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Three-Step Test System for the Identification of Novel $GABA_A$ Receptor Modulating Food Plants

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Abstract Potentiation of γ-amino butyric acid (GABA)-induced $GABA_A$ receptor ($GABA_AR$) activation is a common pathway to achieve sedative, sleep-enhancing, anxiolytic, and antidepressant effects. Presently, a three-component test system was established for the identification of novel $GABA_AR$ modulating food plants. In the first step, potentiation of GABA-induced response of the $GABA_AR$ was analysed by two-electrode voltage clamp (TEVC) for activity on human α1β2-$GABA_AR$ expressed in *Xenopus laevis* oocytes. Positively tested food plants were then subjected to quantification of GABA content by high-performance liquid chromatography with fluorescence detection (HPLC–FLD) to exclude test foods, which evoke a TEVC-response by endogenous GABA. In the third step, specificity of $GABA_A$-modulating activity was assessed by TEVC analysis of *Xenopus laevis* oocytes expressing the homologous glycine receptor (GlyR). The three-component test was then applied to screen 10 aqueous extracts of food plants for their $GABA_AR$ activity. Thus, hop cones (*Humulus lupulus*) and *Sideritis sipylea* were identified as the most potent specific $GABA_AR$ modulators eliciting significant potentiation of the current by 182 ± 27 and 172 ± 19 %, respectively, at the lowest concentration of 0.5 μg/mL. The extracts can now be further evaluated by *in vivo* studies and by structural evaluation of the active components.

Keywords $GABA_A$ receptor · GABA · Glycine receptor · *Sideritis* · Lemon balm leaves · Hop cones

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Introduction

γ-Amino butyric acid (GABA) is a physiologically important amino acid acting as major neurotransmitter in the brain: 40 % of all neurons in the mammalian central nervous system release GABA [1], providing the major inhibitory control and playing a key role in regulating behaviour [2]. When adult neurons are exposed to GABA, the heteropentameric $GABA_A$ receptors ($GABA_ARs$), which belong to the Cys-loop superfamily of ligand-gated ion channels, open an endogenous ion channel pore that is permeable for chloride ions leading to hyperpolarisation of the neuronal membrane. $GABA_ARs$ in humans consist of 19 different subunits (α1–α6, β1–β3, γ1–γ3, ρ1–ρ3, δ, ε, θ, and π) [3]. Depending on their subunit composition, $GABA_ARs$ differ in GABA affinity and pharmacological properties [4]. In molecular-pharmacological studies, α- and β-subunits are often assembled to pentameric $GABA_ARs$ to decrease the number of transfected subunits leading to an almost homogenous receptor population, where the GABA binding site is located at the interface of the α- and β-subunit [5]. Several medical conditions, such as chronic pain, sleep disturbances, anxiety disorders, and epilepsy, are alleviated by drugs enhancing

GABA activity [6, 7]. Thus, $GABA_A$Rs also represent a promising target for neuromodulating foods.

The present study established a three-step test system to identify food plants which may address $GABA_A$Rs as target: in the first step, $GABA_A$R modulation is screened by two-electrode voltage clamp (TEVC) of *Xenopus laevis* oocytes expressing the human α1β2- $GABA_A$R. In positive extracts, endogenous GABA is then quantified by high-performance liquid chromatography (HPLC) to exclude false positive results due to physiologically relevant GABA concentrations. True $GABA_A$R modulating food plants are further subjected to TEVC analysis with *Xenopus laevis* oocytes expressing the homologous glycine receptor (GlyR) to exclude extracts that exert unspecific effects.

The three-step test system was applied to screen 10 aqueous extracts of food plants for their $GABA_A$R modulating activity. For that purpose, food plants were selected for which sedative, anxiolytic, and calming effects *in vivo* have been described, while their physiological targets have not been completely elucidated. For example, sedative effects have been reported for hop cones and lemon balm leaves [8, 9]. Some of the sage (*Salvia*) species evoke anticonvulsant, antiepileptic, and antidepressant effects, whereas *Sideritis* species are consumed in Mediterranean countries because of their analgesic and anticonvulsant health benefits [10, 11]. Animal studies demonstrated that some natural tea components prolong the sleeping time through the potentiation of $GABA_A$R response [12]. Further, L-theanine, which is present in green tea leaves, displays anxiolytic activity in humans [13].

Materials and Methods

Chemicals

Unless noted otherwise, chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany) and all solvents for HPLC analyses from Fisher-Scientific (Schwerte, Germany).

Plant Material and Preparation of Extracts

Natural convective-dried samples of *Sideritis* species *S. arguta*, *S. condensata*, and *S. stricta* were supplied by courtesy of Akdeniz University, Antalya, Turkey. *S. sipylea* was purchased from an Internet shop (enexia.de). The identity of *Sideritis* species was verified as described before [14]. *Sideritis* species was coarsely ground before extraction. Sage leaves (*Salvia officinalis*), lavender flowers (*Lavendula officinalis*), chamomile flowers (*Matricaria chamomilla*), green tea leaves (*Camellia sinensis*), hop cones (*Humulus lupulus*), and lemon balm leaves (*Melissa officinalis*) were obtained in crushed form from a local pharmacy and used directly. Their identity was verified macro- and microscopically as well as by thin-layer chromatography (TLC) analyses following the respective monographs [15, 16] (Suppl. Figure 1). After adding 100 mL of boiling water to 2.5 g of test material, the mixtures were stirred for 15 min at room temperature. Each mixture was filtered (Whatman filter paper) and the filtrates were lyophilized. Before use, the lyophilized samples were dissolved in water (1 mg/mL).

Expression of Receptors in Xenopus Oocytes

Defollicularized oocytes from *Xenopus laevis* were obtained from the FAU Institute of Cellular and Molecular Physiology. The cDNA of human α1- and β2-$GABA_A$R subunits were subcloned into the pcDNA 3.1 vector. The DNA for each subunit was linearised by digestion with Bgl II. Then the cRNA for each subunit was synthesised from the linearised DNA using the mMessage mMachine T7 RNA polymerase kit (Ambion, Austin, TX, USA). The cRNA of human $GABA_A$R α1- and β2-subunits were mixed in a ratio of 1:1 and 17–28 ng of the mixture was microinjected into the oocytes to express the human $GABA_A$R. The cDNA of the α1-subunit of human GlyR was subcloned into the pNKS 2 vector. cRNA was performed on HindII linearised plasmid DNA using the SP6 RNA polymerase mMessage mMachine kit (Ambion, Austin, TX, USA). Approximately 0.5 pg of cRNA was injected into the oocytes to express human GlyR. After injection, the oocytes were kept in ND96 medium (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM HEPES, pH 7.4) with gentamycin at 18 °C for 24–72 h.

Electrophysiology: TEVC Measurement

Effects of the test plant extracts on the current responses of ionotropic $GABA_A$Rs and GlyRs were examined by TEVC. Two days after cRNA injection, the maximal current responses in the processed oocytes were measured upon administration of GABA or glycine with or without test extracts. For that purpose, the oocytes were placed individually into a small chamber and pierced by two glass microelectrodes filled with 3 M KCl for simultaneous recording of the membrane potential and current delivery. During the recording, membrane potential was clamped at −50 mV using a Turbo Tec-03× npi amplifier (npi electronic GmbH, Tamm, Germany) and continuously superfused with ND96 solution. For the preparation of test solutions, GABA or glycine was dissolved in ND 96 buffer either separately or in combination with the respective plant extracts. Non-transfected oocytes, which were used as negative control, did not elicit a response to any of the test solutions. The average of 1 μM-GABA currents before and after the application of extracts was taken as 100 %.

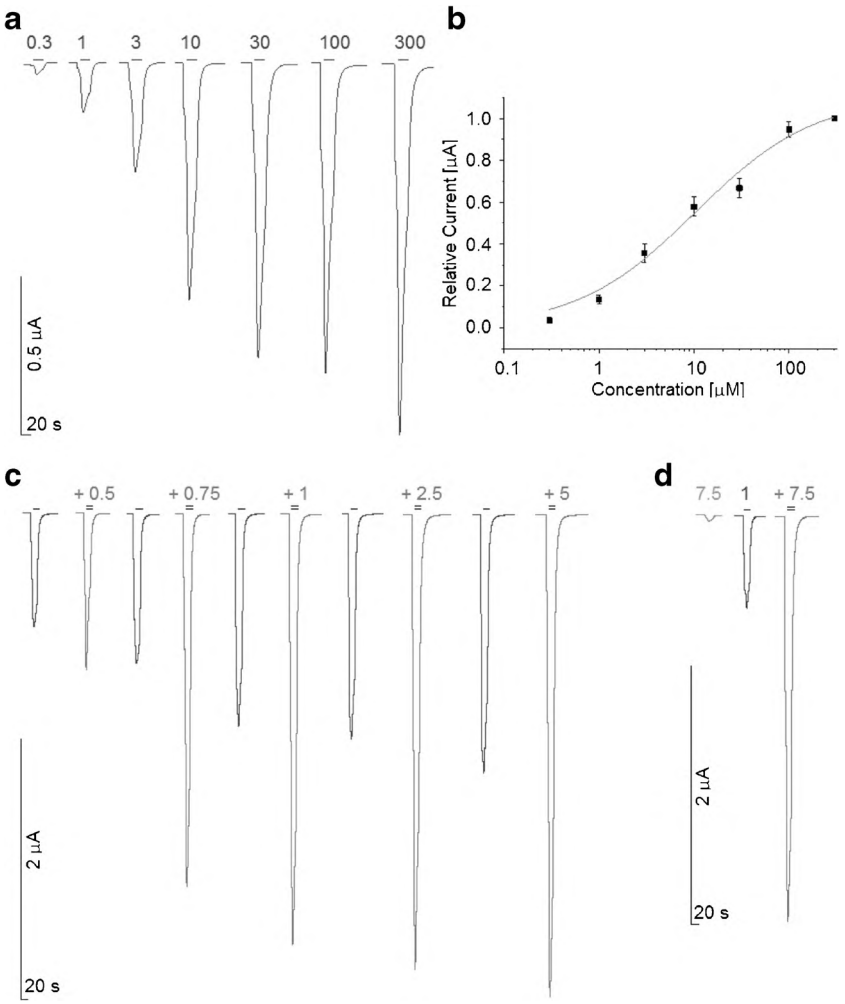

Fig. 1 Modulation of human α1β2-GABA$_A$Rs expressed in *Xenopus laevis* oocytes **a)** Typical traces for responses to increasing concentrations of GABA (0.3–300 µM); **b)** Dose–response curve for agonist GABA fitted with Hill equation; the highest response was set to 1; **c)** Potentiation of GABA-induced current by various concentrations of chamomile flower extract (0.5–5 µg/mL, every second trace "+ concentration value"), control responses to pure 1 µM GABA ("-"); **d)** Current responses for chamomile flower extract with and without GABA: activation by extract (7.5 µg/mL) without GABA (first trace), by 1 µM GABA without extract (second trace) and by GABA plus extract (third trace)

GABA Determination by HPLC–Fluorescence Light Detection (FLD)

HPLC analysis of GABA was performed after derivatization with ortho-phenylenediamine (OPA) according to literature [17] with some modifications. Aqueous plant extracts were prepared in various concentrations (10–40 mg/mL) allowing the detection of GABA. Aliquots of 0.4 mL of the plant extracts were mixed with 0.2 mL of NaOH (1 N) and 0.02 mL of OPA (10 mg/mL in MeOH) each and reacted at room temperature for 2 min. Derivatisation was stopped by adding 0.4 mL of HCl (0.7 N). The solution (50 µL) was injected to a Zorbax Eclipse XDB-C8 column (4.6 × 150 mm, 5 µm, Agilent Technologies, Waldbronn, Germany). Mobile phase A consisted of 90 mM sodium acetate in 7 % acetonitrile, pH 6.5, mobile phase B of 80 % acetonitrile. The elution profile was as follows: 0–10 min 100 % A; 10–15 min, 100–85 % A; 15–16 min, 85–5 % A; 16–21 min, 5 % A; 21–22 min, 5–100 % A; 22–25 min 100 % A with a flow speed of 1 mL/min. The HPLC unit (Jasco, Groß-Umstadt, Germany) was equipped with autosampler (AS-1555), degasser (DG 1580–53), pump (PU-1580), and fluorescence detector (FP 920). Fluorescence light detection (FLD) was carried out at an excitation wavelength of 230 nm and an emission of 450 nm. GABA concentration in plant extracts was calculated using an external calibration curve, which was established from the mean peak areas of triplicate analysis of seven concentrations (1, 2.5, 5, 10, 15, 20, and 25 µg/mL) of a GABA-standard. The coefficient of determination (R^2) of the calibration curve was 0.9988, the method's limit of detection 0.6 µg/mL and limit of quantification 1.7 µg/mL. The measured GABA content of the differently diluted extracts was normalized by calculation to an extract concentration of 1 mg/mL.

Data Analysis

Electrophysiological data were expressed as means ± standard error of the mean (SEM) and concentration–response data for agonist-evoked currents were fitted with the Hill equation using Origin 9.1 (OriginLab, Northampton, MA, USA). The results of HPLC analysis were expressed as means ± standard

deviation (SD). Data were statistically analysed using repeated measures two-way ANOVA. The Holm-Sidak multiple comparisons test was used as follow-up to ANOVA with significance levels $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Statistical analysis was performed using GraphPad Prism 6 (GraphPad Software, La Jolla, USA).

Results and Discussion

The present study established a three-step test system to identify novel food plants, which potentiate $GABA_AR$ response by an allosteric mechanism. In the first step, screening for the desired $GABA_AR$ activity is carried out to identify candidates. In the second and third steps, false positive test results are identified by checking for common interferences, such as the presence of native GABA or unspecific reaction. The workflow is summarized in Supplementary Scheme 1.

Step 1: Screening for Potentiation of $GABA_AR$ Response

The electrophysiological response of the α1β2-heteropentameric $GABA_AR$ expressed in *Xenopus* oocytes was measured by TEVC. First, the $EC_{5–15}$ range of the agonist GABA was determined, because small modulatory effects are observed most sensitively at low ligand concentrations [18]. For this purpose, a GABA dose–response curve was recorded using GABA in concentrations of 0.3–300 μM (Fig. 1). Consequently, the concentration of 1 μM GABA was chosen for co-application with the plant extracts.

Subsequently, the effects of aqueous extracts of several food plants (*Sideritis* species, sage leaves, lavender flowers, chamomile flowers, green tea leaves, hop cones, and lemon balm leaves) on the response of the $GABA_ARs$ were investigated by administering the test extracts (0.5–5 μg/mL) in combination with 1 μM GABA. As shown for chamomile flower extract in Fig. 1c, GABA-evoked currents increased with increasing extract concentrations. In a similar way, all other plant extracts increased the GABA-gated response in a dose-dependent manner. At the highest concentration, all tested extracts showed highly significant enhancement of $GABA_AR$ activity (Fig. 2a).

At the lowest concentration, a significant potentiation was caused by sage leaves, lavender flowers, *S. sipylea,* hop cones, and lemon balm leaves. Here, the strongest effect was observed for hop cones (182 ± 27 % potentiation) and *S. sipylea* (173 ± 18 %). A detailed summary of potentiating effects of the test plant extracts on GABA-mediated currents is presented in Supplementary Table 1**.** From these results it can be deduced that the modulation of $GABA_ARs$ is a common mechanism of sedative food effects.

Step 2: Quantification of Endogenous GABA in the Test Food Plants and Evaluation of its Potential to Induce $GABA_AR$ Response

The presence of GABA, the natural agonist of the $GABA_AR$, in various foods has been shown before [19–21]. To determine if the observed effects resulted from endogenous GABA in the plant extracts, their GABA concentrations were analysed by HPLC-FLD after derivatisation with OPA. The results are shown in Table 1. Although GABA was detected in all analysed plant extracts, the concentrations were very low so that it is highly unlikely that the potentiation observed by TEVC was caused by the extracts' natural GABA content. To further confirm this conclusion and to investigate if other components showing agonistic activity might be present, the plant extracts were tested by TEVC without GABA addition. Even at extract concentrations of 7.5 μg/mL, which exceeded the highest concentration used for co-application experiments, response to the pure plant extracts only reached 1–19 % of the current evoked by 1 μM GABA (= 100 %; Fig. 1d, Table 1). Thus, it can be concluded that the observed activity of the plant extracts to potentiate GABA-induced $GABA_AR$ response was not caused by endogenous GABA content or by other natural GABA agonists.

Step 3: GlyR Modulation by the Test Extracts to Assess the Specificity of $GABA_AR$ Interaction

To determine if the observed activity was indeed specific to $GABA_ARs$, the effects of the test plant extracts on GlyR were also investigated. GlyRs belong, similar to $GABA_ARs$, to the Cys-loop superfamily of ligand-gated ion channels and show high homology [22]. First, GlyR affinity was tested and a dose-dependent curve of the agonist glycine was obtained. The concentration of 30 μM glycine, which was in the $EC_{5–15}$ range, was chosen for further experiments. In the absence of glycine, none of the food plant extracts evoked a current. Although GlyRs show distinct sequence homology with $GABA_AR$ subunits, the extracts did not potentiate the glycine-mediated currents (Fig. 2b). The effects detected for the extracts of *S. sipylea, S. arguta,* lavender flowers, and hop cones were not significantly different from control, whereas the extracts of chamomile flowers, lemon balm leaves, *S. stricta*, green tea leaves, *S. condensata*, and sage leaves even caused small, but significant decreases of glycine-evoked currents. Therefore, it can be concluded that the test extracts of food plants specifically modulate $GABA_AR$ function.

Diverse effects on GlyR have been observed before for substances addressing $GABA_AR$. Similar to the present results, Weir et al. [23] reported that 5β-pregnan-3α-ol-20-one, a steroid anaesthetic, enhanced the activity of α1β2γ2L-$GABA_AR$ to 722 %, but inhibited the glycine-evoked response of α1-GlyR to 57 %. Other steroid anaesthetics such as alphaxalone and 5α-pregnan-3α-ol-20-one, in

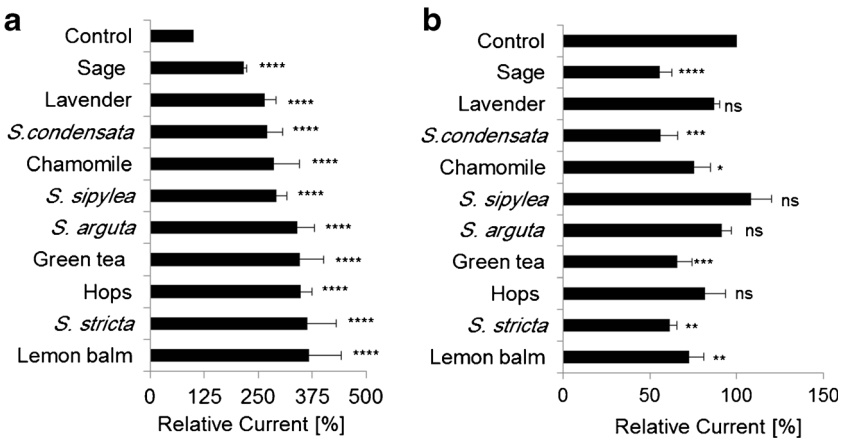

Fig. 2 Potentiating effects of plant extracts at 5 μg/mL on the maximum current (I_{max}) response of (**a**) α1β2-$GABA_AR$ elicited by 1 μM GABA (1 μM GABA without addition =100 %) and (**b**) hα1-GlyR elicited by 30 μM glycine (30 μM glycine without addition =100 %). Mean current amplitudes compared to control ± SEM from 5 to 11 oocytes; ns, not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$

contrast, increased both $GABA_AR$ and GlyR activity [23]. Simultaneous activation of $GABA_AR$ and GlyR may either point towards a dual mechanism, but could also reflect unspecific effects. Our data suggest that the tested plant extracts show specific positive modulatory activity towards the $GABA_AR$, but no or rather inhibitory modulation of GlyR. The present screening assay revealed that lavender flowers, sage leaves, *S. sipylea*, and hop cones potentiate GABA-induced currents at the lowest concentration range rendering those promising candidates for food plants addressing the $GABA_AR$. In concentrations of 0.75 μg/mL or higher, also the extracts of the other *Sideritis* species, chamomile flowers, green tea leaves, and lemon balm leaves potentiated the $GABA_ARs$ response significantly. Further research is now necessary to determine if the concentration of active components is sufficient for $GABA_AR$ modulation *in vivo*. However, several studies have shown that the investigated food plants exert sedative, analgesic, anxiolytic, and antidepressant effects *in vivo* indicating that the extracts may also be able to address $GABA_ARs$ *in vivo* [8–11, 24–26].

Table 1 Endogenous GABA contents of plant extracts and current amplitudes of α1β2- $GABA_AR$ responses to plant extracts without GABA addition

Plant extract	GABA content [μg/mL][a]	I_{rel} [%][b]	n[c]
Sage leaves	0.33 ± 0.05	6 ± 1	7
Lavender flowers	0.22 ± 0.03	3 ± 1	6
S. condensata	0.38 ± 0.01	12 ± 2	6
Chamomile flowers	0.81 ± 0.03	15 ± 1	4
S. sipylea	0.23 ± 0.06	7 ± 2	5
S. arguta	0.21 ± 0.01	3 ± 1	7
Green tea leaves	0.14 ± 0.01	1 ± 0.2	5
Hop cones	0.44 ± 0.02	19 ± 4	5
S. stricta	0.45 ± 0.05	7 ± 1	6
Lemon balm leaves	0.61 ± 0.03	12 ± 2	5

[a] Endogenous GABA contents of plant extracts (1 mg/mL) determined by HPLC–FLD. Values are the mean ± SD from triplicate analysis

[b] Current amplitudes of α1β2-$GABA_AR$ responses to plant extracts without GABA addition at extract concentrations of 7.5 μg/mL. Mean current amplitudes (I_{rel}) compared to the response to 1 μM pure GABA (100 %) ± SEM are shown from at least two independent batches of oocytes

[c] Number of oocyte recordings

It was shown before that volatile extracts of lavender flowers and *Sideritis* species enhanced the GABA-gated Cl^--channel currents in different cell expression systems [14], and some terpenoids, which are positive modulators of $GABA_ARs$, were identified in volatile extracts of *Sideritis* species [27]. In the present study, the aqueous extracts of four *Sideritis* species and also of lavender flowers increased the activity of $GABA_ARs$ even at concentrations as low as 0.5 or 0.75 μg/mL, so that it can be assumed that non-volatile components of *Sideritis* species and lavender flowers may be important factors for physiological effects of these plants.

Hossain et al. [18] reported an aqueous extract of green tea leaves to inhibit the response of $GABA_ARs$. In contrast to this finding, we observed significantly elevated $GABA_AR$ activity up to 3.5-fold (compared to pure GABA) at the highest green tea concentration of 5 μg/mL. This discrepancy may be caused, for example, by the different extraction processes applied in both studies. Green tea leaves contain several physiologically active compounds with diverging effects on $GABA_AR$ activity. For instance, caffeine inhibited the ionotropic $GABA_AR$ response, whereas green tea alcohols, such as leaf alcohol or linalool, potentiated the response [18]. Thus, it can be speculated that different extraction procedures may shift the concentration profiles of inhibitory or activating components in the extracts. Furthermore, the investigated $GABA_ARs$ were composed of different subunits from different species: Hossain et al. [18] expressed bovine α1β1-subunits in *Xenopus laevis* oocytes, whereas the present study used human α1β2-subunits. Some compounds have been reported to induce subunit-selective modulation of $GABA_ARs$, such as salicylidene, which selectively inhibited $GABA_ARs$ containing β1-subunits, but not β2- or β3-subunits [28].

Conclusions

The three-step test system established in the present study proved to be suitable to screen extracts of food plants and to identify specific allosteric $GABA_AR$ modulators. Initially, an effect-guided selection of test extracts was carried out, leading to a high number of positive extracts indicating that the modulation of $GABA_AR$ activity is a common mechanism of sedative or anxiolytic effects of food plant extracts. In future studies, the test system can also be applied for unbiased screening, which may lead to the discovery of novel lead plants. Aqueous extracts of hop cones, *S. sipylea* and, although to a lower extent, of sage leaves and lavender flowers showed specific allosteric $GABA_AR$ modulating activity even at the lowest concentration. Thus, *in vivo* experiments will now be required to understand if the activity observed *in vitro* translates into corresponding behavioural changes. Furthermore, the active components of the extracts have to be determined.

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Compliance with Ethical Standards

Conflict of Interest The scholarship grant by the Turkish Ministry of National Education for Sümeyye Sahin is gratefully acknowledged.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects.

References

1. Granger RE, Campbell EL, Johnston GA (2005) (+)- and (−)-borneol: efficacious positive modulators of GABA action at human recombinant alpha1beta2gamma2L GABA(a) receptors. Biochem Pharmacol 69(7):1101–1111
2. Mohler H (2006) GABAA receptors in central nervous system disease: anxiety, epilepsy, and insomnia. J Recept Signal Transduct Res 26(5–6):731–740
3. Olsen RW, Sieghart W (2008) International Union of Pharmacology. LXX. Subtypes of gamma-aminobutyric acid(a) receptors: classification on the basis of subunit composition, pharmacology, and function. Update. Pharmacol Rev 60(3):243–260
4. Karim N, Wellendorph P, Absalom N, et al. (2012) Low nanomolar GABA effects at extrasynaptic alpha4beta1/beta3delta GABA(a) receptor subtypes indicate a different binding mode for GABA at these receptors. Biochem Pharmacol 84(4):549–557
5. Krasowski MD, Harrison NL (2000) The actions of ether, alcohol and alkane general anaesthetics on GABAA and glycine receptors and the effects of TM2 and TM3 mutations. Br J Pharmacol 129(4): 731–743
6. Sigel E, Steinmann ME (2012) Structure, function, and modulation of GABA(a) receptors. J Biol Chem 287(48):40224–40231
7. Steiger A (2010) Sleep and its modulation by substances that affect $GABA_A$ receptor function. In: Monti JM, Pandi-Perumal SR, Mohler H (eds) GABA and sleep - molecular, functional and clinical aspects. Springer, Basel, pp. 121–146
8. Franco L, Sanchez C, Bravo R, et al. (2012) The sedative effects of hops (*Humulus lupulus*), a component of beer, on the activity/rest rhythm. Acta Physiol Hung 99(2):133–139
9. Emamghoreishi M, Talebianpour MS (2009) Antidepressant effect of *Melissa officinalis* in the forced swimming test. Daru 17(1):42–47
10. Naderi N, Akhavan N, Aziz Ahari F, et al. (2011) Effects of hydroalcoholic extract from *Salvia verticillata* on pharmacological models of seizure, anxiety and depression in mice. Iran J Pharm Res 10(3):535–545
11. Gonzalez-Burgos E, Carretero ME, Gomez-Serranillos MP (2011) *Sideritis* spp.: uses, chemical composition and pharmacological activities–a review. J Ethnopharmacol 135(2):209–225
12. Hossain SJ, Aoshima H, Koda H, et al. (2004) Fragrances in oolong tea that enhance the response of GABAA receptors. Biosci Biotechnol Biochem 68(9):1842–1848
13. Higashiyama A, Htay HH, Ozeki M, et al. (2011) Effects of L-theanine on attention and reaction time response. J Funct Foods 3(3):171–178
14. Kessler A, Villmann C, Sahin-Nadeem H, et al. (2012) $GABA_A$ receptor modulation by the volatile fractions of Sideritis species used as 'Greek' or 'Turkish' mountain tea. Flavour Fragr J 27:297–303
15. Council of Europe (2004) European pharmacopoeia. EDQM, Strasbourg
16. Wichtl M (2004) Herbal drugs and phytopharmaceuticals. CRC Press, Boca Raton FL, USA
17. Zhao M, Ma Y, Wei ZZ, et al. (2011) Determination and comparison of gamma-aminobutyric acid (GABA) content in pu-erh and other types of Chinese tea. J Agric Food Chem 59(8):3641–3648
18. Hossain SJ, Hamamoto K, Aoshima H, et al. (2002) Effects of tea components on the response of GABA(a) receptors expressed in Xenopus oocytes. J Agric Food Chem 50(14):3954–3960
19. Limon A, Gallegos-Perez J-L, Reyes-Riuz JM, et al. (2014) The endogenous GABA bioactivity of camel, bovine, goat and human milks. Food Chem 145:481–487
20. Penas E, Diana M, Frias J, et al. (2015) A multistrategic approach in the development of sourdough bread targeted towards blood pressure reduction. Plant Foods Hum Nutr 70(1):97–103
21. Penas E, Limon RI, Martinez-Villaluenga C, et al. (2015) Impact of elicitation on antioxidant and potential antihypertensive properties of lentil sprouts. Plant Foods Hum Nutr 70(4):401–407
22. Collingridge GL, Olsen RW, Peters J, et al. (2009) A nomenclature for ligand-gated ion channels. Neuropharmacology 56(1):2–5
23. Weir CJ, Ling AT, Belelli D, et al. (2004) The interaction of anaesthetic steroids with recombinant glycine and GABAA receptors. Br J Anaesth 92(5):704–711
24. Vasilopoulou CG, Kontogianni VG, Linardaki ZI, et al. (2013) Phytochemical composition of "mountain tea" from *Sideritis clandestina* subsp. clandestina and evaluation of its behavioral and oxidant/antioxidant effects on adult mice. Eur J Nutr 52(1):107–116
25. Woronuk G, Demissie Z, Rheault M, et al. (2011) Biosynthesis and therapeutic properties of Lavandula essential oil constituents. Planta Med 77(1):7–15
26. Zanoli P, Avallone R, Baraldi M (2000) Behavorial characterisation of the flavonoids apigenin and chrysin. Fitoterapia 71:117–123
27. Kessler A, Sahin-Nadeem H, Lummis SC, et al. (2014) GABA(a) receptor modulation by terpenoids from Sideritis extracts. Mol Nutr Food Res 58(4):851–862
28. Thompson SA, Wheat L, Brown NA, et al. (2004) Salicylidene salicylhydrazide, a selective inhibitor of beta 1-containing GABAA receptors. Br J Pharmacol 142(1):97–106