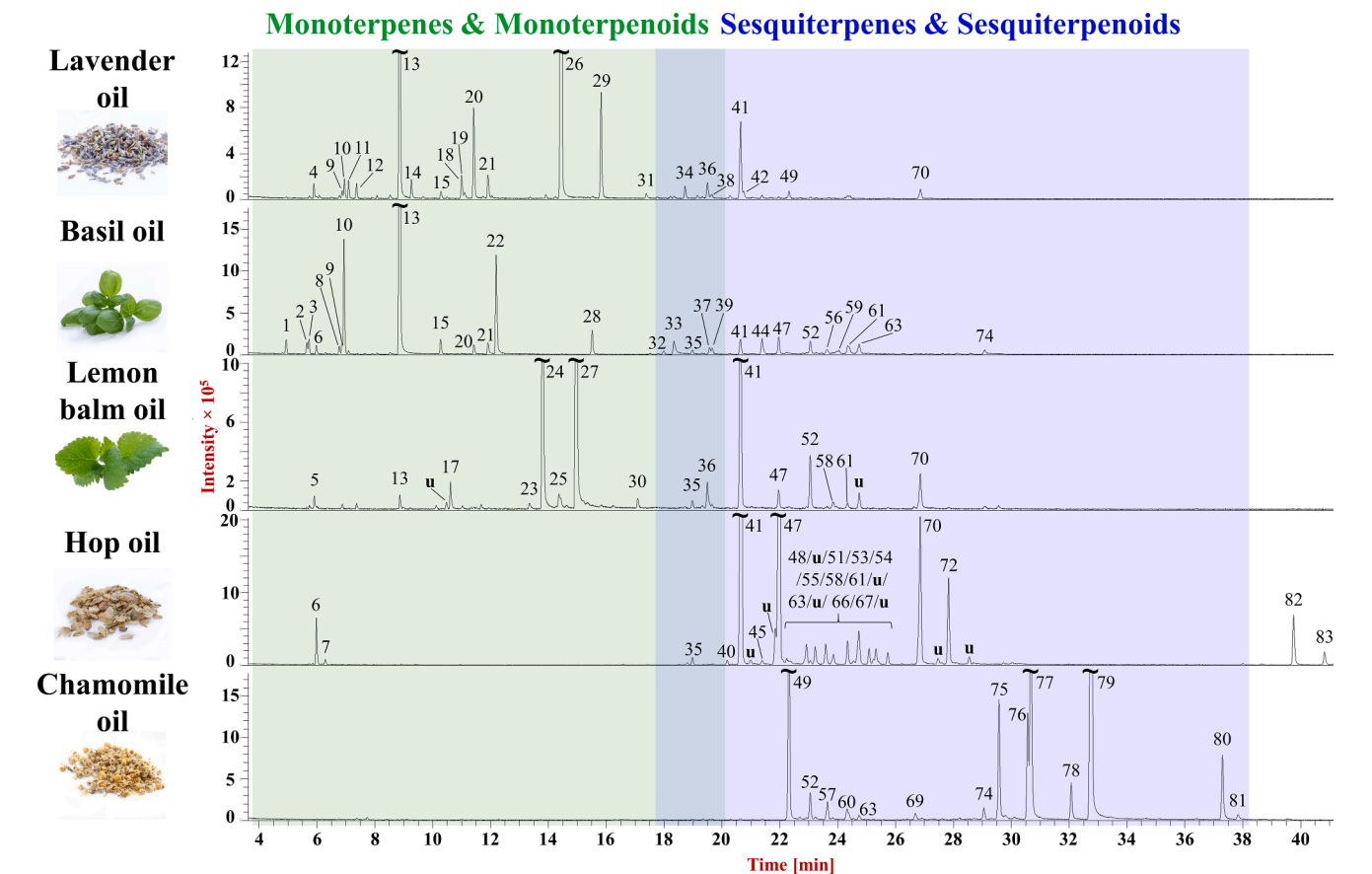


Fig. 1. Obtained GC–MS TIC chromatograms from the EOs measured on a HP-5 ms column and detected with a mass selective detector. The chromatograms show the total ion current (intensity) with the corresponding retention time (time) of the individual compounds in the respective EO. From top to bottom: lavender, basil, lemon balm, hops, and chamomile. The peaks are assigned to the corresponding compounds according to the numbers (No.) in Table 1 (u: unknown). The y-axis shows the signal intensity and the x-axis the time in min. Green: Range of MOTs (from 4 to 20 min). Blue: range of SQTs (from 18 to 38 min).

only found in basil. In contrast, 18 terpenes (Table 2) were detected in at least two different oils (total number of EO given in brackets): myrcene (2), limonene (2), 1,8-cineole (2), camphor (2), terpinen-4-ol (2), α -terpineol (2), geranyl acetate (2), (*E*)- β -farnesene (2), α -muurolene (2), *epi*- α -cadinol (2), linalool (3), α -copaene (3), α -humulene (3), germacrene D (3), γ -cadinene (3), δ -cadinene (3), caryophyllene oxide (3), caryophyllene (4). Interestingly, no compound was characteristic for all five EOs. The most intense MS peaks belonged to linalool and the corresponding acetic acid ester linalyl acetate (lavender), linalool (basil), neral, geranial, and caryophyllene (lemon balm), caryophyllene and α -humulene (hops) and (*E*)- β -farnesene, α -bisabolol and α -bisabolol

oxide A (chamomile; Fig. 1).

Modulatory effects of EOs on GABA_ARs

The modulatory effect of the five EOs which have previously been determined to share sedative and anxiolytic potential was investigated on recombinant GABA_AR containing the $\alpha 1\beta 2$ or $\alpha 1\beta 2\gamma 2$ subunits in transfected HEK293 cells (Fig. 2, 3). The used EOs possess distinct MOT and SQT profiles (Fig. 1). GABA_AR $\alpha 1\beta 2\gamma 2$ are the most common form in synapses of the central nervous system (Nusser et al., 1998). To investigate a possible contribution of the $\gamma 2$ subunit on GABA modulation by

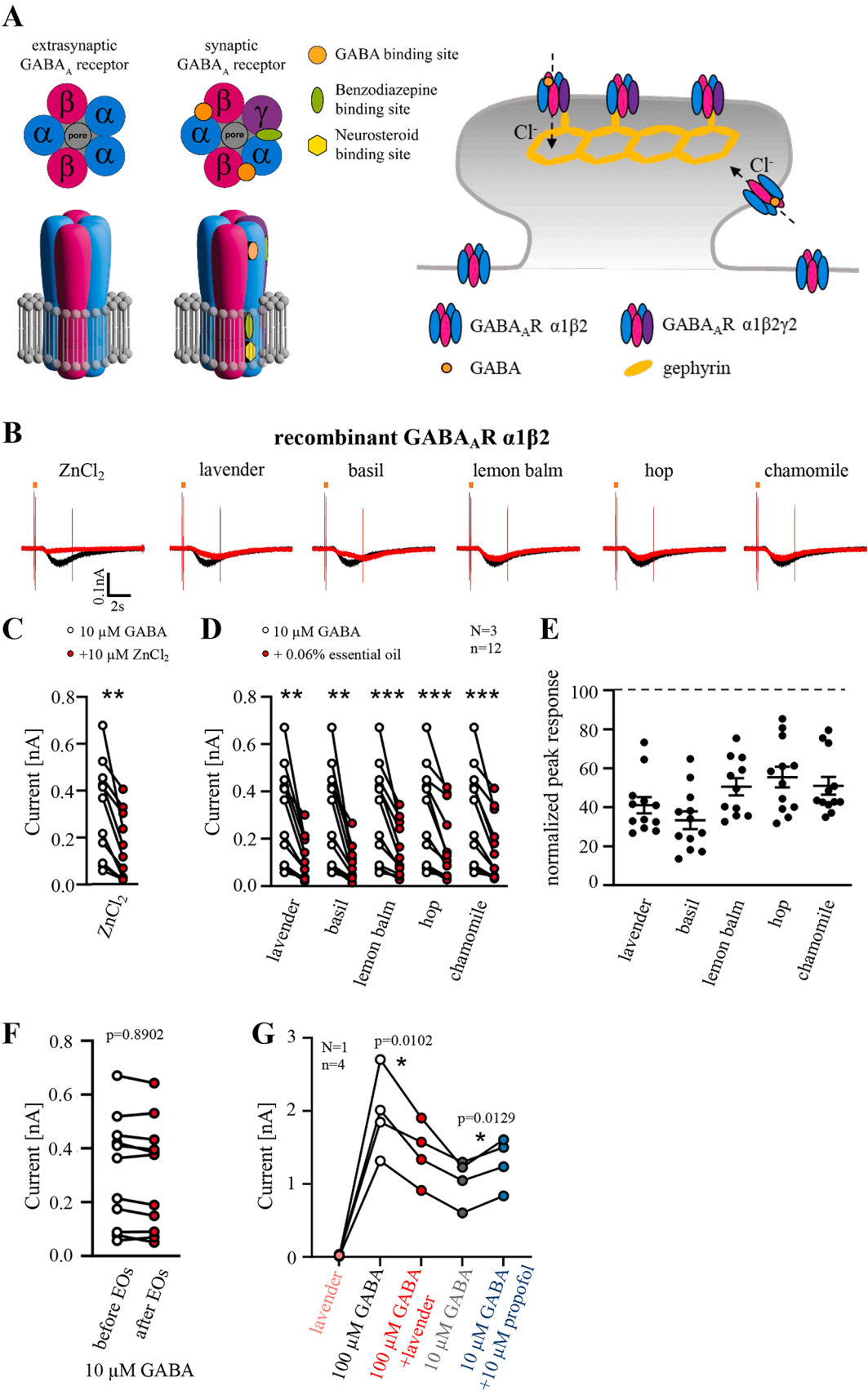
Table 2
EO components present in different oils.

No.	Compound	Structure	Classification	EO*				
				Lavender	Basil	Lemon balm	Hops	Chamomile
1	Myrcene		acyclic monoterpene		x		x	
2	Limonene		monocyclic monoterpene	x	x			
3	1,8-Cineole		bicyclic monoterpene ether	x	x			
4	Camphor		bicyclic monoterpene ketone	x	x			
5	Terpinen-4-ol		monocyclic monoterpene alcohol	x	x			
6	α -Terpineol		monocyclic monoterpene alcohol	x	x			
7	Geranyl acetate		acyclic monoterpene ester	x		x		
8	(E)- β -Farnesene		acyclic sesquiterpene	x				x
9	α -Murolene		bicyclic sesquiterpene			x	x	
10	<i>epi</i> - α -Cadinol		bicyclic sesquiterpene alcohol		x			x
11	Linalool		acyclic monoterpene alcohol	x	x	x		
12	α -Copaene		tricyclic sesquiterpene		x	x	x	
13	α -Humulene		monocyclic sesquiterpene		x	x	x	
14	Germacrene D		monocyclic sesquiterpene		x	x		x
15	γ -Cadinene		bicyclic sesquiterpene		x	x	x	
16	δ -Cadinene		bicyclic sesquiterpene		x		x	x
17	Caryophyllene Oxide		tricyclic sesquiterpene ether	x		x	x	
18	Caryophyllene		bicyclic sesquiterpene	x	x	x	x	

EOs, the modulatory potential of EOs was compared to the $\alpha 1\beta 2$ receptor composition. For electrophysiological measurements, 10 μ M GABA was applied to the cells alone or in combination with 0.06 % of the EOs basil, hops, chamomile, lavender or lemon balm. 10 μ M GABA has been determined previously a suitable GABA concentration where GABA_AR modulation can be reliably detected (Janzen et al., 2021; Milanos et al., 2017). These recordings were always done from the same cell with GABA application alone as the positive control for membrane surface expression of the receptor. Since EOs were diluted in ethanol with a final concentration of 0.6 %, the same amount of ethanol was added to the GABA solution to generate the control condition for electrophysiological measurements. To verify the GABA_AR subtype composition, we co-applied 10 μ M GABA together with 10 μ M ZnCl₂ to the transfected cells. Since 10 μ M ZnCl₂ selectively blocks $\alpha 1\beta 2$ containing receptors

and shows no effect on GABA_AR $\alpha 1\beta 2\gamma 2$ (Gingrich and Burkat, 1998; Korpi et al., 2002), we distinguished GABA_AR with and without the $\gamma 2$ subunit by their decreasing or unchanged/increasing current responses to ZnCl₂ (Fig. 2B, C and 3B).

GABA_AR $\alpha 1\beta 2$ have been shown at peri- and extrasynaptic sites (Mortensen and Smart, 2006; Sieghart and Sperk, 2002). Application of 10 μ M GABA to heterologously expressed recombinant $\alpha 1\beta 2$ receptors, resulted in currents of 0.29 ± 0.06 nA ($n = 12$, Fig. 2D, Supplemental Table 1). Co-application of GABA with each EO significantly decreased current responses to ~ 33 –56% with the highest reduction for basil (0.09 ± 0.02 nA, $p = 0.0013$) and the lowest reduction for hops (0.17 ± 0.04 nA, $p = 0.0010$) (Fig. 2). In contrast, GABA application before and after EO administration was unchanged ($p = 0.8902$, Fig. 2F). The EO lavender did not exhibit any agonistic activity. Following application of a



(caption on next page)

Fig. 2. Effects of EOs on recombinant $\alpha 1\beta 2$ GABA_ARs. A) Pentameric GABA_ARs $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ (top and side view) with GABA (orange circle), benzodiazepine (green cylinder) and neurosteroid-binding (yellow hexagon) sites indicated (left). Scheme of postsynapse with GABA_AR containing $\alpha 1\beta 2$ or $\alpha 1\beta 2\gamma 2$ subunits, respectively (with α cyan, β magenta, γ purple). Synaptic GABA_AR are anchored to the postsynaptic area via the scaffold protein gephyrin (hexagonal lattice, dark yellow; right image). B–E) GABAergic currents of GABA_AR containing $\alpha 1\beta 2$ subunits ($N = 3$, $n = 12$). B) Exemplary traces of patch-clamp measurements on $\alpha 1\beta 2$ for GABA alone (black) or in combination with EOs (red). GABA application is marked in orange. C) ZnCl₂ control to verify inhibition of $\alpha 1\beta 2$ containing GABA_AR. D) Effect of EOs on GABA currents of GABA_AR $\alpha 1\beta 2$. E) Percentage change of GABA currents after application of EOs. Gray dotted line marks 100 %. Data are shown as mean $\pm$ SEM and normalized individual values. F) GABA application before and after application of EOs. G) GABAergic currents following application of 0.06 % lavender alone followed by saturating concentration of GABA (100 μ M) and in presence of lavender, GABA 10 μ M and GABA in presence of propofol (10 μ M). Note, in G all measurements have been performed continuously on one cell ($N = 1$, $n = 4$). Level of significance: ** $p < 0.01$, *** $p < 0.001$.

saturating GABA concentration of 100 μ M, lavender still inhibited the GABAergic current ($p = 0.0102$, Fig. 2G). Positive allosteric modulation by propofol application was however preserved after washout of high GABA concentration (Fig. 2G).

For GABA_AR containing $\alpha 1\beta 2\gamma 2$, GABA-induced currents were of larger amplitudes with 1.23 ± 0.23 nA ($n = 12$, Fig. 3C, Supplemental Table 1). Here, EOs showed a positive modulation of the $\alpha 1\beta 2\gamma 2$ receptors in contrast to $\alpha 1\beta 2$ pointing to a strong contribution of the $\gamma 2$ subunit to the observed effects. Currents were increased to 133–198 % (Fig. 3C and D). While hop was the only EO with no modulatory effect on $\alpha 1\beta 2\gamma 2$ GABA_AR, and lemon balm only exhibited a tendency for increased GABA current, the positive modulation was significant using basil (1.55 ± 0.27 nA, $p = 0.0049$), chamomile (1.92 ± 0.32 nA, $p = 0.0001$), and lavender (2.0 ± 0.33 nA, $p = 0.0002$). The GABAergic current was however unaffected when GABA application before and after EO treatment was compared (Fig. 3E). As a single concentration of 0.06 % EO was used for recordings, stronger positive or negative modulation at other concentrations cannot be excluded.

The EO of lavender behaved differently from the other EOs in terms of changing the kinetics of the GABAergic responses. While lavender had no agonistic potential, the positive modulation at low GABA concentration (10 μ M) was no longer observed at saturating GABA concentrations (100 μ M). The modulation of the GABAergic response was however reversible demonstrated by subsequent application of propofol (Fig. 3F). Investigating the deactivation kinetics of lavender at $\alpha 1\beta 2\gamma 2$ GABA_AR configurations (Fig. 3G, H, Supplemental Table 2), a long ion channel opening with no recovery during a deactivation period was obtained for all cells recorded from different independent biological replicates. Thus, we investigated the current fraction of deactivation and the decay time during deactivation in the presence of the EO with GABA-activated currents in the absence of the oil but presence of the solvent ethanol. Interestingly, a significantly decreased fraction of the normalized deactivating current of 0.52 ± 0.12 ($p = 0.0113$) compared to GABA application alone and an increased decay time of 3.05 ± 0.42 s ($p = 0.0297$) was observed suggesting a stabilization of the open pore configuration most likely by direct binding to the receptor ($n = 11$, Fig. 3G, H).

Taken together, while $\alpha 1\beta 2$ GABA_ARs showed a negative modulation of GABA currents by the here tested EOs, GABA_ARs containing the $\gamma 2$ subunit were positively modulated.

Discussion

In the present study, the composition of five EOs was characterized by GC–MS and functionally investigated using electrophysiological readouts. The EOs were selected due to their use in human diet and descriptions on their calming, relaxing, or sedative effect in the traditional medicine. Previously, single components of EOs have been tested for their physiological effects on GABA_A receptors, however, EOs themselves have only sparsely been investigated on various GABA_A receptor subtypes to characterize their modulatory potential. EOs used in the present study were rich in MOTs and SQTs. Due to the complex structures of SQTs, only very few standards are commercially available, and isolation or synthesis is time consuming and labor intensive. This is a well-known challenge in research with complex natural products. Nonetheless, the majority of detected compounds was (at least

tentatively) identified (Table 1). The composition of the EOs has also been investigated in earlier studies such as (Bocquet et al., 2018; De Martino et al., 2009) but has not yet been analyzed with respect to GABA_ARs modulation.

Lavender and basil had the most substances in common with six MOTs and one SQT (limonene, 1,8-cineole, camphor, terpinen-4-ol, α -terpineol, linalool and caryophyllene; for the exact classification see Table 2). In contrast, chamomile shared only one compound (corresponding compound in brackets) with each of the following three oils: lavender ((*E*)- β -farnesene), lemon balm (germacrene D) and hop (δ -cadinene; Table 2). Based on the substances' similarity of the EOs, the oils of lavender and basil may be supposed to exert the most similar modulatory effect on GABA_ARs, as was observed for $\alpha 1\beta 2$ GABA_ARs revealing similar negative modulations. The receptor composition of GABA_ARs $\alpha 1\beta 2\gamma 2$ was however strongly positively modulated by lavender as well as basil most likely due to their similar EO compositions. A similarly large positive modulation was detected for chamomile despite being strongly divergent in chemical composition (predominantly SQTs in chamomile versus MOTs in lavender). In general, both MOTs and SQTs have previously been demonstrated to alter GABAergic activity. While MOTs from lavender have been shown to positively modulate GABA_ARs (Milanos et al., 2017), similar effects have also been exhibited for single SQTs (Janzen et al., 2021). Mechanistically, it is assumed that single components of EOs e.g. MOTs and SQTs interact with GABA_ARs via an allosteric binding site within the transmembrane region due to their lipophilic character. Molecular modeling and docking studies indicate that sesquiterpenoids in particular could bind to the neurosteroid-binding site located in this region (Janzen et al., 2021; Kessler et al., 2014). However, the modulatory potential might largely differ if instead of single substance application, EOs are supplemented carrying a combination of terpenes. Due to the large number of single components within EOs, determining a binding site at the GABA_AR is complicated. Strategies to identify essential binding sites at the receptor can be either (i) mutating amino acid residues in a suggested possible binding site followed by functional determination of the modulatory potential at distinct GABA_AR subunit combinations or (ii) separate in vitro expression of a large number of possible GABA_AR subunit combinations known from the brain to determine subtype specificity of EOs.

The terpene (*E*)- β -farnesene is a substance common in both EOs, lavender and chamomile. Janzen et al. found a significant reduction in the GABA-induced currents when $\alpha 1\beta 2$ GABA_ARs were treated with GABA and (*E*)- β -farnesene (Janzen et al., 2021). Albeit, there is no study, to the best of our knowledge, that has investigated the modulatory effect of (*E*)- β -farnesene on the synaptic GABA_AR composition $\alpha 1\beta 2\gamma 2$. The strong positive modulation of chamomile oil might thus not be assigned to (*E*)- β -farnesene alone but rather to a synergism with other compounds such as *epi*- α -cadinol, germacrene D, δ -cadinene, α -bisabolol, α -bisabolol oxide A and α -bisabolol oxide B.

Overall, the identified MOTs are structurally divers and comprise aliphatic-, monocyclic and bicyclic hydrocarbons, acyclic alcohols, acyclic aldehydes, acyclic esters, monocyclic alcohols, bicyclic ethers, bicyclic alcohols, bicyclic ketones, and bicyclic esters. The same holds true for the SQTs identified here with additional tricyclic structures. Noteworthy, Kessler et al. observed structural similarities for GABA_AR active MOTs in case of a cyclic structural ground motif (both mono- or bicyclic) containing a hydroxyl group (Kessler et al., 2014). The authors

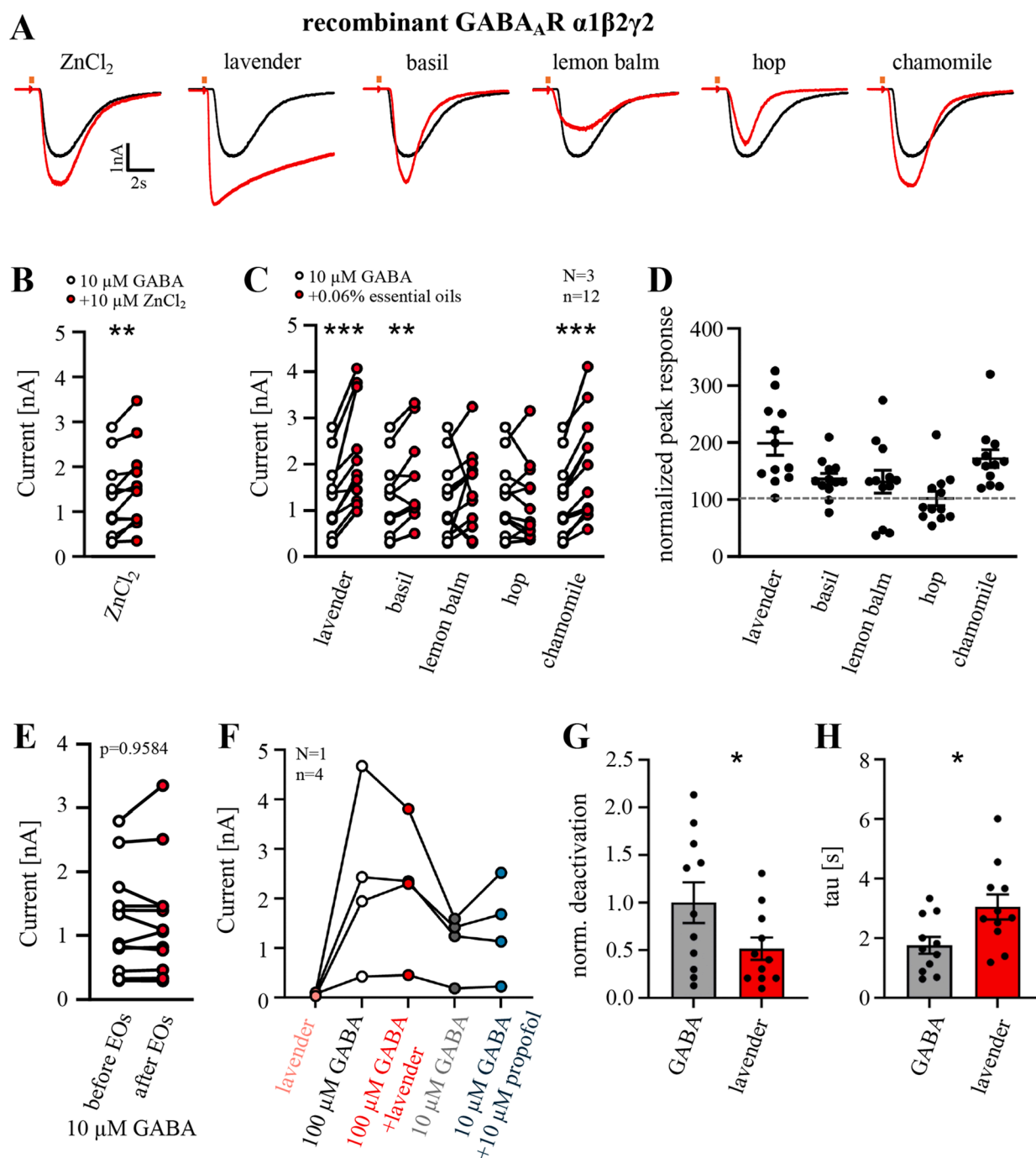


Fig. 3. Effects of EOs on $\alpha 1\beta 2\gamma 2$ GABA_ARs. A-D) Currents of GABA_AR containing $\alpha 1\beta 2\gamma 2$ subunits ($N = 3$, $n = 12$). A) Exemplary traces of patch-clamp measurements on $\alpha 1\beta 2\gamma 2$ for GABA alone (black) or in combination with EOs (red). GABA application is marked in orange. B) GABAergic current of $\alpha 1\beta 2\gamma 2$ receptors upon co-application of ZnCl₂ together with GABA (each 10 μ M). C) Effect of EOs on GABA currents of $\alpha 1\beta 2\gamma 2$ GABA_AR. D) Percentage change of GABA currents after application of EOs. Gray dotted line marks 100 %. Data are shown as mean $\pm$ SEM and normalized individual values. E) 10 μ M GABA application before and after EO measurements with no significant change in GABAergic currents. F) GABAergic currents following single application of 0.06 % lavender to check for agonistic activity followed by saturating concentration of GABA (100 μ M) and in presence of lavender, GABA 10 μ M and GABA in presence of propofol (10 μ M). Note, in F all measurements have been performed continuously on one cell ($N = 1$, $n = 4$). Kinetic properties G) deactivation and H) decay time of GABA_AR $\alpha 1\beta 2\gamma 2$ currents in response to 10 μ M GABA alone and with 0.06 % lavender ($n = 11$). Data are shown as mean $\pm$ SEM and individual values. Level of significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

further showed that almost all terpenoids that were neither cyclic nor contained a hydroxyl group showed a lack of modulatory potential on GABA_ARs $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ subtypes. In a recent review about structure-activity relationships it was pointed out that especially bicyclic monoterpene alcohols like (+)-, (-)-borneol or isoborneol harbor pronounced modulating activity on GABA_ARs (Cicek, 2018).

Nevertheless, general conclusions are still in the process of being established concerning common structural motifs of GABA_AR being targeted by SQTs. Janzen *et al.* showed a significant modulatory activity of humulol and β -caryolanol, two cyclic sesquiterpene alcohols (mono- and tricyclic) (Janzen *et al.*, 2021). Accordingly, borneol, terpinene-4-ol and α -terpineol, which are identified in the present study, might be

likewise effective in the analyzed oils. Those three cyclic monoterpene alcohols were found to be present in lavender and two of them in basil suggesting a contribution of those compounds to the significant positive modulation of $\alpha 1\beta 2\gamma 2$ GABA_ARs. Moreover, chamomile contained five cyclic (mono-, bi- and tricyclic) sesquiterpene alcohols: spathulenol, *epi*- α -cadinol, α -bisabolol oxide B, α -bisabolol and α -bisabolol oxide A, which might point to a positive modulation of GABA_ARs with $\alpha 1\beta 2\gamma 2$ subunit compositions by cyclic sesquiterpene alcohols. Spathulenol and the representatives of the α -bisabolol series, as sole constituents of the EO of chamomile, have previously been found to reveal modulatory activity on $\alpha 1\beta 2$ GABA_ARs (Janzen et al., 2021).

The EOs of hop and lemon balm, containing neither cyclic monoterpene alcohols nor cyclic sesquiterpene alcohols, gave no significant positive modulation of $\alpha 1\beta 2\gamma 2$ GABA_ARs. These two oils contain terpenes belonging to aliphatic-, monocyclic-, bicyclic- and tricyclic hydrocarbons, cyclic ethers, acyclic alcohols, acyclic aldehydes and acyclic esters e.g. myrcene, pseudolimonene, α -muurolene, α -copaene, carophyllene oxide, linalool, citronellal or methyl geranate (Table 2). α -Muurolene was present in these two oils only. Accordingly, the lack of those two subgroups of terpene alcohols may explain the lack of GABA_AR modulation observed in this study.

Additionally, two cyclic sesquiterpene alcohols *epi*- α -cadinol and *epi*-cubebol have been identified in basil oil, which have not been reported previously with respect to GABA_AR modulation. However, these cyclic sesquiterpene alcohols represent promising candidates for further investigation.

We could show that EOs can be strong positive modulators of $\alpha 1\beta 2\gamma 2$ GABA_ARs containing $\gamma 2$ as a key subunit in this modulation, and that this evidently relates to their high content of MOTs and SQTs. A limitation of the present study is however the use of mainly a single GABA concentration together with a single concentration of an EO which may rather underestimate the modulation by the EO. The modulation of the GABA_ARs by the EO seems to be concentration-dependent on the coapplied concentration of the agonist GABA as the positive modulation by lavender was no longer present at a saturating concentration of GABA. Such modulatory differences of EOs at low and high neurotransmitter concentrations needs to be further explored to discover physiological consequences for both, tonic and phasic GABAergic neurotransmission. In addition, ethanol as solvent may lead to an enhancing effect of the EO modulation similar to a previously described combination of very low concentrations of ethanol and a neuroactive steroid which did not facilitate GABAergic currents on their own but had an additive effect when combined (Akk and Steinbach, 2003).

Due to the complex composition of EOs, further research is needed, also with respect to the mode of application (e.g. direct acute application compared to long-term application), bioavailability in vivo and potential metabolic conversion of substances during their passage through the human body (Kornbausch et al., 2022). Hence, further studies on transfected cells with distinct subunit compositions of GABA_A receptors known to exist in the brain are required to nail down the modulatory potential of EOs. Second, in vitro recordings from primary neurons rich in GABAergic synapses, e.g. hippocampal or cortical neurons, are essential to further evaluate the modulatory potential of EOs in a more physiological situation. In primary neurons, the GABA_ARs are anchored via the scaffold protein gephyrin to synaptic sites, but also harbor auxiliary subunits (Shisa7, TMEM132B) (Han et al., 2019; Wang et al., 2024). Auxiliary subunits have been demonstrated to also modulate the deactivation kinetics of GABA_ARs. Thus, the whole protein complex of GABA_ARs subunits, the scaffold proteins, and auxiliary subunits need to be taken into account to fully explore the modulatory effects of EOs.

Unravelling the major components of EOs responsible for GABA modulation (e.g. different MOTs, SQTs) is a first step for clarification. Additionally, a synergistic effect apparently plays an important role in EO application which appears to be also the case for GABA modulatory effects (Bakkali et al., 2008; Bunse et al., 2022). In general, a similar substance composition may thus elicit similar physiological effects, even

if the EOs originate from different biological sources, on condition that qualitative as well quantitative composition harbor generally resembling motifs. Following identification of compound compositions and their concentration profiles within EOs and definition of ratios between single components that harbor the modulatory potential and estimation of a possible cytotoxicity, in vivo experiments can follow in a controllable manner to fully explore the therapeutic potential of EOs in various disease models.

Conclusion

Our results revealed that negative or positive allosteric modulation of GABA_ARs by EOs depends on the presence of the GABA_AR $\gamma 2$ subunit, demonstrating a possible physiological modulation of synaptic GABAergic neurotransmission by mixtures of MOTs or SQTs in relation to the chemical composition of the respective EOs. Quantitative data on defined mixtures of MOTs and SQTs and further sub-fractionations of EOs are thus promising future targets in this field of research.

Data availability

Data are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

Christian Böhm: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Anna-Lena Wiessler:** Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation. **Dieter Janzen:** Validation, Methodology, Formal analysis, Data curation. **Julian Nausester:** Data curation, Formal analysis, Validation, Visualization, Methodology. **Benedikt Slavik:** Methodology, Data curation. **Helene M. Loos:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Carmen Villmann:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Andrea Buettner:** Writing – review & editing, Supervision, Software, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors state that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phyplu.2025.100852](https://doi.org/10.1016/j.phyplu.2025.100852).

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