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Effect of 158 herbal remedies on human TRPV1 and the two-pore domain potassium channels KCNK2, 3 and 9

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ABSTRACT

Background and aim: Herbal medicines are used to treat a broad number of maladies. However, the pharmacological profile of most remedies is poorly understood. We investigated the effect of herbal remedies from kampo, traditional Chinese medicine (TCM) and other phytotherapies on human two-pore domain potassium channels (KCNK channels; TREK-1, TASK-1 and TASK-3) as well as the human TRPV1 channel. KCNK channels are responsible for the background potassium current of excitable cells, thus essential for the maintenance of the resting membrane potential. Hence, modulators of KCNK channels are of medical significance, e.g. for the treatment of sleep disorders and pain. The transient receptor potential channel TRPV1 is a pain detector for noxious heat. Agonists of this receptor are still used for the treatment of pain in ectopic applications.

Experimental procedure: We evaluated the effect of 158 herbal remedies on these channels in a heterologous expression system (*Xenopus laevis* oocytes) using the two-electrode voltage-clamp technique with the aim of increasing the comprehension of their pharmacological profile.

Results and conclusion: Some remedies with modulating effects were identified such as *Angelica pubescens* (radix), which inhibit TASK-1 and TASK-3 channels. Furthermore, the modulatory effects of the most effective remedies on the two TASK family members TASK-1 and TASK-3 correlate positively, reflecting their close relation. For the TRPV1 channel *Terminalia chebula* and *Alchemilla xanthochlora* were identified as potentiators. This study identifies a variety of herbal remedies as modulators of human K2P and TRPV1 channels and gives new insights into the pharmacological profile of these herbal remedies.

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1. Introduction

Transient receptor potential (TRP) channels are non-selective cation channels, which mediate the transmembrane flux of cations like Na⁺ and Ca²⁺, leading to cellular depolarization and the

activation of Ca²⁺-sensitive effector proteins. TRP channels are tetramers with four-fold symmetry and each subunit possesses six transmembrane α -helices (S1 – S6) and a loop between S5 and S6.¹ The polymodal channel TRPV1, which acts as a pain detector for noxious heat, is gated among others by high temperatures (>42 °C) and the spicy tasting natural product capsaicin.² Agonists like capsaicin were shown to ameliorate pain in ectopic applications,³ which is explained by functional desensitization of sensory neurons.⁴ Beside capsaicin, further TRPV1 activating phytochemicals like resiniferatoxin, vanillin, eugenol and piperine were identified.⁵

Two-pore domain potassium (K2P, KCNK) channels, are potassium-selective channels with basal activity that are

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responsible for the leak potassium current present in excitable cells.⁶ They help to maintain the resting membrane potential close to the reversal potential of potassium ions. KCNK channels are dimers and each subunit consists of 4 transmembrane domains (M1 – M4) and two-pore forming loops (P1 and P2) between M1 and M2 (P1) and M3 and M4 (P2).⁷ 18 KCNK family members are described (KCNK1–18) and therefrom 15 are present in the human genome.⁸ KCNK channels are involved in many physiological processes like ion homeostasis, hormone secretion and regulation of neuronal excitability and they could be associated with pathologies like vascular hypertension, arrhythmia, depression and nociception.^{8,9} In this study the human K2P channels TREK-1 (KCNK2), TASK-1 (KCNK3) and TASK-3 (KCNK9), all highly expressed in the brain, were investigated. These channels are targets of volatile anesthetics and contribute to the sedative effects via KCNK channel mediated neuronal hyperpolarization.^{9,10} TREK-1 channels are positively modulated by high temperatures, similar to TRPV1 channels.¹¹ Moreover, both channels are coexpressed within nociceptive C-fibres and TREK-1 leak current was shown to counterbalance the strong depolarizing current generated by TRPV1.¹² TASK-1 and TASK-3 are critical targets of angiotensin II, which modulates aldosterone secretion due to leak current inhibition dependent stimulation of adrenal cells in the zona glomerulosa.¹³ Hence, they possess great importance in the regulation of blood volume and pressure. All in all, KCNK channels are clinically relevant targets and therefore KCNK channel modulators are of great therapeutic purpose. By now a multitude of K2P channel modulators are identified. For example, pungent substances from pepper and chilli (e.g. piperine, hydroxy- α -sanshool, capsaicin and polygodial) negatively modulate KCNK channel activity.^{14,15}

Herbal medicines are based on medicative plants or particular parts of such plants. They have been used for thousands of years in a variety of cultures. Nowadays, herbal medicines, which are typically used as complementary or alternative therapies for western medicine, gain popularity. E.g. traditional Chinese medicine (TCM) is a regular feature of the Chinese health care system. The mode of action was investigated for numerous herbal remedies and a plethora of active principles from natural sources is still identified such as artemisinin (*Artemisia annua* L.) or magnolol from *Magnolia* species.¹⁶ However, the pharmacological profile of various herbal remedies is poorly understood and less is known concerning the effect of herbal remedies on KCNK and TRPV1 channels. Therefore, we evaluated the effect of ethanol tinctures of 158 herbal remedies on heterologously expressed ion channels of the KCNK and TRP family in an electrophysiological screening with the aim of increasing the comprehension of their pharmacological activity on these clinically important ion channels. On that account, human TRPV1 as well as TREK-1, TASK-1 and TASK-3 were heterologously expressed in *Xenopus laevis* oocytes and the effect of herbal remedies was evaluated using the two-electrode voltage-clamp technique (TEVC). In this study, the majority of the tested remedies (121) are originated from the traditional Japanese phytomedicine kampo, which emerged from TCM. Another 15 herbal remedies come from TCM and a number of 22 remedies are originated from other phytotherapies (Supplementary Table 1). This screening revealed a variety of herbal remedies as TRPV1 and KCNK channel modulators. Hence, our study gives new insights into the pharmacological profile of a variety of commonly used herbal remedies.

2. Materials and methods

2.1. In vitro transcription

Heterologous expression in *Xenopus* oocytes requires the injection of RNA. For that point, the receptor coding cDNA was

linearized and transcribed into RNA with the AmpliCap-T7 Message Maker kit (Epicentre, Madison, USA). RNA transcripts were purified by ammonium acetate precipitation and dissolved in nuclease free water. Detailed informations regarding the used plasmids were previously published¹⁷ (TRPV1),¹⁸ (TREK-1 (K2P2.1)),¹⁴ (TASK-1 (K2P3.1) and TASK-3 (K2P9.1).

2.2. Expression system

Xenopus laevis oocytes were obtained as previously described.¹⁷ For heterologous expression of human KCNK and TRPV1 channels, receptor coding cRNA was injected (30–50 nl) using an injection-setup from WPI (Nanoliter 2000, Micro4) (TRPV1 = 10–20 ng/oocyte; KCNK channels = 3 ng/oocyte). Injected oocytes were stored at 17 °C in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM HEPES, 200 U/ml penicillin and 200 µg/ml streptomycin; pH 7.2). Measurements were performed one to five days after cRNA injection.

2.3. Electrophysiology

The electrophysiological recordings were performed using the two-electrode voltage-clamp technique (TEVC) as previously described.¹⁹ Recorded signals were amplified with the Turbo Tec-03X amplifier (npi, Tamm, Germany) and computed by Cellworks 6.1.1 software (npi instruments, Tamm, Germany). Borosilicate glass electrodes (0.5–2 M Ω), pulled with a vertical puller (LM-3P-a List medical), were filled with 3 M KCl. Oocytes were placed in a chamber and constantly washed with normal frog ringer (NFR), containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl₂, 10 mM HEPES (pH 7.2). For the application of test substances (automatic pipette (Eppendorf 1 Research pro) the constant flow was interrupted until the maximal response/effect was transcended/reached.

For the recordings of the stimuli gated receptor TRPV1 and the leaky KCNK channels different recording protocols were used. For TRPV1, oocytes were clamped at a constant membrane potential of –40 or –60 mV and capsaicin was used to verify receptor expression and normalize responses induced/modulated by the tinctures of the investigated remedies (evaluation of modulatory effects = 3 µM capsaicin (close to the EC₅₀ of capsaicin on TRPV1 under our experimental conditions; compare Supplementary Figs. 1) and 10 µM capsaicin for the investigation of direct activating activities). The analysis is based on the current peak amplitude. KCNK channels were investigated as previously described.¹⁴ In short, recordings were performed with a voltage ramp series protocol and the modulation of the current amplitude of the ramps was used to determine the modulatory effect of test substances on the basal activated KCNK channels. The modulation was calculated as the ratio of the current prior and during the application of the tested substances/tinctures (modulation = $I_{\text{application}}/I_{\text{reference}}$). KCNK channel expression was verified on the basis of increased background current and KCNK IV characteristics. Moreover, the recently identified KCNK channel antagonist piperine was used as a positive control for our readout (Fig. 1B).¹⁴

2.4. Tinctures and chemicals

The 158 tinctures of herbal remedies were obtained from Dr. Peter Lepke (Kronen Apotheke Wuppertal, Germany). Tinctures were made as previously described.²⁰ In short, 200 g of crushed plant material were inlaid in 1 l ethanol (45–90% v/v) for 10 days (room temperature). Afterwards, the supernatant was filtrated to get the final tincture. The investigated herbal remedies, which were selected among frequently used remedies, are listed in

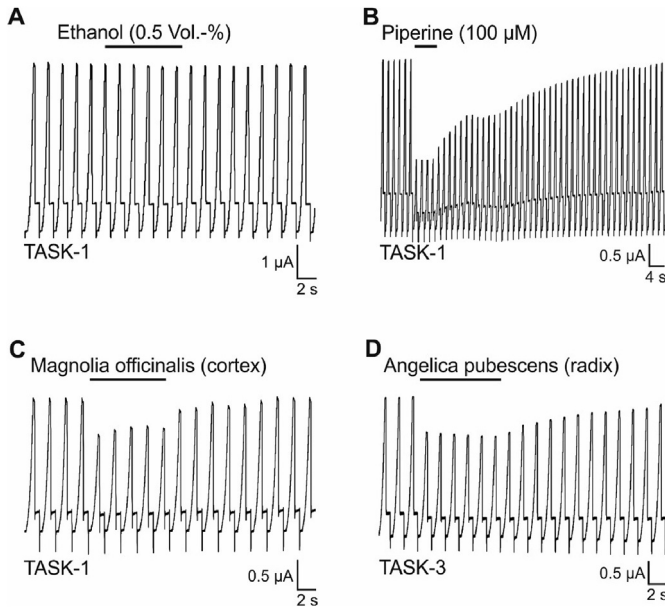


Fig. 1. Exemplary recordings with the ramp series protocol for ethanol (A), the control application of piperine (B) and the negative modulation by *Magnolia officinalis* (cortex) on TASK-1 (C) as well as *Angelica pubescens* (radix) on TASK-3 (D). Recordings were done with a voltage ramp series protocol (ramp duration = 700 ms, voltage range: 100 to 50 mV). Applications are indicated by the application bars above the trace. Ethanol (0.5 vol.-%) exerts no effect (A). The control application of piperine (100 µM) caused a reversible inhibition of the currents. The applications of the tinctures (0.1 vol.-%) reversibly inhibits TASK-1 (C) and TASK-3 (D) currents.

Supplementary Table 1. To avoid misunderstandings due to the bulk of synonyms commonly used for the denomination of herbal remedies, we used the official accepted name from the online database theplantlist (www.theplantlist.org) with the used plant component in brackets behind it. All tinctures were tested in a 1:1000 dilution due to the lack of unspecific effects on uninjected oocytes at this concentration.²¹ Moreover, the tinctures doesn't alter pH of test applications in a 1:1000 dilution. Effects via ethanolic residuals in the diluted tinctures can be excluded due to the absence of ethanol effects in our paradigm (Fig. 1A). Chemicals were purchased from Sigma-Aldrich in a purity >95% (magnolol, osthole, glycyrrhizin, liquiritigenin, tannic acid) > 98% (rosmarinic acid, sclareol, honokiol, aucubin, andrographolide, schizandrin) or > 99% (capsaicin, phorbol 12-myristate 13-acetate (PMA), ferulic acid) and pre-diluted in DMSO or ethanol. Neither ethanol, nor DMSO revealed effects at uninjected oocytes as well as TRPV1 and KCNK channel expressing oocytes in the final concentration.

2.5. Data analysis

Electrophysiological data were collected using the Cellworks Reader 3.7 software (npi instruments, Tamm, Germany) and analyzed with Clampfit v10.2.0.14 (MDS Analytical Technologies, Sunnyvale, USA). The dose-response data were fitted with the Hill equation using OriginPro v.9 (OriginLab Corporation, Northampton, USA). Data are shown as means ± SEM (standard error of the mean). Student's t-test served for the calculation of statistical significance (*p < 0.05; **p < 0.005; ***p < 0.001) and Bonferroni-correction was applied for multiple comparisons. The direct activating effects of the tinctures on TRPV1 were compared with control NFR applications. Modulations were tested for statistical significance against the effect of ethanol (0.1 vol.-%). Correlations were done and checked for significance with STATISTICA 13.0 (StatSoft (Europe) GmbH).

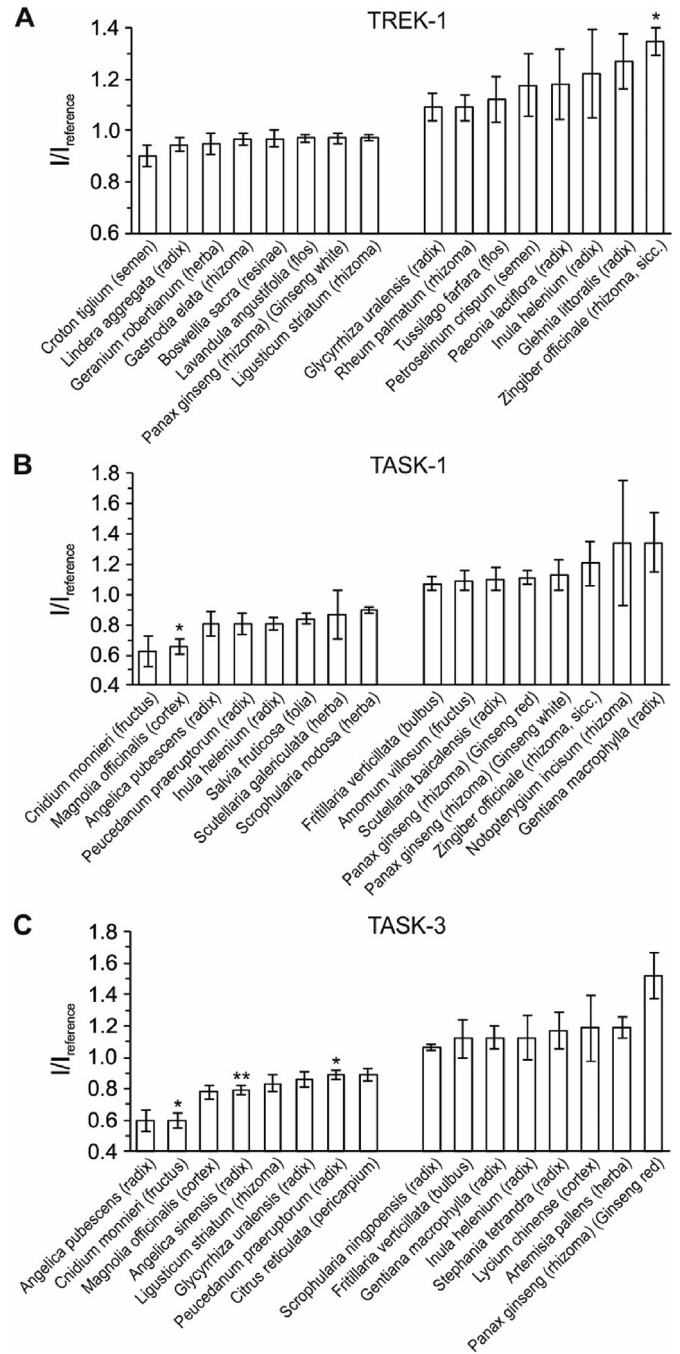


Fig. 2. Best eight negative and positive KCNK channel modulators of 158 herbal remedies for TREK-1 (A), TASK-1 (B) and TASK-3 (C). KCNK channel modulation is calculated as the ratio of the current amplitudes during and prior to the application of the tincture (modulation = $I_{\text{application}}/I_{\text{reference}}$). Data are shown as mean ± SEM (n = 3–9 (A), n = 3–13 (B), n = 3–12 (C) *: p < 0.05; **: p < 0.005; ***: p < 0.001; Student's t-test (Bonferroni corrected)).

3. Results

3.1. Effect of herbal remedies on KCNK2, 3 and 9

The modulatory effect of herbal remedies on KCNK channels was assessed by the modulation of the IV current amplitudes of the voltage ramp series protocol. For all three KCNK channels tinctures with modulatory action were identified. For TASK-1 and TASK-3 the tinctures of *Magnolia officinalis* (cortex), *Angelica pubescens* (radix)

and *Cnidium monnieri* (fructus) were identified as the best inhibitors, reaching an inhibition of approximately 40% (Figs. 1 and 2). The tincture of *Peucedanum praeruptorum* (radix) was a further common inhibitor of TASK-1 and TASK-3 channels among the best eight inhibitory tinctures. For the TREK-1 channel the strongest inhibitory effect was observed by the tinctures of *Croton tiglium* (semen) and *Lindera aggregata* (radix) (Fig. 2). However, the inhibition was around 10%, which is lower compared to the strongest inhibitory effects observed for TASK-1 and TASK-3. Moreover, we identified herbal remedies with potentiating effects, such as *Zingiber officinale* (rhizoma, sicc.) and *Glehnia littoralis* (radix) (TASK-1), *Gentiana macrophylla* (radix) and *Notopterygium incisum* (radix) (TASK-1) as well as *Panax ginseng* (rhizoma) (Ginseng red) and *Artemisia pallens* (herba) for TASK-3 (Fig. 2). A direct comparison of the effects of all tested remedies on the four investigated channels is shown in Supplementary Table 2.

Among the best eight positive and negative modulators some coincidences occurred between the TASK family members TASK-1 and TASK-3, especially for the inhibitory effects (Fig. 2). We asked whether the coincidences occurred just by chance or if our screening reflects the close relation between both TASK family members. For that purpose we correlated the effects of the remedies shown in Fig. 2 for each KCNK channel combination (Fig. 3). For the TASK family members a significant positive correlation was detected (Fig. 3A, p-value = 0.024). However, neither the effects of TASK-1 nor that of TASK-3 correlated with the effects of TREK-1 (Fig. 3B and C; TASK-1 vs. TREK-1: p-value = 0.582; TASK-3 vs. TREK-1: p-value = 0.434). Therefore, our screening reflects the stronger homology of the investigated human TASK family members compared to TREK-1 (amino acid sequence identity: TASK-1 vs. TASK-3 = 74.5%; TASK-1 vs. TREK-1 = 30.5%; TASK-3 vs. TREK-1 = 29.9%).

Furthermore, we evaluated the effect of some secondary herbal metabolites present in the identified modulatory remedies, such as osthole from *Cnidium monnieri* (fructus)²² or magnolol and honokiol from *Magnolia officinalis* (cortex)²³ at a concentration of 1 mM. This approach led to the identification of leonurine as a natural 5-HT_{3A} receptor antagonist.²¹ With the exception of magnolol, which slightly inhibits TASK-3 channels (Supplementary Fig. 5), none of the tested substances (rosmarinic acid, honokiol, sclareol, aucubin, andrographolide, osthole, Phorbol 12-myristate 13-acetate (PMA), ferulic acid, schizandrin, glycyrrhizin, tannic acid and liquiritigenin) revealed significant effects on the tested KCNK channels (data not shown).

3.2. Effect of herbal remedies on TRPV1

In contrast to KCNK channels TRPV1 has low basal activity. Hence, direct activating effects and modulatory effects can be distinguished. First of all, the direct activating properties of the tinctures, which are indicative for TRPV1 agonists within the remedy, were checked. An additional capsaicin containing control tincture for TRPV1 (*Capsicum annuum* (fructus)) was tested. The 1:1000 dilution used in this screening evoked saturating TRPV1 responses and even the 1:10000 dilution reliably activated TRPV1 channels (Supplementary Fig. 2), confirming the feasibility of this approach. However, no significant direct activating herbal remedies were identified in our screening in a 1:1000 dilution (Fig. 4).

In contrast to that, some remedies with modulatory action were found. The negative modulation was weak compared to the positive modulation observed for a few remedies. The strongest inhibitory effect was caused by the tincture of *Carthamus tinctorius* (flos), reaching an inhibition of capsaicin-induced currents of 20% (Fig. 5A, C). Pronounced potentiations were observed for *Alchemilla xanthochlora* (herba) (275%; Fig. 5B, D) and *Terminalia chebula* (fructus)

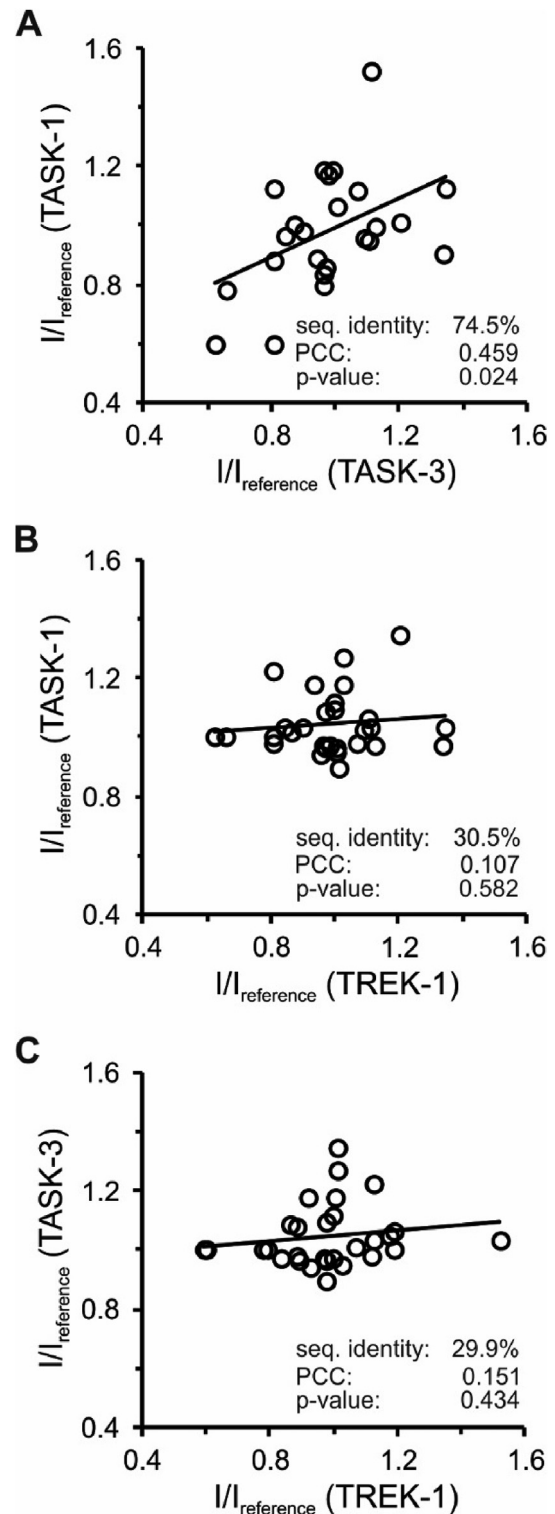


Fig. 3. Screening reflects close relation of the TASK family members TASK-1 and TASK-3. To test the hypothesis of relation dependent coincidences in our screening between the two TASK family members TASK-1 and TASK-3, we correlated the effect of the best eight modulators shown in Fig. 2 for all three channel combinations. Linear regression was performed and the Pearson correlation coefficient (PCC) as well as the significance of this correlation (p-value) are shown in each panel. Moreover, sequence similarity of each channel combination is depicted. A) Screening results of TASK-1 and TASK-3 channels possess a significant positive correlation and 74.5% sequence identity ($n = 24$). B, C) Neither the combination of TASK-1 nor that of TASK-3 with TREK-1 shows a correlation of the strongest modulatory effects ($n = 29$). Furthermore, sequence identity is low for this combinations (TASK-1 vs. TREK-1 = 30.5%, TASK-3 vs. TREK-1 = 29.9%).

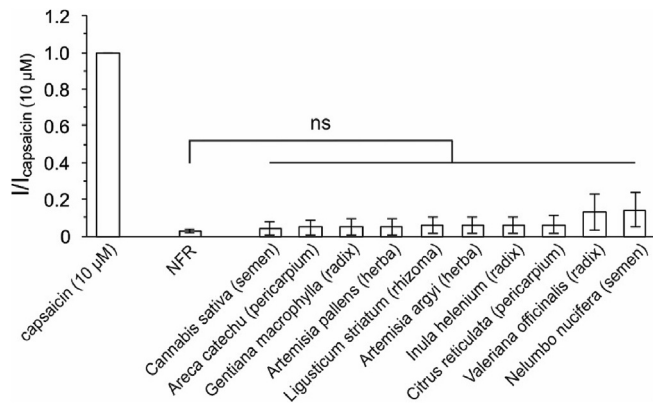


Fig. 4. Strongest ten direct activating remedies for the TRPV1 channel. Direct activating effects were normalized to the amplitude of capsaicin-induced currents (10 µM capsaicin). Data are shown as mean ± SEM. None of the remedies possesses direct activating effects different from NFR application, which mimics the process of application mechanically (ns: $p > 0.05$; Student's t-test (Bonferroni corrected), $n = 3-5$).

(869%; Fig. 5B, E). Interestingly, the tincture of *Croton tiglium* (semen) was inconspicuous in the screening, where the modulations of the capsaicin responses due to the presence of the tinctures were assessed. However, subsequent capsaicin responses were positively modulated, reaching a potentiation of 393% (Fig. 5B, F).

4. Discussion

A plethora of herbal remedies is commonly used in alternative medicines. The variety of ingredients with potential pharmacological action within a single remedy would be able to cause complex physiological interactions. The combination of several remedies in some applications like rikkunshito leads to further increment of pharmacological complexity.²⁰ Detailed knowledge regarding the pharmacological profile of herbal remedies is crucial for the comprehension of their mode/s of action. However, less is known about the effect of herbal remedies on clinically relevant KCNK and TRPV1 channels. This study gives new insights into the pharmacological profile of a broad spectrum of herbal remedies on KCNK2, 3, 9 and TRPV1 channels and could therefore indicate new physiological meanings.

4.1. Effect of herbal remedies on TREK-1, TASK-1 and TASK-3

KCNK channels are of pharmacological interest, e.g. for the treatment of cardiovascular diseases and neurological disorders like pain and depression.⁶ Hence, great effort was done to identify KCNK channel modulators and to determine the underlying modulatory mechanisms. Braun et al. revealed the polycationic KCNK channel inhibitor ruthenium red as a blocker of the extracellular ion pathway.²⁴ In addition to such direct mechanisms, an indirect mechanism for negative TREK channel modulation, including the K2P channel specific extracellular cap as the ligand binding site, is described.²⁵ Moreover, intracellular binding sites are known, such as the binding site of volatile anaesthetics which encloses the C-terminal domain as well as the intracellular loop between M2 and M3.²⁶

Our screening revealed a variety of herbal remedies with modulatory action on the investigated KCNK channels. For example the tincture of *Magnolia officinalis* (cortex), traditionally used for the treatment of seizures, anxiety, depression and sleep disorders,²⁷ inhibited TASK-1 and TASK-3. However, most effects were previously shown to be based on the potentiation of GABA_A

receptors via the lignans magnolol and honokiol.^{21,28} Magnolol slightly inhibited TASK-3 channels in our experiments (Supplementary Fig. 5). However, the observed inhibition by *Magnolia officinalis* (cortex) are scarcely reasoned by magnolol alone and the contribution of KCNK channel inhibition to the beneficial effects of *Magnolia officinalis* (cortex) remains elusive. *Angelica pubescens* (radix) also inhibits both TASK channels (Figs. 1 and 2). It's traditionally used for the treatment of arthritic disease and extracts of the root of *Angelica pubescens* possess anti-inflammatory and analgesic effects.^{29,30} Ingredients like caffeic acid, umbelliferone and osthole were responsible for these effects. Isoimperatorin, a component which was recently shown to ameliorate osteoarthritis, reduced pain in behavioural experiments.²⁹ However, the observed inhibition of TASK-1 and TASK-3 seems contradictory to analgesic effects due to the resulting excitation of nociceptive fibers and there is no hint for KCNK channel relevance in arthritis that could be indicative that the inhibition contributes to the effects of *Angelica pubescens* (radix) traditional use. Beside inhibitory effects, potentiating tinctures were identified. For example the tinctures of *Panax ginseng* (rhizoma) potentiated both investigated TASK family members (KCNK3 and 9). The rhizoma of ginseng is used among others, for the treatment of anxiety and insomnia and possesses sedative effects.^{31,32} Steroid glycosides, called ginsenosides, are thought to be responsible for these effects because of their ability to activate GABA_A receptors.³¹ If the identified potentiation of TASK channels contributes to the effects of ginseng is hard to determine and needs further investigation. *Syzygium aromaticum* (flos) also possesses sedative effects. Nevertheless, no effect on KCNK channels was observed in this study. Hence, the sedative effect seems to be KCNK channel independent and is primary caused by the previously described effect on GABA_A receptors (Hoffmann et al., 2016). Interestingly, the screening results reflect the closer relation of both TASK family members TASK-1 and 3 (Fig. 3). This is most likely explained by higher sequence similarity and therefore more structural accordances regarding the ligand docking sites between TASK-1 and TASK-3.

4.2. Effect of herbal remedies on TRPV1

TRPV1 channels are present in some nociceptive fibers and pivotal for the detection of noxious heat.² Agonists of TRPV1 receptors, such as capsaicin, ameliorate pain in ectopic applications like plasters via functional desensitization of nociceptive fibers.^{3,4} However, none of the tested remedies activated TRPV1 in our screening (Fig. 4) with the exception of the *Capsicum annum* (fructus) control tincture, which leads to saturating TRPV1 responses in a 1:1000 dilution (Supplementary Fig. 2), illustrating the sensitivity of our screening. However, it's noteworthy that the tinctures of *Zingiber officinalis* (rhizoma) failed to elicit responses despite their pungent sensory impression and their TRPV1 activating ingredients gingerol and zingerone.³³ This might be explained by lower concentrations of the active principles within the tincture. Moreover, [8]-gingerol, the best TRPV1 agonist identified in the study from Dedov et al., activated TRPV1 with an approximately 20-fold lower potency as capsaicin and the potency of [6]-gingerol was nearly 200-fold lower.³³

The TRPV1 receptor activity can be modulated by pH and phosphorylation.³⁴ The latter plays an important role in inflammatory hyperalgesia, which is caused by inflammatory mediator triggered PKC activation in nociceptive fibers.³⁵ Moreover, inflammatory hyperalgesia is absent in TRPV1 knockout mice.³⁶ PKC ϵ driven phosphorylation increases TRPV activity by two mechanisms. On the one hand it results in improved membrane trafficking.³⁷ On the other hand, phosphorylation leads to potentiation

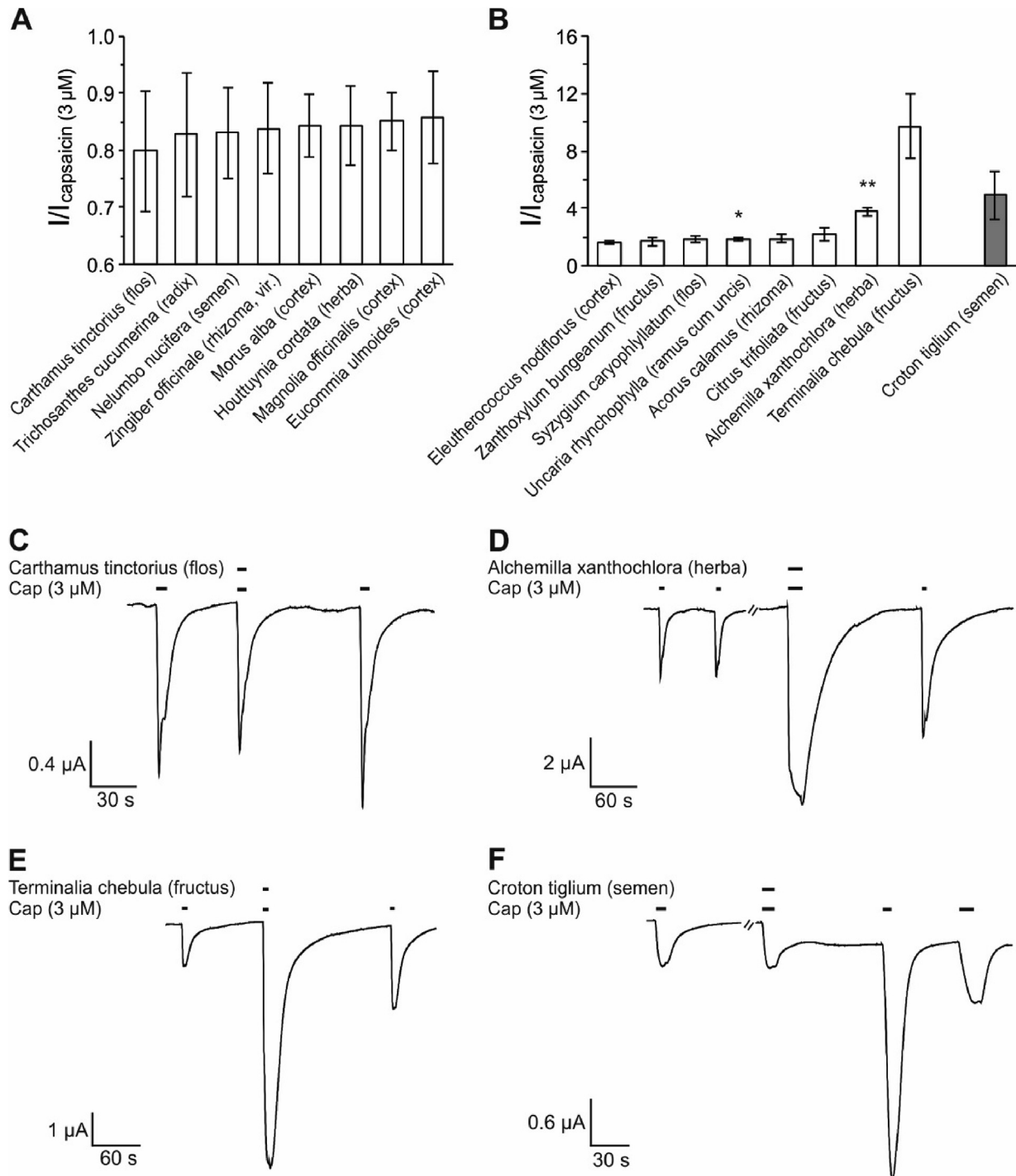


Fig. 5. Best eight negative (A) and positive (B) modulators of the TRPV1 channel. For the assessment of modulatory effects, capsaicin (3 μM) was alternately applied with capsaicin (3 μM) and 0.1 vol.-% of the tincture. The strongest inhibitory effect was observed for *Carthamus tinctorius* (flos) with 20% inhibition (A, C). Potentiating effects (B) were stronger, e.g. for *Alchemilla xanthochlora* (herba) (D) and *Terminalia chebula* (fructus) (E). The tincture of *Croton tiglium* (semen) potentiated capsaicin-induced currents not immediately. However, subsequent capsaicin responses were dramatically potentiated (gray bar in B, F). A, B) Data are shown as mean ± SEM (n = 3–6 (A), n = 3–8 (B), *: p < 0.05; **: p < 0.005; Student's t-test (Bonferroni corrected)). C, D, E, F) Original traces recorded at a potential of –60 mV.

of TRPV1 currents by increasing the channel opening probability.^{35,38} Furthermore, TRPV1 modulation was observed for small molecules like MRS1477, which exerts positive allosteric modulation of TRPV1.³⁹ In this study, positive modulators were hypothesized as drugs for selective analgesia. In contrast to TRPV1 agonists, which cause unselective defunctionalization via general TRPV1 activation, positive modulators could lead to specific

defunctionalization of previously highly activated and hence pain related nociceptors. Our screening revealed potentiating remedies for the TRPV1 receptor. Pronounced effects were observed for *Alchemilla xanthochlora* (herba) and *Terminalia chebula* (fructus) (Fig. 5). *Alchemilla xanthochlora* (herba) is used among others for the treatment of inflammation, diarrhoea, ulcers, menstruation disorders and oedema.⁴⁰ Moreover, it's methanol extract possesses

blood pressure lowering properties and contains a high amount of flavonoids.⁴⁰ Quercetin 3-arabinopyranoside was shown to be a major flavonoid component of *Alchemilla xanthochlora*⁴¹ and there is accumulating evidence for antihypertensive and beneficial cardiovascular effects of quercetin.⁴² Moreover, a contribution of TRPV1 to cardiovascular physiology is discussed.⁴³ *Alchemilla xanthochlora* is traditionally used for the treatment of ulcers and beneficially effected aphthous stomatitis in a clinical trial.⁴⁴ Capsaicin was also shown to act antiulcerogenic.⁴⁵ Interestingly, *Terminalia chebula* (fructus) showed antiulcerogenic activity in experimentally induced ulcer in rats, too.⁴⁶ Future studies should clarify the relevance of TRPV1 potentiation in the antihypertensive and antiulcerogenic effects of *Alchemilla xanthochlora* and *Terminalia chebula* (fructus). The tincture of *Croton tiglium* (semen) didn't show direct modulation of capsaicin-induced responses. However, it potentiated subsequent TRPV1 currents (Fig. 5B, F). *Croton tiglium* (semen) is traditionally used for the treatment of constipation, gastrointestinal disorders, inflammation, peptic ulcer, visceral pain, and headache and is a natural source for phorbol and phorbol esters such as phorbol-12-myristate-13-acetate (PMA).^{47,48} PMA activates PKC, which potentiates TRPV1 function via phosphorylation.⁴⁹ Hence, the observed potentiation of TRPV1 channels through PMA and the tincture of *Croton tiglium* (semen) (Fig. 5B, F; Supplementary Figs. 3 and 4) is indirect and not caused via direct TRPV1 modulation.

5. Conclusion

In this study, we investigated the effect of 158 herbal remedies on heterologously expressed human TREK-1, TASK-1, TASK-3 as well as TRPV1 channels with the aim of increasing the comprehension of their pharmacological action on these clinical relevant targets. A variety of remedies exhibited modulatory effects, like *Magnolia officinalis* (cortex), which inhibits the activity of TASK-1 and TASK-3. For the human TRPV1 receptor, we detected *Alchemilla xanthochlora* (herba) and *Terminalia chebula* (fructus) as positive modulators. This potentiation could be responsible for the antihypertensive and antiulcerogenic effects of these remedies. However, the active principles of the identified active remedies are unknown and the physiological relevance of the observed effects on KCNK and TRPV1 channels should be assessed in future studies. All in all, this study gives new insights into the pharmacological effects of herbal remedies on KCNK and TRPV1 channels.

Author contributions

RH, LB, PZ, HH, KS, MW and GG conceived and designed the experiments. RH, LB, PZ, ST, KL, AG, KH, JL and MB performed the experiments. RH, LB, PZ and GG wrote the paper.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtcme.2020.04.005>.

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