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2 ***In vitro* screening of herbal medicinal products for their supportive**  
3 **curing potential in the context of SARS-CoV-2**

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24

## 25 Abstract

26 **Background:** Herbal medicinal products have a long-standing history of use in the therapy of  
27 common respiratory infections. In the COVID-19 pandemic, they may have the potential for  
28 symptom relief in non-severe or moderate disease cases. Here we describe the results derived by  
29 *in vitro* screening of five herbal medicinal products with regard to their potential to i) interfere  
30 with the binding of the human Angiotensin-converting enzyme 2 (ACE2) receptor with the SARS-  
31 CoV-2 Spike S1 protein, ii) modulate the release of the human defensin HBD1 and cathelicidin LL-  
32 37 from human A549 lung cells upon Spike S1 protein stimulation and iii) modulate the release of  
33 IFN- $\gamma$  from activated human peripheral blood mononuclear cells (PBMC). The investigated  
34 extracts were: Sinupret extract (SINx), Bronchipret thyme-ivy (BRO TE), Bronchipret thyme-  
35 primrose (BRO TP), Imupret (IMU), and Tonsipret (TOP).

36 **Methods:** The inhibitory effect of the herbal medicinal products on the binding interaction of  
37 Spike S1 protein and the human ACE2 receptor was measured by ELISA. The effects on  
38 intracellular IFN- $\gamma$  expression in stimulated human PBMCs were measured by flow cytometry.  
39 Regulation on HBD1 and LL-37 expression and secretion was assessed in 25d long-term cultured  
40 human lung A549 epithelial cells by RT-PCR and ELISA.

41 **Results:** IMU and BRO TE concentration-dependently inhibited the interaction between spike  
42 protein and the ACE2 Receptor. However, this effect was only observed in the cell-free assay at a  
43 concentration range which was later on determined as cytotoxic to human PBMC. SINx, TOP and  
44 BRO TP significantly upregulated the intracellular expression of antiviral IFN $\gamma$  from stimulated  
45 PBMC. Co-treatment of A549 cells with IMU or BRO TP together with SARS-CoV-2 spike protein  
46 significantly upregulated mRNA expression (IMU) and release (IMU and BRO TP) of HBD1 and LL-  
47 37 (BRO TP).

48 **Conclusions:** The *in vitro* screening results provide first evidence for an immune activating  
49 potential of some of the tested herbal medicinal extracts in the context of SARS-CoV-2. Whether  
50 these could be helpful in prevention of SARS-CoV-2 invasion or supportive in recovery from SARS-  
51 CoV-2 infection needs deeper understanding of the observations.

## 52 Introduction

53 All over the world the population is struggling with the outbreak of COVID-19. Effective  
54 antiviral therapies are unavailable so far, comprehensive vaccination has yet to be achieved and  
55 various mutants of the enveloped, single-stranded RNA virus SARS-CoV2 have emerged (Greaney  
56 et al., 2021). Thus it is of critical importance to search for compounds, which could help in fighting  
57 the virus pandemic. Testing the effectiveness of different drug types used previously in the  
58 treatment of other diseases is here one strategy to accelerate the development. Herbal medicinal  
59 products have the potential to interfere with various steps of the viral replication cycle (Brendler  
60 and Al-Harrasi, 2020; Mani et al., 2020). Besides, they have been reported to exhibit an anti-  
61 inflammatory and immune modulating potential and thus may also be supportive in prevention  
62 or attenuation of mild to moderate SARS-CoV-2 infections (Glatthaar-Saalmüller B, 2011; Seibel  
63 et al., 2015; Seibel et al., 2018). In this pilot screening study, five herbal medicinal products,  
64 marketed for the treatment against respiratory infections (Bachert, 2020; Rossi et al., 2012; Seibel  
65 et al., 2015; Seibel et al., 2018), were explored for their potential to i) interfere with the binding  
66 of the human Angiotensin-converting enzyme 2 (ACE2) receptor with the SARS-CoV-2 spike  
67 protein. The ACE2 receptor found in various organs including type I and II pneumocytes,  
68 endothelial cells, oral and nasal mucosa but also intestinal tissues, liver, kidney, or brain  
69 (Hamming et al., 2004; Xu et al., 2020) has been identified as the key cellular receptor, which  
70 facilitates uptake of the SARS-CoV-2 virus into the host cell (Ortiz et al., 2020). Drugs targeting the  
71 interaction between the spike protein receptor binding domain of SARS-CoV-2 and ACE2 receptor  
72 may thus offer some protection against this novel viral infection (Hu et al., 2020) (Yang et al.,  
73 2020). ii) We investigated whether the extracts can modulate the release of the defensin HBD1  
74 and cathelicidin LL-37 from human A549 lung cells upon SARS-CoV-2 spike protein stimulation.  
75 In the innate immune system, these antimicrobial peptides (AMPs), have a non-enzymatic  
76 inhibitory effect on a broad spectrum of microorganisms (Wilson et al., 2013). Human beta  
77 defensins (HBDs) have known antiviral effects on both enveloped and non-enveloped viruses  
78 (Ding et al., 2009). Due to the relative nonspecificity of the targets of defensins compared to those

79 of the adaptive arm, antiviral applications of defensins are conceptually ideal for protection  
80 against different viral infections (Holly et al., 2017; Wilson et al., 2013). iii) We investigated  
81 whether the plant extracts could modulate the activated immune system in terms of regulating  
82 anti-viral interferon gamma (IFN- $\gamma$ ) production. This type II interferon is produced by T  
83 lymphocytes and NK cells and essential for antiviral defense. It suppresses virus replication and  
84 activates T cell cytokine production (Levy and García-Sastre, 2001).

85

## 86 **Methods**

### 87 **Extracts**

88 All plant extract mixtures were provided by Bionorica SE either in dried or fluid form. The dried  
89 extracts were used in different dilutions. The following extracts (Bionorica SE, Neumarkt in der  
90 Oberpfalz, Germany) were investigated:

91 Bronchipret® thyme-ivy (BRO TE), an extract of thyme herb (*Thymus vulgaris* L. or *Thymus zygis*  
92 L.) and ivy leaves (*Hedera helix* L.). BRO-TE is a mixture of fluid extracts of thyme herb (extractant:  
93 ammonia solution 10% (m/m) / glycerol (85%) (m/m) / ethanol 90% (v/v) / water  
94 (1:20:70:109); drug-extract ratio (DER): 1:2–2.5) and ivy leaves (extractant: ethanol 70% (v/v);  
95 DER: 1:1) as contained in Bronchipret® syrup with a thyme/ivy fluid extract ratio of 10:1. In order  
96 to minimise ethanol content in the test system the extract mixture was dealcoholised by rotary  
97 evaporation to a final ethanol content of 1% (v/v). To control for loss of volatile ingredients,  
98 specific identity tests were performed with the concentrate.

99 Bronchipret® thyme-primrose (BRO TP), an extract of thyme herb (*Thymus vulgaris* L. or *Thymus*  
100 *zygis* L.) and primrose root (*Primula veris* L. or *Primula elatior* (L.) HILL). BRO-TP is a mixture of  
101 genuine dry extracts of thyme herb (extraction solvent: ethanol 70% (v/v); DER: 6–10:1) and  
102 primrose root (extractant: ethanol 47% (v/v); DER 6–7:1) as contained in Bronchipret® TP film-  
103 coated tablets without excipients and with a final thyme/primrose dry extract ratio of 2.67:1. A  
104 stock solution of 100 mg/ml was prepared in 50% EtOH

105 Imupret® (IMU), 100 g Imupret oral drops contain: 29 g of an ethanolic-aqueous extract  
106 (extraction solvent: Ethanol 59 Vol.-%) out of Marshmallow root (*Altheae officinalis* L.) 0.4 g,  
107 Chamomille flowers (*Matricaria recutita* L.) 0.3 g, Horsetail herb (*Equisetum avense* L.) 0.5 g,  
108 Walnut leafs (*Juglans regia* L.) 0.4 g, Yarrow herb (*Achillea millefolium* L.) 0.4 g, Oak bark (*Quercus*  
109 *robur* L.) 0.2 g, Dandelion herb (*Taraxacum officinale* F.H. WIGGERS) 0.4 g. Total-ethanol 19 %  
110 (v/v).. In order to minimise ethanol content in the test system the extract mixture was  
111 dealcoholised (> 0.5% (v/v)) by rotary evaporation. The content quality of the dealcoholized test  
112 item complied with Imupret® oral drops as checked by identity tests and quantitative analysis.  
113 Sinupret® extract (SINx), combined genuine dry extract (BNO 1011) of gentian root (*Gentiana*  
114 *lutea* L.), primrose flower (*Primula veris* L.), sorrel herb (*Rumex crispus* L.), elder flower  
115 (*Sambucus nigra* L.) and verbena herb (*Verbena officinalis* L.) with a ratio of 1:3:3:3:3 (extraction  
116 solvent: ethanol 51% (v/v); DER 3–6:1) as contained in Sinupret® extract coated tablets without  
117 excipients.  
118 Tonsipret® (TOP), homeopathic dilution for tonsillitis tablets containing 37.5% Dilution Capsicum  
119 D3 (*Capsicum annuum* L.), 37.5% Dilution Guaiacum D3 (*Guaiacum officinale* L./ *Guaiacum*  
120 *sanctum* L.) and 25.0% mother tincture Phytolacca (*Phytolacca americana* L.). In order to minimise  
121 ethanol content in the test system the mixture was dealcoholized (> 0.5% (v/v)) by rotary  
122 evaporation. The quality of the dealcoholised test item complied with the corresponding  
123 manufacturing stage of the herbal medicinal product Tonsipret® as checked by identity analyses.  
124 All extracts were centrifuged (16.000 x g, 3 min, RT) and the supernatant was used for the  
125 experiments. The final concentration of the solvent (ethanol) was less than 0.5% in all assays.

126

### 127 **Human A549 lung cell line and primary human PBMC**

128 The human lung adenocarcinoma A549 cell line was purchased from DSMZ (Germany). For the  
129 experiments, cells were used at low passage number after thawing. Cells were cultured in RPMI  
130 medium containing 10% heat inactivated FCS, 1% penicillin/streptomycin and 1% L-glutamine  
131 for 25 days for differentiation according to the protocol by Cooper et al., 2016(Cooper et al., 2016).

132 After 25 days, cells were exposed to the extracts for different time points either with or without  
133 co-treatment with SARS-CoV-2 spike protein (Trenzyme, Germany).

134 For PBMC isolation, blood was taken in Li-Heparin vacutainers from healthy volunteers at the  
135 University of Freiburg - Medical Center after written informed consent. The study was approved  
136 by the Ethics Committee of the University of Freiburg and carried out according to their guidelines  
137 and regulations (ethical vote 373/20). PBMC isolation was done by centrifugation on a  
138 LymphoPrep™ gradient using SepMate centrifugation tubes (Stemcell Technologies, Germany),  
139 cells were then washed twice with PBS and viability and concentration was determined using the  
140 trypan blue exclusion test.

141

#### 142 **Assessment of SARS-CoV-2 Spike-ACE2 binding inhibition**

143 The capacity of extracts to inhibit the SARS-CoV-2-Spike-ACE2 binding was tested using the SARS-  
144 CoV-2 (COVID-19) Inhibitor screening kit (BioCat GmbH, Heidelberg, Germany) according to the  
145 manufacturer's instructions. This colorimetric ELISA assay measures the binding between  
146 immobilized SARS-CoV-2 Spike protein RBD and biotinylated human ACE2 protein. The  
147 colorimetric detection is done using streptavidin-HRP followed by TMB incubation.

148

#### 149 **Quantification of human defensins by qRT-PCR**

150 A549 cells were treated with extracts with/without Sars-CoV-2 Spike S1 protein (Trenzyme,  
151 Germany) for different time points. Total RNA was isolated from the cells using the RNeasy mini  
152 isolation kit from Qiagen (Germany) with a purification step using the RNase-free DNase kit  
153 (Qiagen) according to the manufacturer's instructions. The quantity and quality of RNA were  
154 determined by a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Germany). 25 ng  
155 RNA was used for qPCR reaction. Changes in mRNA levels were measured by qRT-PCR as  
156 described previously (Nam et al., 2006; Pierson et al., 2013).

157

#### 158 **Quantification of HBD1 and LL-37 peptide release from A549 cells**

159 HBD1 and LL-37 peptide release was quantified in supernatants from 25 day long-term cultured  
160 A549 cells using a human BD-1 Standard ABTS ELISA Development Kit (PeproTech, Germany) and  
161 LL-37 human ELISA kit (HycultBiotech, Germany) according to the manufacturer's instructions.

162

### 163 **Flow cytometric analysis of intracellular IFN-gamma expression**

164 PBMC were treated with extracts for 6h and co-stimulated with 50 ng/ml phorbol 12-myristate  
165 13-acetate (PMA), 1 µg/ml ionomycin, and a SARS-CoV-2 S peptide pool (Miltenyi Biotec,  
166 Germany) for immune reaction together with 10 µg/ml brefeldin A. Cells were then collected and  
167 processed for intracellular staining with anti-IFN-γ-FITC monoclonal antibody (Miltenyi Biotec,  
168 Germany). Changes in the subset of lymphocyte cells and cytokine production (IFN-γ) were  
169 assessed using flow cytometry (FACS Calibur, BD, Germany).

170

### 171 **Determination of cell viability**

172 The LIVE/DEAD Fixable Far Red Dead Cell Stain Kit (Thermo Fisher Scientific, Germany) and the  
173 LDH-Glo Cytotoxicity Assay (Promega, Germany) were used to determine cytotoxicity of extracts  
174 in PBMC and A549 cells, respectively after 24 hour exposure, according to the manufacturer's  
175 instructions.

176

### 177 **Statistics**

178 Results were analysed using the GraphPad Prism 6.0 software (La Jolla, California, USA). Data  
179 were presented as means + SD. Statistical significance was determined by the one way ANOVA test  
180 followed by Holm-Sidak's correction. P values <0.05 (\*) were considered statistically significant  
181 and <0.01 (\*\*) were considered highly statistically significant.

182

183

## 184 Results

### 185 Effect of extracts on SarsCoV2-Spike-ACE2 binding inhibition

186 The inhibitory potential of the extracts between the SARS-CoV-2 spike protein and the ACE2  
187 receptor was tested in a cell free assay. A concentration-dependent inhibitory effect on spike  
188 binding to ACE2 was seen with extract IMU (maximum of  $86\% \pm 1.3$ ), extract BRO TP (maximum  
189 of  $44\% \pm 16.8$ ) and extract BRO TE (maximum of  $77\% \pm 10.8$ ). For extracts TOP, no relevant effect  
190 (data not shown) and for SINx minor effects were observed (figure 1).

191

### 192 Effect of extracts on IFN- $\gamma$ intracellular expression in activated human PBMC

193 First, cytotoxicity of the extracts was quantified after 24 h exposure in human PBMC. Relevant  
194 toxicity (i. e.  $>10\%$ ) was evident for extract IMU at  $\geq 1:40$  dilution, for extract BRO-TP at  $\geq 0.25$   
195 mg/ml and for BRO-TE at  $\geq 1:200$  dilution. For TOP and SINx, no relevant toxicity could be seen  
196 in the applied *in vitro* model. Upon stimulation with a mixture of SARS-CoV-2 S peptide pool, PMA  
197 and ionomycin, a significant increase in intracellular IFN- $\gamma$  expression (in range of 7-15%) could  
198 be seen (see figure 2). Compared to control cells, a significant increase in IFN- $\gamma$  expression could  
199 be seen in stimulated PBMC with extract TOP ( $145\% \pm 27$  at a 1:60 dilution), extract SINx ( $169\%$   
200  $\pm 26$  at 1.25 mg/ml) and extract BRO TE ( $157\% \pm 31$  at a 1:1000 dilution) (figure 2). For extract  
201 IMU and BRO TP, no additional increase in IFN- $\gamma$  expression was seen. In contrast, at  $\geq 0.167$   
202 mg/ml, extract BRO TP had an inhibitory effect on INF- $\gamma$ , which is likely due to beginning  
203 cytotoxicity.

204

### 205 Effects of extracts on mRNA expression and peptide release of HBD1 and LL-37

206 For first insights into the effects of the test items on cellular defense mechanisms we analysed the  
207 mRNA expression and secretion of HBD-1 and LL-37 in human A549 cells. Based on the findings  
208 in human PBMC, the absence of relevant cytotoxicity (i. e.  $> 10\%$ ) upon extract exposure was first  
209 confirmed in 25d long-term cultured A549 cells using the LDH-Glo cytotoxicity assay (data not  
210 shown). We then treated the cells with Sars-CoV-2 Spike S1 protein together with the extracts for

211 24 h. Thereafter, a significant increase in HBD1 mRNA expression was measured for IMU (154%  
212  $\pm$  33 at 1:100 dilution) and extract SINx (144%  $\pm$  27 at 1.67mg/ml) as compared to control (figure  
213 3). A similar trend was observed in LL-37 regulation upon co-treatment with IMU (184% $\pm$ 68 at  
214 1:60 dilution), BRO-TP (161%  $\pm$  17 at 0.167 mg/ml) or BRO-TE (130%  $\pm$  2 at 1:400 dilution)  
215 together with the SARS-CoV-2 spike protein. Additionally, the extract-induced secretion of HBD1  
216 and LL37 was analysed by ELISA in presence and absence of the spike protein at different time  
217 points (figure 4). Both extracts, IMU and BRO TP, could significantly trigger also the secretion of  
218 HBD1 peptide with or without S1 spike protein co-treatment from A549 cells. Extract BRO TP  
219 could also significantly trigger the secretion of LL-37 either with S1 spike protein co-treatment or  
220 without. This was not the case for extract IMU.

221

222

## 223 Discussion

224 Herbal extracts are known to induce diverse cellular defense mechanisms following viral infection  
225 of human cells (Khare et al., 2020; Kim et al., 2010). Using different screening assays, we show  
226 here that extracts of marketed herbal medicinal products elicit potential beneficial effects *in vitro*  
227 in terms of cellular defense activation upon challenge with SARS-CoV-2 spike protein or peptide  
228 mix.

229 The first step of viral infection is the interaction of the virus with the host cell. In case of SARS-  
230 CoV-2 the spike protein interacts with the ACE2 receptor on the surface of epithelial cells such as  
231 from in the oral cavity or respiratory tract (Mani et al., 2020; Tay, 2020; Xu et al., 2020). In our  
232 study, extracts from IMU and BRO TE were able to interfere with the binding between the S1 spike  
233 protein and the human ACE2 receptor. Computational binding studies show that common herbal  
234 secondary metabolites like luteolin or quercetin could be able to bind and block the ACE2 receptor  
235 (Khare et al., 2020; Mani et al., 2020; Muchtaridi et al., 2020), and also bind the SARS-CoV-2 spike  
236 protein. A demonstration of interference with SARS-CoV-2 spike –ACE2 binding using cell-free or  
237 *in vitro* assays is missing for most of them, though. So, for quercetin and its metabolites, inhibition  
238 of recombinant human ACE2 (rhACE2) activity has been reported *in vitro* (Liu et al., 2020). The  
239 rhACE2 activity was then inhibited by rutin, quercetin-3-O-glucoside, tamarixetin, and 3,4-  
240 dihydroxyphenylacetic acid by 42–48%. With an IC<sub>50</sub> of 4.48  $\mu$ M, quercetin was the most potent  
241 rhACE2 inhibitor tested in this study. The herbal extracts investigated here contain high amounts  
242 of these plant metabolites (Assessment report on *Quercus robur* L.; EMA, 2013;  
243 EMA/HMPC/136583/2012 Assessment report on *Primula veris* L. and/or *Primula elatior* (L.) Hill,  
244 2012; EMA/HMPC/342334/2013 Assessment report on *Thymus vulgaris* L. and L., 2012; Seibel  
245 et al., 2015; Seibel et al., 2018). Thus, these constituents could account or add to the inhibitory  
246 effect observed by the two herbal extract mixtures IMU and BRO TE and it will be important to  
247 further ascertain this hypothesis in ongoing studies. The products are mixtures made from several  
248 medicinal plants, thus it might also be helpful to determine the plant with the largest share on this  
249 effect. This is the more important as the inhibition was observed at a concentration range which

250 turned out to be cytotoxic to human PBMC and which is not relevant for the direct use in practice.  
251 But, IMU and BRO TP also activated the human innate immune defense by increasing the level of  
252 defensin HBD1 and/or cathelicidin LL37 upon SARS-CoV-2 spike protein stimulation and this was  
253 evident at much lower concentrations. By regulating chemokine and cytokine production, both  
254 AMPs help to maintain homeostasis of the immune system and display antiviral properties, as for  
255 example evidenced by gene regulation upon viral challenge or expression in cells involved in viral  
256 defense (Wilson et al., 2013). LL-37, consisting of 37 amino acids and an overall positive net  
257 charge (+6) can also eliminate microbes directly by electrostatic binding to negatively charged  
258 molecules on microbial membranes (PrePrint Roth et al., 2020). In secretions from lung and nose  
259 it was found that LL-37 could reach high concentrations indicating to a relevant role in lung  
260 immune defense mechanisms (Kim et al., 2003; Mansbach et al., 2017; Schaller-Bals et al., 2002).  
261 Defensins can be detected in the mucosa of all respiratory tissues, including pharynx (22, 23). The  
262 effect of AMPs on virus infections appear to be specific to the virus, AMP, and also target cell and  
263 to our knowledge, a direct antiviral efficacy of HBD-1 or LL-37 against SARS-CoV-2 has not been  
264 shown so far (Ding et al., 2009). However, interestingly, for LL-37 a binding to the SARS-CoV-2  
265 spike protein and an inhibitory action of LL-37 on the spike protein to its entry receptor have been  
266 reported using binding competition studies (PrePrint Roth et al., 2020). In another study, high  
267 structural similarity of LL-37 to the N-terminal helix of the receptor-binding domain of SARS-  
268 CoV-2 was reported (PrePrint Kiran Bharat Lokhande, 2020). There is also currently  
269 speculation that vitamin D, by upregulation of LL-37, could be beneficial on the course of COVID-  
270 19 disease (Crane-Godreau et al., 2020) and LL-37 even has been proposed by some researchers  
271 for treatment of COVID-19 patients (PrePrint Zhang et al., 2020). Thus, stimulating LL37  
272 expression, as observed here *in vitro* with BRO TP, might have several advantages during early  
273 phase of SARS-CoV-2 entry, but this hypothesis, again, needs further verification.  
274 BRO TE and SINx extracts could be shown in the present study to further boost the immune  
275 response of PBMC in terms of intracellular IFN- $\gamma$  expression, which was activated by a mixture  
276 including SARS-CoV-2 spike peptides. Once a virus has entered the cell and is replicated, a host

277 immune response starts to combat viral infection (Tay, 2020). An early interferon response of the  
278 host has been reported to be essential for an effective defense against SARS-CoV-2 infection (Liao  
279 et al., 2020; Tay, 2020). In turn, a decrease of IFN $\gamma$  positive T-helper cells might increase the risk  
280 for severe courses of COVID-19 (Pierce et al., 2020; Sattler et al., 2020). The here reported *in vitro*  
281 findings provide a first hint that BRO TE and SINx may help in further IFN $\gamma$  expression during  
282 infection. On the one hand, INF- $\gamma$  is essential for the antiviral defense, but on the other hand  
283 persistent high levels of INF- $\gamma$  have been reported to worsen the systemic inflammation,  
284 intensifying tissue injury and organ failure (Wang et al., 2020) during COVID-19 disease. This yet  
285 known ambiguous role of IFN- $\gamma$  in the course of SARS-CoV-2 infection here also asks for special  
286 attention.

287 In conclusion, the marketed herbal medicinal products tested in this study demonstrate a range  
288 of potential support of the human immune defense against SARS-CoV-2 infection. Whether the  
289 observed effects could be relevant for the systemic use in man or limited to local effects e. g. in the  
290 oral cavity, needs to be investigated. Further confirmatory mechanistic studies are necessary to  
291 gain a deeper understanding of the reported observations and the transferability of the *in vitro*  
292 results to the clinical situation needs to be tested.

293

## 294 List of Abbreviations

ACE2	Angiotensin-converting enzyme 2
AMP	Antimicrobial peptide
BRO-TE	Bronchipret® thyme-ivy
BRO-TP	Bronchipret® thyme-primrose
CC <sub>50</sub>	Cytotoxic concentration
COVID-19	Coronavirus disease 2019
DER	Drug-extract ratio
IMU	Imupret®

PMA	Phorbol-12-myristat-13-acetat
PBS	Phosphate-buffered saline
FCS	Fetal calf serum
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SD	Standard Deviation
SINx	Sinupret extract®
TOP	Tonsipret®

295

## 296 **Declarations**

### 297 **Ethics approval and consent to participate**

298 The study on human PBMC was approved by the Ethics Committee of the University of Freiburg  
299 and carried out according to their guidelines and regulations (ethical vote 373/20).

300

### 301 **Consent for publication**

302 Not applicable. This article does not contain data from any individual person.

303

### 304 **Availability of data and material**

305 The datasets used and/or analyzed during the current study are available from the  
306 corresponding author on request.

307

### 308 **Competing interests**

309 H.T.T. and E.L. report no competing interests

310 P.P., J.S. and DAS are employees of Bionorica SE

311

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313 The study was funded by a grant from Bionorica SE.

314 **Authors' contributions**

315 Study design: E.L..All authors contributed to the study conception, Design of experiments, data

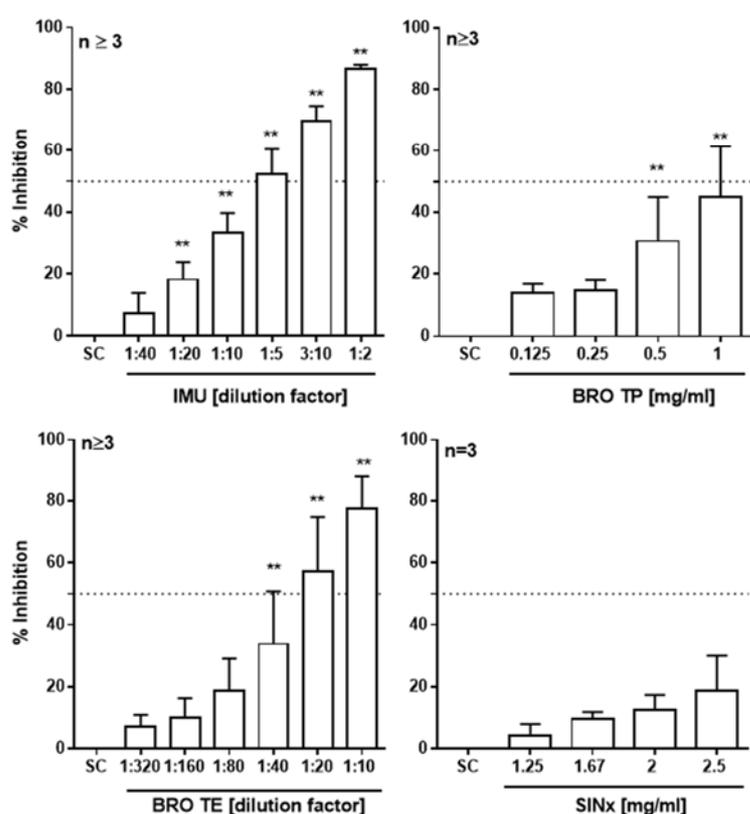
316 acquisition, analysis of data: H.T.T, E. L. The first draft of the manuscript was written by H.T.T.

317 and E.L. All authors commented on previous versions of the manuscript.

318

319

Figure 1:



320

321 **Figure1: Efficacy of Spike-ACE2 binding inhibition by the tested extracts.** The SARS-CoV-2 S

322 protein RBD coated plate was incubated with plant extracts and biotinylated human ACE2 for 1 h.

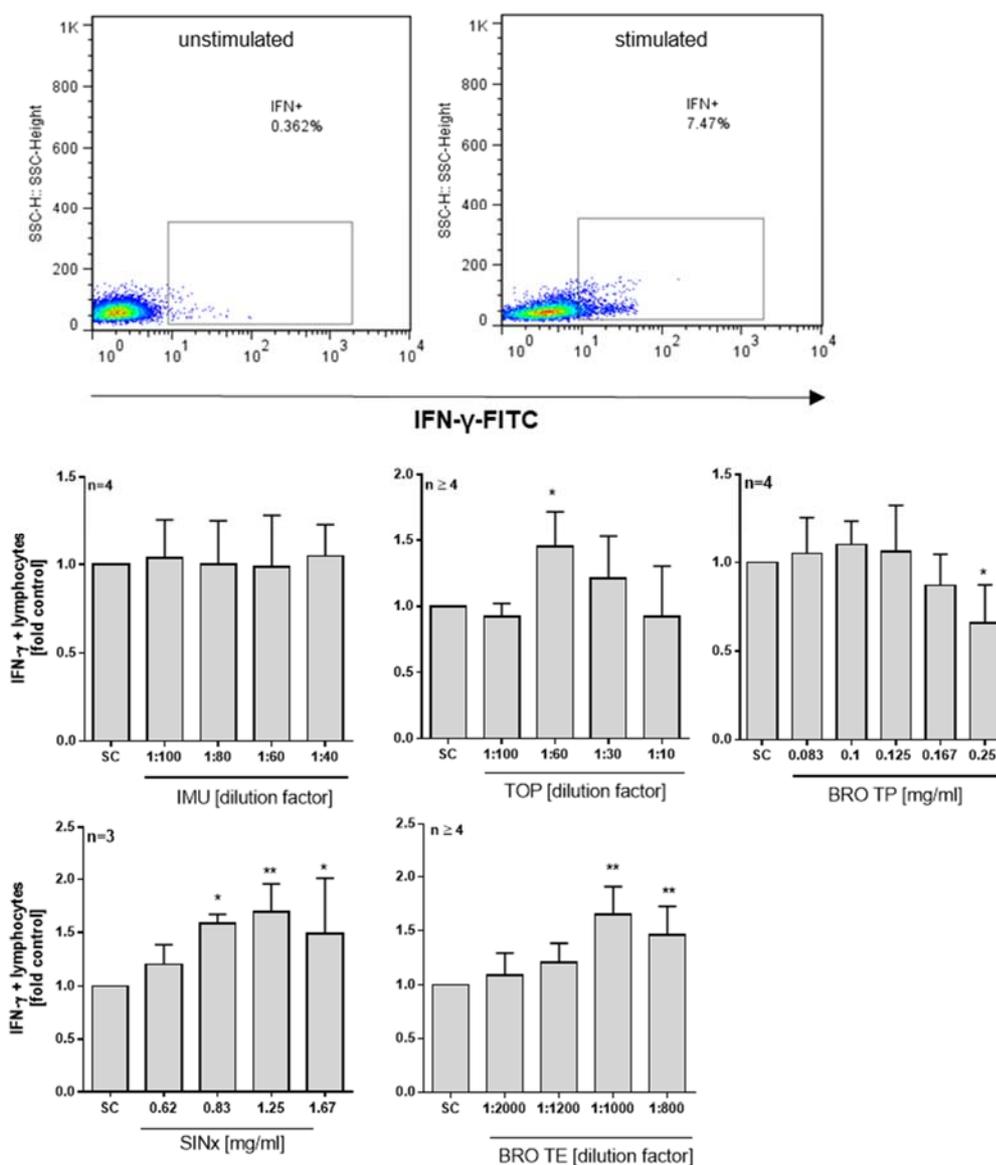
323 Thereafter, the plate was washed thoroughly and incubated with Streptavidin- HRP followed by

324 colorimetric detection using a multiplate reader from Tecan (Germany). Bars are means +SD of at

325 least three independent experiments.

326

Figure 2:



327

328 **Figure 2: Intracellular IFN- $\gamma$  expression from stimulated human PBMC upon extract**

329 **treatment.** Cells were stimulated with a mixture of SARS-CoV-2 S peptide pool, PMA and

330 ionomycin and treated with the extracts for 6 hours. Brefeldin A was used to enhance

331 intracellular cytokine staining signals. Representative scattergrams of intracellular IFN- $\gamma$

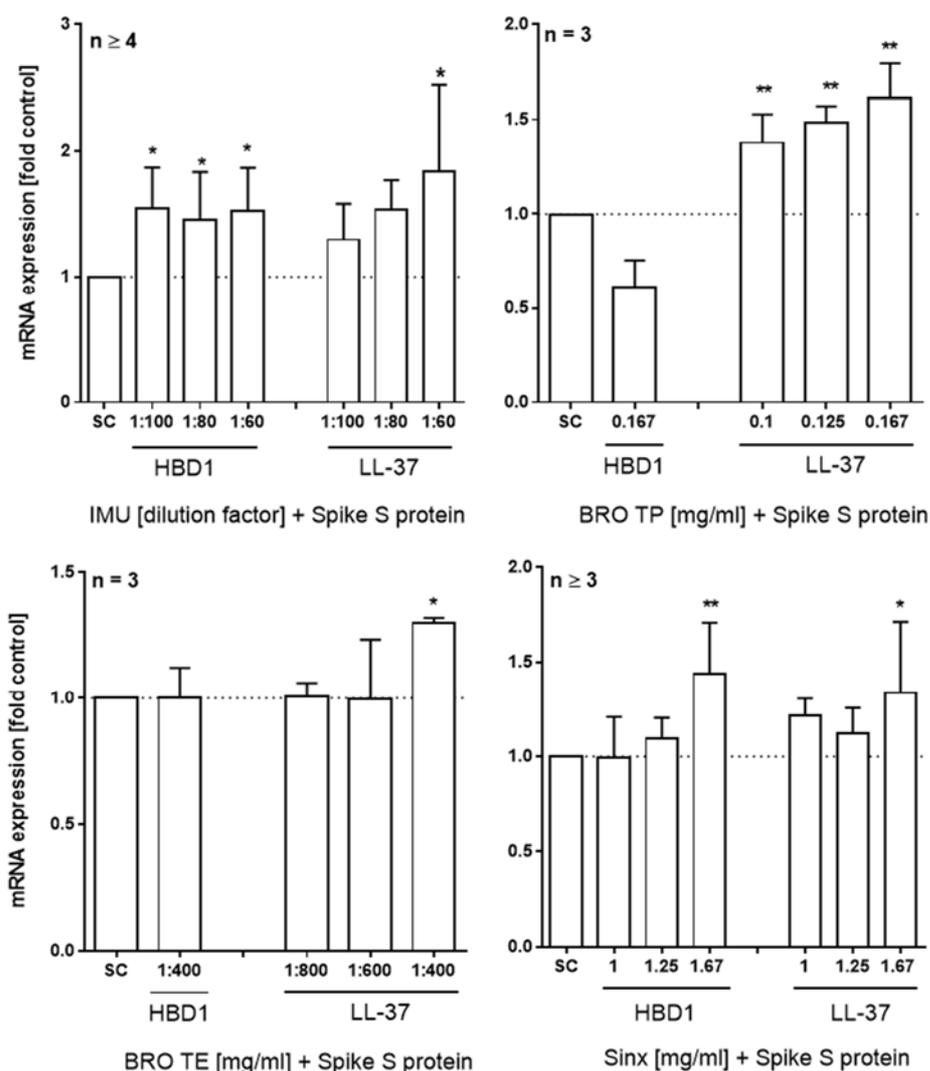
332 staining without (left) and after (right) stimulation are given. Bars are means +SD of at least

333 three independent experiments.

334

335

Figure 3:



336

337

338 **Figure 3: Effect of extracts on HBD1 and LL-37 mRNA expression in Spike protein**

339 **stimulated A549 lung cells.** A549 cells were cultured for 25 days and co-treated with extracts

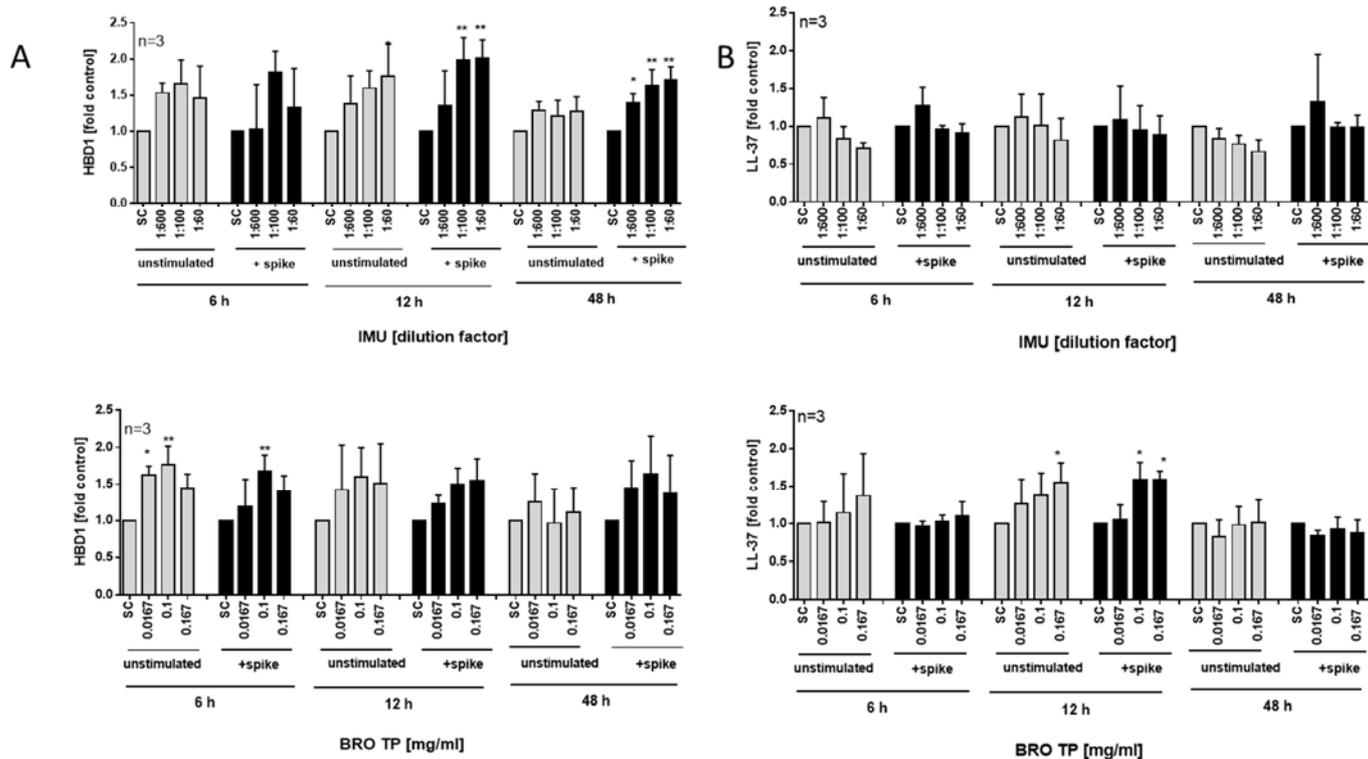
340 and SARS-CoV-2 Spike S1 protein for 24h. Bars are mean values +SD of at least three independent

341 experiments.

342

343

Figure 4:



344

345

346 **Figure 4: Effects of extracts on HBD1 and LL-37 peptide secretion measured by ELISA**

347 **technique.** Human A549 lung cells were cultured for 25 days and exposed to A) IMU or B) BRO

348 TP for different time points with and without Sars-CoV-2 Spike S1 protein stimulation. Bars are

349 mean values +SD of at least three independent experiments.

350

351

352 **Literature**

- 353 Assessment report on *Quercus robur* L., *Q.p.M.L.*, *Quercus pubescens* Willd., cortex  
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