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Botanical Medicines with Activity against Stationary Phase *Bartonella henselae*

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29 **ABSTRACT**

30

31 *Bartonella henselae* is a Gram-negative, facultative intracellular bacterium which is the causative
32 agent of cat scratch disease. In humans, infections with *B. henselae* can result in acute or chronic
33 systemic infections with various clinical symptoms including local skin lesions, malaise, aches,
34 chills, lymphadenopathy, endocarditis, or meningoencephalitis. The current treatment for
35 *Bartonella* infections with antibiotics such as doxycycline and rifampin is not always effective
36 presumably due to bacterial persistence. There have been various anecdotal reports of herbal
37 extracts used for treating patients with persistent *Bartonella* infections but their activity on *B.*
38 *henselae* is unknown. To test the potential antimicrobial activity of botanical or herbal medicines
39 and develop better therapies for persistent *Bartonella* infections, in this study, we screened an
40 herbal product collection against stationary phase *B. henselae in vitro* using SYBR Green I/
41 propidium iodide (PI) viability assay. These herbal medicines were selected by the fact that they
42 are commonly used to treat Lyme and co-infections by patients and herbalists, and as a follow-up
43 to our recent study where these herbs were tested against *B. burgdorferi*. We identified five
44 herbal product extracts that had high activity against stationary phase *B. henselae* at 0.5% (*v/v*),
45 including *Cryptolepis sanguinolenta*, *Juglans nigra*, *Polygonum cuspidatum*, *Scutellaria*
46 *baicalensis*, and *Scutellaria barbata*. Among them, *Cryptolepis sanguinolenta*, *Juglans nigra*,
47 and *Polygonum cuspidatum* could eradicate all stationary phase *B. henselae* cells within 7 days at
48 0.25% (*v/v*) in drug exposure time-kill assay, whereas *Scutellaria baicalensis* and *Scutellaria*
49 *barbata* showed relatively poor activity. The minimum inhibitory concentration (MIC)
50 determination of these top hits indicated they were not only active against stationary phase non-
51 growing *B. henselae* but also had good activity against log phase growing *B. henselae*. Our
52 findings may help to develop more effective treatments for persistent *Bartonella* infections.

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60 **INTRODUCTION**

61

62 *Bartonella* species are fastidious Gram-negative facultative intracellular pathogens with a unique
63 intraerythrocytic lifestyle, which usually require an obligately bloodsucking arthropod vector and
64 a mammalian host during their life cycle [1]. At least thirteen *Bartonella* species are known to be
65 pathogenic in humans, leading to either acute or chronic infections with known diseases such as
66 cat scratch disease, trench fever, Carrion's disease, and bacillary angiomatosis [2]. Infection
67 typically begins with cutaneous inoculation and the pathogenesis of *Bartonella* involves
68 establishing a primary niche in the endothelium [3] before seeding into the blood stream leading
69 to intraerythrocytic infection [4-6]. *Bartonella* has been described as a "stealth pathogen" [7]
70 due to its ability to cause persistent bacteremia, evade the immune system, and cause varying
71 types and severity of symptomatology [6-10]. *Bartonella* can induce vasoproliferative tumor
72 formation [11] and a recent review of the public health implications of bartonellosis discussed
73 the need to research *Bartonella*'s potential role in breast cancer tumorigenesis [12]. Preliminary
74 data have also implicated bartonellosis in neuropsychiatric manifestations including Autism
75 Spectrum Disorder [13], homicidality [14], and hallucinations [15].

76

77 *Bartonella henselae* is the most common causative agent of cat scratch disease, with symptoms
78 of malaise, decreased appetite, aches, headache, chills, arthritis, and lymphadenopathy that can
79 persist for several months [16]. In certain situations, disseminated *B. henselae* may lead to the
80 development of serious complications including ocular manifestations including neuroretinitis
81 [17], neurologic manifestations including encephalopathy, seizures, cerebral vasculitis,
82 meningoencephalitis [18][19][20], visceral organ involvement including hepatomegaly and/or
83 splenomegaly [21], and cardiac manifestations including endocarditis [2][22], which can have a
84 particularly high mortality [18]. Case studies have also implicated *B. henselae* in monoclonal and
85 biclonal gammopathy [23] and various auto-immune manifestations including pediatric acute-
86 onset neuropsychiatric syndrome (PANS) [24], transverse myelitis [25,26], and autoimmune
87 thyroiditis [27].

88

89 Emerging evidence suggests *B. henselae* may also serve as a co-infective pathogen in patients
90 with other vector-borne diseases [12]. In particular, ticks are known to be polymicrobially

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91 infected with multiple pathogens [28] including *Borrelia burgdorferi,* the most common etiologic
92 agent in Lyme disease. Although the ability of ticks to serve as a competent vector for
93 transmitting *B. henselae* to humans is debated, ticks have been documented to carry *B. henselae*
94 [12] and case reports link *B. henselae*-related disease following a tick attachment [29-31].
95 Multiple papers document *B. henselae* exposure in patients with Lyme disease and patients with
96 co-infections may experience more severe and protracted clinical manifestations [31-34].
97
98 Currently there is no single treatment effective for all *Bartonella*-associated diseases, with
99 different antibiotics recommended for use according to different presentations [35]. The first-line
100 antibiotics for the treatment of *Bartonella*-associated infection include doxycycline,
101 erythromycin, azithromycin, gentamicin, rifampin, ciprofloxacin, and tetracycline as well as
102 some drug combinations such as doxycycline plus gentamicin or doxycycline plus rifampin
103 [35][36]. However, the recommended treatment of systemic *B. henselae* infections is based on
104 limited trial data [37] with various treatment failures, probably related to antibiotic resistance and
105 bacterial persistence [36][38]. Therefore, identifying novel antimicrobials targeting persistent
106 *Bartonella* pathogens could significantly assist in development of improved therapeutic protocols
107 for the treatment of *Bartonella*-associated diseases.
108
109 Botanical medicines have a long history of documented use since ancient Mesopotamia, ancient
110 China and ancient India. The safety and efficacy of these medicines is supported by centuries of
111 use in various traditional medicine systems such as Ayurveda and Traditional Chinese Medicine
112 [39-41]. Several recent reports have concluded that the frequency of severe adverse effects
113 related to the use of botanical medicine is rare [42-44]. Unlike conventional antibiotics, which
114 can have a detrimental impact on the microbiome and increase microbial resistance [45, 46],
115 botanical medicines may have a beneficial impact on the microbiome [47]. Many botanical
116 extracts have been reported to have antimicrobial activities, for example, *Laserpitium zernyi* herb
117 extracts were active against different bacterial species including *Pseudomonas aeruginosa*,
118 *Micrococcus luteus*, *Enterococcus faecalis* and *Bacillus subtilis*, and different extracts of *Ononis*
119 *arvensis* showed antimicrobial activity against *Escherichia coli*, *P. aeruginosa*, *Salmonella*
120 *typhimurium*, *Staphylococcus aureus* and *Candida albicans*. Some Mediterranean herb extracts
121 could inhibit growth of representative oral microorganisms and biofilm formation of

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122 *Streptococcus mutans* [48-50]. Members of our group previously documented 32 essential oils
123 derived from botanical medicines that were found to have better activity against stationary phase
124 *B. henselae* compared to antibiotic controls [51]. It is becoming increasingly important to study
125 botanical medicines with potential antimicrobial activity for improved treatment of infections
126 due to antibiotic resistant bacteria. According to the World Health Organization, antibiotic
127 resistance is currently "one of the biggest threats to global health, food security, and development"
128 [52].
129
130 Previously, we have developed a rapid high-throughput drug screen method with the help of
131 SYBR Green I/ propidium iodide (PI) viability assay, leading to successful identification of
132 various active drugs as well as drug combinations against stationary phase *Borrelia burgdorferi*
133 as a surrogate model of persister bacteria [53-56]. In two recent studies, we screened the FDA-
134 approved drug library and a collection of essential oils using this method to identify active drug
135 candidates with potential for treating *Bartonella* infections [51][57]. In this current study, we
136 adapted the same methodology to screen an herbal product collection which we used recently on
137 *Borrelia burgdorferi* [56] and successfully identified various botanical extracts with activity
138 against stationary phase *B. henselae*, which has successfully been used as a model of persister
139 drug screens [53-56] because of its higher content of persister cells than the log phase culture
140 [58]. The implications of these findings for better therapy of persistent *Bartonella*-associated
141 infections are discussed.
142
143 **MATERIALS AND METHODS**
144
145 **Bacterial strain, culture media and culture conditions**
146 *Bartonella henselae* JK53 strain was obtained from BEI Resources (ATCC), NIAID, NIH. *B.*
147 *henselae* JK53 was cultured in Schneider's Drosophila medium (Life Technologies Limited,
148 Paisley, UK) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Co., St. Louis,
149 MO, USA) and 5% sucrose (Fisher Scientific, New Jersey, USA) as described previously
150 [57][59]. Cultures were incubated in sterile 15 mL or 50 mL polypropylene conical tubes
151 (Corning, New York, USA) in microaerophilic incubator without shaking at 37 °C, 5% CO_2.
152 Based on our previous study, the one-day-old and five-day-old cultures were considered as log

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153 phase and stationary phase, respectively [57]. The Columbia sheep blood agar (HemoStat
154 Laboratories, Dixon, CA, USA) was used to perform the colony count in drug exposure assay,
155 which was also cultured at 37 °C, 5% CO_2.
156
157 **Drugs and herbal products**
158 A panel of herbal products was purchased from KW Botanicals Inc. (San Anselmo, CA, USA)
159 and Heron Botanicals (Kingston, WA, USA) [56]. These botanical products were chosen based
160 on significant antimicrobial activity against other bacterial pathogens shown by previous studies
161 [60-64], anecdotal clinical usage reported by patients, favorable safety profiles, and the ability to
162 be absorbed systemically. Most of the botanicals were identified via macroscopic and
163 organoleptic methods with voucher specimens on file with the respective production facilities.
164 Additional details on sourcing, testing and validation of botanical and natural medicines used are
165 summarized in Table S1. Most botanical extracts were provided as alcohol extracts in 30%, 60%,
166 and 90% alcohol dilutions, and the alcohol used was also tested separately as a control in the
167 same dilutions of 30%, 60%, and 90%. Herbal products were dissolved in dimethyl sulfoxide
168 (DMSO) at 5% (*v/v*), followed by dilution at 1:5 into five-day-old stationary phase cultures to
169 achieve 1% final concentration. To make further dilutions for evaluating anti-*Bartonella* activity,
170 the 1% herbal products were further diluted with the same stationary phase cultures to achieve
171 desired concentrations. Antibiotics including azithromycin (AZI), daptomycin (DAP),
172 doxycycline (DOX), gentamicin (GEN), methylene blue, miconazole, and rifampin (RIF)
173 included as controls were purchased from Sigma-Aldrich (USA) and dissolved in appropriate
174 solvents [65] to form 10 mg/mL stock solutions. All the antibiotic stocks were filter-sterilized by
175 0.2 μm filters except the DMSO stocks and then diluted and stored at -20 °C.
176
177 **Microscopy techniques**
178 *B. henselae* cultures treated with different herbal products or control drugs were stained with
179 SYBR Green I (10 × stock, Invitrogen) and propidium iodide (PI, 60 μM, Sigma) mixture dye
180 and then examined with BZ-X710 All-in-One fluorescence microscope (KEYENCE, Inc., Osaka,
181 Japan). The SYBR Green I/PI pre-mixed dye was added to the sample in a 1:10 ratio of the dye
182 against the sample volume and mixed thoroughly, followed by incubating in the dark at room
183 temperature for 15 minutes. SYBR Green I is a green permeant dye that stains all cells whereas

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184 propidium iodide (PI) as an orange-red impermeant dye stains only dead or damaged cells with a
185 compromised cell membrane. Thus, live cells with intact membrane will be stained only by
186 SYBR Green I as green cells, while damaged or dead cells with a compromised cell membrane
187 will be stained orange-red by PI. The residual bacterial viability could then be assessed by
188 calculating the ratio of green/red fluorescence, respectively, as described previously [53]. The
189 stained samples were confirmed by analyzing three representative images of the same bacterial
190 cell suspension using fluorescence microscopy. BZ-X Analyzer and Image Pro-Plus software
191 were used to quantitatively determine the fluorescence intensity.
192
193 **Screening of herbal product collection against stationary phase *B. henselae* using SYBR**
194 **Green I/PI viability assay**
195 For the primary herbal products screening, each product was assayed in two concentrations, 1%
196 (*v/v*) and 0.5% (*v/v*). A five-day-old stationary phase *B. henselae* culture was used for the
197 primary screen. Firstly, 40 μL 5% herbal product DMSO stocks were added to 96-well plates
198 containing 160 μL *B. henselae* culture, respectively, to obtain the desired concentration of 1%.
199 Then the 0.5% concentration was obtained by mixing 100 μL 1% treatment with 100 μL *B.*
200 *henselae* culture. Antibiotics including AZI, DAP, DOX, GEN, methylene blue, miconazole and
201 RIF were used as control drugs at their Cmax. Cmax is a pharmacokinetic measure referring to
202 the maximum serum concentration that a drug achieves in a specified compartment such as blood
203 after the drug has been administered. Control solvents including DMSO, 30%, 60%, and 90%
204 alcohol were also included. Plates were sealed and placed in a 37°C incubator without shaking
205 over a period of three days. SYBR Green I/ PI viability assay was used to assess the live/dead
206 cell ratios after drug exposure as described [53]. Briefly, the SYBR Green I/PI dye was added to
207 the sample followed by incubation in the dark for 15 minutes. Then the plate was read by
208 microplate reader (HTS 7000 plus Bioassay Reader, PerkinElmer Inc., USA). The green/red (538
209 nm/650 nm) fluorescence ratio of each well was used for calculating the residual viability
210 percentage, according to the regression equation of the relationship between residual viability
211 percentage and green/red fluorescence ratio obtained by least-square fitting analysis as described
212 [57]. All tests were run in triplicate.
213
214

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215 **Drug exposure assay of active hits**

216 The active hits from the primary screens were further confirmed by drug exposure assay. The
217 five-day-old stationary phase *B. henselae* culture was used for drug exposure experiments and
218 was treated with 0.25% (*v/v*) active herbal products respectively. Control antibiotics including
219 AZI, DAP, DOX, GEN, methylene blue, miconazole and RIF were used at their Cmax. Control
220 solvents were also included. The drug exposure assay was carried out in 15 mL polypropylene
221 conical tubes over the course of seven days at 37°C, 5% CO_2 without shaking. At each time point,
222 a portion of bacterial cells was collected by centrifugation and rinsed twice with fresh
223 Schneider's medium followed by re-suspension in fresh Schneider's medium. Then the cell
224 suspension was serially diluted and plated on Columbia blood agar plates for viable bacterial
225 counts (colony forming unit, CFU). The plates were incubated at 37 °C, 5% CO_2 until visible
226 colonies appeared and the CFU/mL was calculated accordingly. All tests were run in triplicate.

227

228 **Minimum inhibitory concentration (MIC) determination of active hits**

229 The standard microdilution method was used to measure the minimum inhibitory concentration
230 (MIC) of each herbal product needed to inhibit the visible growth of *B. henselae* after a five-day
231 incubation period as described [51][57]. The diluted one-day-old *B. henselae* log phase culture
232 was used for MIC determination. The 5% herbal product stocks were added into 96-well plates
233 containing 1×10^6 bacteria per well with fresh modified Schneider's medium, respectively, to
234 achieve 1% final concentration. Other lower concentrations were obtained by mixing 1%
235 treatment with diluted one-day-old log phase *B. henselae* culture. Plates were sealed and
236 incubated at 37 °C without shaking for five days. Then the bacterial cell proliferation was
237 assessed using the SYBR Green I/PI assay and the bacterial counting chamber. The MIC is the
238 lowest concentration of the herbal product that prevented the visible growth of *B. henselae*. All
239 tests were run in triplicate.

240

241 **Statistical analysis**

242 The statistical analysis was performed using two-tailed Student's *t*-test. Mean differences were
243 considered statistically significant if P value was < 0.05. Analyses were performed using Image
244 Pro-Plus, GraphPad Prism, and Microsoft Office Excel.

245

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246 **RESULTS**

247

248 **Screening the herbal product collection to identify herbs active against non-growing**
249 **stationary phase *B. henselae***
250 In this study, we adapted the SYBR Green I/PI viability assay developed previously [51][57] to
251 identify herbal products with significant activity against stationary phase *B. henselae* compared
252 to antibiotic controls. As described above, we tested a panel of botanical extracts and their
253 corresponding controls against a five-day-old stationary phase *B. henselae* culture in 96-well
254 plates incubated for 3 days. For primary screens, all the herbal products were applied at two
255 concentrations, 1% (*v/v*) and 0.5% (*v/v*), respectively. Meanwhile, the currently used antibiotics
256 for bartonellosis treatment such as doxycycline, azithromycin, gentamicin, and rifampin were
257 included as control drugs for comparison, as well as the previously identified FDA-approved
258 drugs effective against *B. henselae* including daptomycin, methylene blue and miconazole [57]
259 (Table 1). All of the pharmaceutical antibiotics were used at their Cmax. In the primary screens,
260 four different alcohol extracts of *Juglans nigra*, three different alcohol extracts from *Cryptolepis*
261 *sanguinolenta*, one alcohol extract of *Polygonum cuspidatum*, one glycerite extract of
262 *Polygonum cuspidatum*, one glycerite extract of *Scutellaria baicalensis* (huang qin), and one
263 glycerite extract of *Scutellaria barbata* (ban zhi lian) at both 1% (*v/v*) and 0.5% (*v/v*) were found
264 to have strong activity against stationary phase *B. henselae* compared to the control antibiotics
265 including AZI, DOX, GEN, and RIF according to the plate reader results (Table 1). In contrast,
266 *Andrographis paniculate, Stevia rebaudiana, Artemisia annua, Uncaria rhynchophylla, Uncaria*
267 *tomentosa, Rhizoma coptidis, Citrus paradise, Dipsacus fullonum, Campsiandra angustifolia,*
268 *Otoba parvifolia* and colloidal silver did not show significant activity against stationary phase *B.*
269 *henselae* (Table S2). Therefore, the following active herb extracts were selected as top hits,
270 including *Juglans nigra* 30%, 45%, 60%, and 90% alcohol extract, *Polygonum cuspidatum 30%*
271 alcohol extract, *Polygonum cuspidatum* glycerite extract, *Cryptolepis sanguinolenta* 30%, 60%,
272 and 90% alcohol extract, *Scutellaria baicalensis* glycerite extract, and *Scutellaria barbata*
273 glycerite extract. These top hits were chosen based on the fact that a lower percentage of viable
274 cells remained compared to the other natural antimicrobials used in this study as well as
275 exhibiting significantly better activity compared to the current antibiotics used to treat *Bartonella*
276 infections, including AZI, DOX, GEN, and RIF, as indicated by the statistically analysis that the

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277 P value was < 0.05. Our previous experience showed some compounds in the herbal extracts
278 could interfere with the SYBR Green I/PI assay due to their autofluorescence. To eliminate this
279 impact, we checked the residual cell viability by examining microscope images of the herbal
280 extract treated samples to confirm the plate reader results. As shown by fluorescence microscopy,
281 solvents such as DMSO and alcohol did not have significant impact on residual bacterial cell
282 viability compared to the drug free control (Figure 1 and Table 1), as the P value was > 0.05.
283 Clinically used antibiotics against *Bartonella* infections such as AZI and DOX only showed
284 weak activity when used at their Cmax (residual viability above 60%) (Table 1). Antibiotics
285 reported to have a clinical improvement for *Bartonella* infections including GEN and RIF
286 showed relatively better activity (residual viability below 50%) against stationary phase *B.*
287 *henselae* than AZI and DOX. FDA-approved drugs that we identified as effective against
288 stationary phase *B. henselae* (DAP, methylene blue, and miconazole) [57] had better activity
289 (residual viability below 40%) than the other antibiotics tested. Among the five top herbal hits
290 that had better activity (residual viability between 0% and 16%) against stationary phase *B.*
291 *henselae* than most control antibiotics, the most active herbal products were *Juglans nigra* and
292 *Polygonum cuspidatum* alcohol extracts of different concentrations. However, the fluorescence
293 microscope observation of *Juglans nigra* 30% and 45% alcohol extracts, and *Polygonum*
294 *cuspidatum* glycerite extract at 0.5% treatment exhibited significantly higher percentage of green
295 (live) cells compared with the plate reader results (Figure 1 and Table 1), as indicated by the
296 statistically analysis that the P value was < 0.05, which were also higher than that of most control
297 antibiotics, indicating the relatively poor accuracy of the plate reader results and poor activity of
298 these herbal products at these particular concentrations. Therefore, they were excluded from
299 active hits for subsequent MIC testing and drug exposure assay (see below). Alcohol extracts
300 from *Cryptolepis sanguinolenta* of different concentrations also exhibited strong activity against
301 stationary phase *B. henselae* as shown by red (dead) cells in fluorescence microscope
302 observation (Figure 1), which is consistent with the plate reader results. Glycerite extracts from
303 the two *Scutellaria* plants, including *Scutellaria baicalensis* (huang qin) and *Scutellaria barbata*
304 (ban zhi lian), also showed good activity with low percentages of residual viable bacterial cells
305 remaining (Figure 1).

306

307 **Time-kill curves of active hits**

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308 To further demonstrate the efficacy of the active herbal products identified from the primary
309 screens in eradicating persistent *B. henselae* cells, we performed a time-kill drug exposure assay
310 against a five-day-old stationary phase *B. henselae* culture at a lower concentration of 0.25%
311 (*v/v*), along with their corresponding solvent controls. Meanwhile, clinically used antibiotics to
312 treat *Bartonella* infections including AZI, DOX, GEN, and RIF were used at their Cmax as
313 controls. Compared to the drug free control, as shown in Figure 2 and Table 2, some clinically
314 used antibiotics such as AZI and DOX showed poor activity in killing stationary phase *B.*
315 *henselae* partly due to their low Cmax. Other antibiotics such as GEN and RIF exhibited better
316 activity which could eradicate all *B. henselae* cells by day 7 and day 5, respectively. The
317 difference of residual viabilities of stationary phase *B. henselae* after treatment by control
318 solvents and without drug treatment was not statistically significant, as the P value was > 0.05.
319 All three *Cryptolepis sanguinolenta* alcohol extracts of different concentrations were able to
320 eradicate all *B. henselae* cells in the seven-day drug exposure, where *Cryptolepis sanguinolenta*
321 60% alcohol extract was the most active herbal product that killed *B. henselae* with no detectable
322 CFU after five-day exposure. *Juglans nigra* in 60% and 90% alcohol extracts both exhibited
323 good activity that eradicated all *B. henselae* cells without viable cells being recovered after the
324 seven-day drug exposure. *Polygonum cuspidatum* 30% alcohol extract was also effective to kill
325 all *B. henselae* cells by day 7. However, *Scutellaria barbata* (ban zhi lian) and *Scutellaria*
326 *baicalensis* (huang qin) showed poor activity at the concentration of 0.25% (*v/v*) during this
327 seven-day drug exposure, with considerable numbers of viable cells remaining after treatment.
328

329 **Minimum inhibitory concentration (MIC) determination of active hits**

330 The activity of antibiotics against non-growing bacteria is not always correlated with that against
331 growing bacteria [57]. Thus, it was also necessary to determine the MICs of these active herbal
332 products against log phase growing *B. henselae*. The MIC determination of herbal products for *B.*
333 *henselae* was conducted by the standard microdilution method as described [51][57]. As shown
334 in Table 3, *Juglans nigra* 60% extract was the most active herbal product among the top 5 hits,
335 capable of inhibiting visible *B. henselae* proliferation at 0.125%-0.25% (*v/v*). Other herbal
336 products including *Juglans nigra* 90% alcohol extracts, *Polygonum cuspidatum* 30% alcohol
337 extract, *Cryptolepis sanguinolenta* 30%, 60%, and 90% alcohol extracts, *Scutellaria baicalensis*
338 (huang qin), and *Scutellaria barbata* (ban zhi lian) had similar activity against growing *B.*

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339 *henselae* such that they inhibited log phase *B. henselae* proliferation at 0.25%-0.5% (*v/v*). These
340 results indicated that these top hits of herbal products were not only active against non-growing
341 stationary phase *B. henselae*, but also effective against log phase growing *B. henselae*.
342

343 **DISCUSSION**
344

345 In this study, we successfully applied the SYBR Green I/PI viability assay to evaluate a panel of
346 botanical products for their activity against stationary phase *B. henselae* as a model of persister
347 drug screens [51][57]. We identified some herbal products that had high activity at 1% (*v/v*)
348 concentration compared to clinically used antibiotics, including extracts of *Juglans nigra*,
349 *Cryptolepis sanguinolenta*, *Polygonum cuspidatum*, *Scutellaria baicalensis*, and *Scutellaria*
350 *barbata*. Among these top hits, three herbal product extracts could eradicate all stationary *B.*
351 *henselae* cells without CFU being detected within a seven-day drug exposure at a low
352 concentration of 0.25% (*v/v*), including *Cryptolepis sanguinolenta* 30%, 60%, 90% alcohol
353 extracts, *Juglans nigra* 60%, 90% alcohol extracts, and *Polygonum cuspidatum* 30% alcohol
354 extracts. The MIC determination of these active hits showed they were also effective in
355 inhibiting the growth of log phase *B. henselae*.
356

357 These plant species whose extracts we found to be active against *B. henselae* have also been
358 reported to have various biological activities in previous studies. Different parts of various
359 species from genus *Juglans* have shown pain-relieving, antioxidant, antibacterial, antifungal and
360 antitumor activities [66-68]. In particular, *Juglans nigra* exhibited both bacteriostatic activity and
361 bactericidal activity against *Borrelia* based on *in vitro* studies [60][69]. Previous studies have
362 profiled the phytochemicals of *Juglans* plants, including different types of steroids, flavonoid C-
363 glycoside, flavones, essential oil components, and tannins [70]. *Juglans* contain several active
364 constituents with potential importance to human health including juglone, phenolic acids,
365 flavonoids, and catechins (including epigallocatechin) [71-75]. A study comparing leaf essential
366 oils of *J. regia* and *J. nigra* further showed *J. nigra* leaf oil was less phytotoxic [76]. The safety
367 of *Juglans nigra* use in humans has not been adequately studied however it has a long history of
368 anecdotal use and the oral LD50 of juglone in rats is calculated at 112mg/kg [77].
369

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370 *Cryptolepis sanguinolenta* and its constituents were reported to have many biological activities
371 including antibacterial, antifungal, anti-inflammatory, anticancer, antimalarial and anti-amoebic
372 properties [61][78-82]. A recent review has assessed the phytochemistry and pharmacology of
373 *Cryptolepis sanguinolenta* and concluded that although there may be some concern regarding
374 potential reproductive toxicity, it is generally safe at doses below 500mg/kg and may serve as
375 promising source of potential antimicrobial agent(s) [83]. Among constituents and secondary
376 metabolites of the plant identified with antimicrobial activity, an alkaloid called cryptolepine was
377 the most well-studied and considered to be the most important active component. Cryptolepine
378 was reported to have a lytic effect on *S. aureus* as seen in SEM photomicrographs which led to
379 altered cell morphology, and was able to intercalate into DNA at cytosine-cytosine sites or
380 inhibited the activity of topoisomerase causing DNA damage [84-86]. *Cryptolepis sanguinolenta*
381 was reported to be highly effective and non-toxic in the treatment of uncomplicated malaria in a
382 small randomized open trial of 44 patients [87]. *Cryptolepis sanguinolenta* is also used
383 anecdotally to treat a malaria-like tickborne infection called Babesiosis [88]. Given its anecdotal
384 use to treat Babesiosis, the results of the current study on *B. henselae,* and the results of our
385 previous study on *B. burgdorferi* [56]*, Cryptolepis sanguinolenta* represents a unique potential
386 therapeutic agent to treat multiple tickborne infections. Future studies are needed to elucidate
387 more specific antimicrobial mechanisms of cryptolepine as well as other active ingredients
388 against infectious pathogens such as *B. henselae*. *Polygonum cuspidatum* has been documented
389 to have antibacterial effects against *Vibrio vulnificus* [89], *Streptococcus mutans* [90] and
390 *Streptococcus* associated biofilms [91]. Its constituents have also been shown to have
391 antimicrobial, anti-tumor, anti-inflammatory, neuroprotective, and cardioprotective effects [92-
392 96]. One of the most active constituents is a polyphenol called resveratrol, which was reported to
393 be active against log phase *Borrelia burgdorferi* and *Borrelia garinii* by *in vitro* testing [60]. In
394 addition, another active constituent called emodin (6-methyl-1,3,8-trihydroxyanthraquinone) has
395 been shown to have activity against stationary phase *Borrelia burgdorferi* cells [97]. A study
396 unraveling the mechanism of action of *Polygonum cuspidatum* using a network pharmacology
397 approach, suggested that polydatin might play a pivotal role in the therapeutic effects of
398 *Polygonum cuspidatum* [98]. Recent trials using *Polygonum cuspidatum* have not reported
399 significant toxicity [99, 100]. One of the active constituents, resveratrol, has been shown to be
400 rapidly absorbed [101], well-tolerated [102] and associated with minimal toxicity except mild

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401 diarrhea at an oral dose of 2000mg 2x/day for 2 weeks [103].

402

403 Our study is the first to identify the antimicrobial activity of extracts from *Juglans nigra*,
404 *Cryptolepis sanguinolenta*, and *Polygonum cuspidatum* against stationary phase *B. henselae.* In
405 addition, considering the possibility of *B. henselae* coinfection among Lyme and tick-borne
406 disease patients, the overlap of active herbal products against both *B. henselae* identified in our
407 current study and *B. burgdorferi* according to our previous study [56], including *Cryptolepis*
408 *sanguinolenta, Juglans nigra*, and *Polygonum cuspidatum*, should provide a promising strategy
409 for better treatment of coinfections with both pathogens.

410

411 In this current study, clinically used antibiotics for treating *Bartonella*-associated infections
412 including AZI and DOX showed weak activity in eradicating stationary phase *B. henselae* cells
413 (Table 1, Figure 1 and Figure 2). This finding coincides with the reported discrepancies in
414 antibiotic efficacies between *in vitro* MIC data and clinical data from patients [35]. The poor
415 activities of current clinically used antibiotics against stationary phase *B. henselae* as shown in
416 our study could partly explain clinically documented treatment failure and could be in part due to
417 persistent infection. This phenomenon may also be partly due to the limited antibacterial activity
418 of these antibiotics. Doxycycline inhibits bacterial protein synthesis by binding to the 30S
419 ribosomal subunit [104]. Azithromycin could also inhibit bacterial protein synthesis by binding
420 to the 50S ribosomal subunit, and thus prevent bacteria from growing [105]. Although these
421 antibiotics all target growing bacteria, they are not very effective at killing non-growing
422 stationary phase *B. henselae*, and thus could lead to treatment failure in persistent and chronic
423 infections. Conversely, the herbal extracts could be promising candidates for treating persistent *B.*
424 *henselae* because they contain multiple active phytochemicals including steroids, flavones,
425 tannins and more. These compounds have complex and synergistic effects and thus have
426 potentially broader antimicrobial activity. Many of these phytochemicals are lipophilic and could
427 target the bacterial cell membrane, which is an important target of persister drugs like
428 pyrazinamide [106] and daptomycin [107], especially when persistent bacterial cells are
429 aggregated together (Figure 1). The high lipophilicity of these phytochemicals, which could
430 cause bacterial cell membrane damage, could be responsible for the aggregated bacterial forms
431 and also explain for the varying numbers of bacterial cells among different samples seen in
432 microscopic pictures (Figure 1).

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433

434 Despite the promising findings of the herbal extracts active against *B. henselae*, future studies are
435 needed to identify the active ingredients of these herbs and to better understand their specific
436 antimicrobial mechanisms of action. It would also be of interest to test compounds like juglone,
437 cryptolepine, resveratrol, and emodin which are known active components of the *Juglans nigra*,
438 *Cryptolepis sanguinolenta* and *Polygonum cuspidatum* herbs, on *B. henselae* in future studies.
439 Different parts of these plants might have different antimicrobial activities because of varying
440 concentrations of the active compounds they contained, and different solvents used to extract the
441 compounds could also significantly affect their activity. Therefore, the pharmacokinetic profiles
442 of active components should be studied thoroughly in the future, as well as the optimal extraction
443 strategy to obtain the maximally effective ingredients in order to better determine the utility and
444 practicality of these active herbal medicines.

445

446 The present study only tested the activity of herbal products against *B. henselae in vitro* and
447 there are a few points that are important to address. For one, *B. henselae* is a facultative
448 intracellular pathogen and could reside and propagate inside mammalian erythrocytes and/or
449 endothelial cells. Therefore, it will be of interest to assess the activity of these identified
450 candidates against intracellular *B. henselae ex vivo* and *in vivo* in animal models of infection
451 [108] in the future. The host cell can provide the pathogen with protective shelter from the action
452 of drugs and herbal antimicrobials as well as the host immune system, therefore the efficacy of
453 these antimicrobials *in vivo* might differ from that *in vitro* [11]. Additionally, it is important to
454 note that botanical medicines have multiple mechanisms of action beyond the antimicrobial
455 activity that was assessed in the present study. For example, botanicals have been shown to exert
456 effects via multiple mechanisms with potential benefit in *Bartonella* infections including anti-
457 inflammatory activity, immune modulation/stimulation, microbiome modulation, endothelial cell
458 support and biofilm disruption. Future studies are needed to assess the safety and efficacy of
459 these herbal products in appropriate animal models of *Bartonella* infections where broader
460 biologic mechanisms of action including their effects on the host can be evaluated.

461

462 In the future we also hope to test different combinations of active herbal products and their active
463 constituents with and without antibiotics to develop better treatments. As indicated by the Yin-

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464 Yang model, the bacterial pathogen has a heterogeneous population, with persister population
465 (Yin) and growing population (Yang), which are also composed of various subpopulations with
466 varying metabolic or dormant states in continuum [38]. Therefore, it should be reasonable to
467 deploy drug/herb or herb/herb combinations for more effective treatments, with different drugs
468 or herbal medicines targeting different bacterial subpopulations in varying physiological states.
469 Indeed, members of our group recently demonstrated that antibiotic combinations were more
470 effective in eradicating *in vitro* stationary phase and biofilm *B. henselae* compared to single
471 antibiotics [109]. Our goal is to use the herbal medicines we identified in this study to develop
472 more safe and effective treatments for persistent bartonellosis.
473
474 **Conflicts of Interest**
475
476 Jacob Leone ND is owner of two naturopathic medical practices, FOCUS Health Group and
477 Door One Concierge, which provides treatment to patients with tick-borne diseases. Dr. Leone
478 does receive profits from medical services and botanical preparations he exclusively makes
479 available to patients in these two practices and does not currently sell botanical products
480 commercially.
481
482 **Acknowledgments**
483
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485 for evaluation in this study. We thank BEI Resources/ATCC, NIAID, NIH for providing
486 *Bartonella henselae* JK53 strain used in this study. We gratefully acknowledge the support of our
487 work by the Bay Area Lyme Foundation, the Steven & Alexandra Cohen Foundation, LivLyme
488 Foundation, Global Lyme Alliance, NatCapLyme, and the Einstein-Sim Family Charitable Fund.
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497
498 **Table 1. Activity of top active herbal products against stationary phase *B. henselae*** [1]

Herbal Products (HP) and Control Drugs	Trade Names of Herbal Products	Residual Viability (%) after 1% HP or Antibiotic Treatment		Residual Viability (%) after 0.5% HP Treatment	
		Plate Reader[2]	Microscope[3]	Plate Reader[2]	Microscope[3]
Drug free control		70%	75%		
DMSO control		60%	70%		
30% alcohol		70%	78%		
60% alcohol		80%	82%		
90% alcohol		65%	63%		
AZI		70%	65%		
DOX		66%	60%		
GEN		44%	50%		
RIF		31%	45%		
DAP		6%	10%		
Methylene Blue		29%	35%		
Miconazole		40%	50%		
Juglans nigra 45% AE[5]	Hu tao ren	0%[4]	0%	9%	59%
Juglans nigra 60% AE[5]	Black walnut	0%[4]	0%	4%	32%
Juglans nigra 90% AE[5]	Black walnut	0%[4]	7%	6%	30%
Juglans nigra 30% AE[5]	Black walnut	2%	2%	6%	63%
Polygonum cuspidatum 30% AE[5]	Japanese knotweed	6%	0%	8%	21%
Polygonum cuspidatum GE[6]	Hu zhang	8%	18%	13%	70%
Cryptolepis sanguinolenta 30% AE[5]	Cryptolepis	8%	10%	14%	42%
Scutellaria baicalensis GE[6]	Huang qin	8%	12%	11%	25%
Scutellaria barbata GE[6]	Ban zhi lian	8%	50%	2%	48%
Cryptolepis sanguinolenta 60% AE[5]	Cryptolepis	10%	24%	14%	34%
Cryptolepis sanguinolenta 90% AE[5]	Cryptolepis	11%	25%	14%	47%

499 [1] A five-day-old stationary phase *B. henselae* culture was treated with herbal products (1% or 0.5%) (*v/v*)
500 or control drugs for three days. Drug concentrations used in this experiment were based on their Cmax
501 and were as follows: 0.2 μg/mL AZI, 2.4 μg/mL DOX, 10 μg/mL GEN, 7.8 μg/mL RIF, 60 μg/mL DAP,
502 2.9 μg/mL methylene blue, and 6.3 μg/mL miconazole. [2] Residual viability was calculated according to

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503 the regression equation and the ratio of Green/Red fluorescence obtained by SYBR Green I/PI assay. [3]
504 Residual viability was assayed by fluorescence microscope counting. [4] Values of SYBR Green I/PI
505 calculated by the plate reader were lower than 100% dead cells. [5]Abbreviation: AE: alcohol extract; GE:
506 glycerite extract.

507

508 **Table 2. Drug exposure assay of top active herbal products against *B. henselae* stationary**
509 **phase culture** [1]

Herbal Products and Control Drugs[2]	CFU/mL after Drug Exposure			
	1 Day	**3 Day**	**5 Day**	**7 Day**
Drug free control	$1.45 \pm 0.26 \times 10^7$	$9.17 \pm 1.44 \times 10^6$	$1.67 \pm 0.29 \times 10^6$	$1.33 \pm 0.29 \times 10^4$
DMSO	$2.07 \pm 0.12 \times 10^7$	$9.33 \pm 0.58 \times 10^6$	$1.50 \pm 0.50 \times 10^6$	$1.50 \pm 0.50 \times 10^4$
30% alcohol	$4,17 \pm 1.04 \times 10^7$	$9.50 \pm 1.32 \times 10^6$	$1.33 \pm 0.58 \times 10^6$	$1.83 \pm 0.76 \times 10^4$
60% alcohol	$3.33 \pm 0.29 \times 10^7$	$1.02 \pm 0.13 \times 10^7$	$2.50 \pm 1.00 \times 10^6$	$2.17 \pm 0.29 \times 10^4$
90% alcohol	$3.17 \pm 0.29 \times 10^7$	$9.00 \pm 1.50 \times 10^6$	$1.17 \pm 0.29 \times 10^6$	$4.33 \pm 1.15 \times 10^4$
AZI	$1.87 \pm 0.32 \times 10^7$	$7.00 \pm 1.00 \times 10^6$	$8.83 \pm 1.44 \times 10^5$	$2.17 \pm 0.29 \times 10^4$
DOX	$2.50 \pm 1.00 \times 10^7$	$6.17 \pm 1.76 \times 10^6$	$9.67 \pm 2.47 \times 10^5$	$1.83 \pm 1.15 \times 10^4$
GEN	$5.00 \pm 0.00 \times 10^4$	$1.00 \pm 0.00 \times 10^3$	$8.50 \pm 0.87 \times 10^2$	0
RIF	$5.83 \pm 1.76 \times 10^6$	$5.83 \pm 2.57 \times 10^4$	0	0
Juglans nigra 60% AE	$3.67 \pm 0.76 \times 10^6$	$6.67 \pm 2.89 \times 10^4$	$1.02 \pm 0.21 \times 10^5$	0
Juglans nigra 90% AE	$3.83 \pm 3.69 \times 10^6$	$2.67 \pm 0.58 \times 10^6$	$4.83 \pm 1.04 \times 10^2$	0
Cryptolepis sanguinolenta 30% AE	$6.00 \pm 0.87 \times 10^6$	$2.83 \pm 0.76 \times 10^6$	$6.33 \pm 1.26 \times 10^4$	0
Cryptolepis sanguinolenta 60% AE	$5.33 \pm 2.25 \times 10^4$	$2.67 \pm 1.04 \times 10^5$	0	0
Cryptolepis sanguinolenta 90% AE	$7.83 \pm 2.75 \times 10^6$	$9.50 \pm 3.97 \times 10^5$	$2.17 \pm 1.04 \times 10^2$	0
Polygonum cuspidatum 30% AE	$7.17 \pm 1.61 \times 10^6$	$2.33 \pm 0.29 \times 10^6$	$5.50 \pm 3.12 \times 10^2$	0
Scutellaria barbata GE	$1.03 \pm 0.20 \times 10^7$	$3.17 \pm 1.04 \times 10^6$	$3.17 \pm 0.58 \times 10^5$	$3.17 \pm 0.58 \times 10^3$
Scutellaria baicalensis GE	$9.00 \pm 0.50 \times 10^6$	$2.83 \pm 0.76 \times 10^6$	$6.50 \pm 2.78 \times 10^5$	$7.83 \pm 0.29 \times 10^3$

510 [1] A five-day-old stationary phase *B. henselae* culture was treated with herbal products or control drugs.
511 The beginning CFU for the five-day-old stationary phase *B. henselae* culture was about 2×10^7 CFU/mL.
512 At different time points of drug exposure (day 1, day 3, day 5, and day 7), portions of bacteria were
513 removed, washed, and plated on Columbia blood agar for CFU counts. [2] The herbal product concentration
514 used in this experiment was 0.25% (*v/v*). Drug concentrations used in this experiment were based on their
515 Cmax and were as follows: 0.2 μg/mL AZI, 2.4 μg/mL DOX, 10 μg/mL GEN, and 7.8 μg/mL RIF.

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518
519 **Table 3. Minimum inhibitory concentrations (MICs) of top active herbal products against**
520 ***B. henselae***

Herbal Products	MIC (*v/v*)
Juglans nigra 60% AE	0.125%-0.25%
Juglans nigra 90% AE	0.25%-0.5%
Polygonum cuspidatum 30% AE	0.25%-0.5%
Cryptolepis sanguinolenta 30% AE	0.25%-0.5%
Cryptolepis sanguinolenta 60% AE	0.25%-0.5%
Cryptolepis sanguinolenta 90% AE	0.25%-0.5%
Scutellaria baicalensis GE	0.25%-0.5%
Scutellaria barbata GE	0.25%-0.5%

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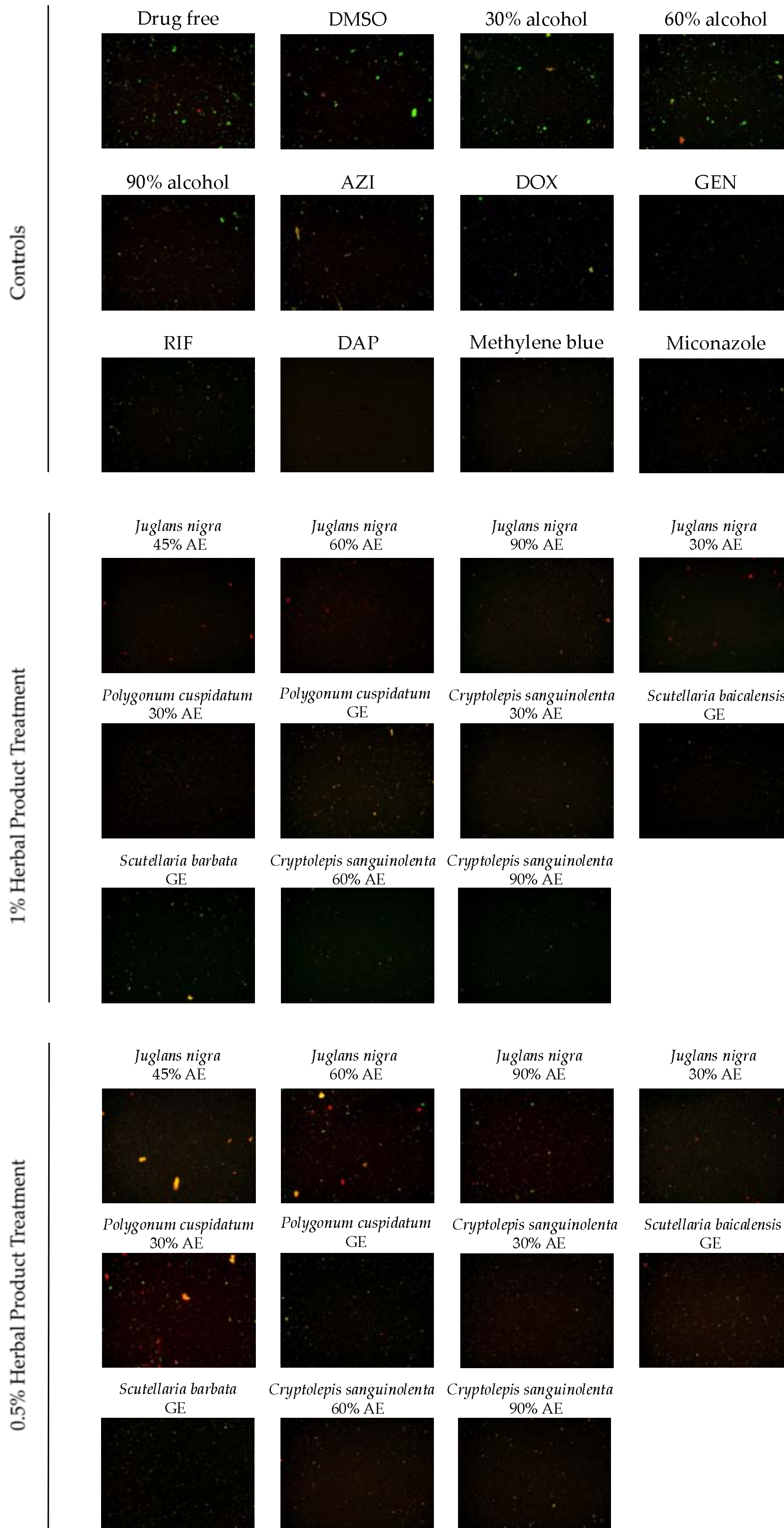

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528
529 **Figure 1. Effect of top hits of herbal products against stationary phase *B. henselae* in comparison**
530 **with control drugs.** A five-day-old stationary phase *B. henselae* culture was treated with 1% (*v/v*) or
531 0.5% (*v/v*) herbal products or control antibiotics for three days followed by SYBR Green I/PI viability
532 assay and fluorescence microscopy (400 × magnification). Drug concentrations used were based on their
533 Cmax and were as follows: 0.2 μg/mL AZI, 2.4 μg/mL DOX, 10 μg/mL GEN, 7.8 μg/mL RIF, 60 μg/mL
534 DAP, 2.9 μg/mL methylene blue, and 6.3 μg/mL miconazole. Green cells represent live cells and red cells
535 represent dead cells.
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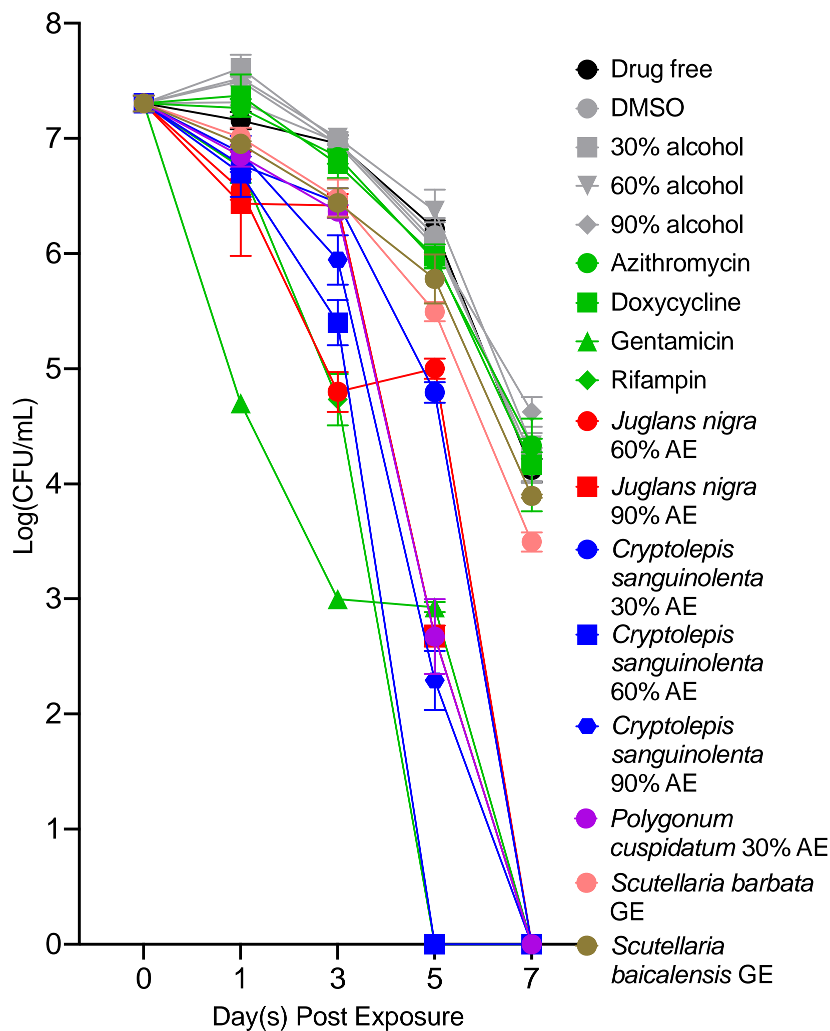

543

544 **Figure 2. Time-kill curves of active herbal product treatment against stationary phase *B. henselae*
545 in comparison with control drugs.** The herbal products or control antibiotics were added to the five-day
546 old stationary phase culture respectively at time point 0, and at different times of drug exposure (day 1,
547 day 3, day 5, and day 7), portions of bacteria were removed and washed and plated on Columbia blood
548 agar plates for CFU counts. The herbal product concentration used in this experiment was 0.25% (*v/v*).
549 Drug concentrations used in this experiment were based on their Cmax and were as follows: 0.2 μg/mL
550 AZI, 2.4 μg/mL DOX, 10 μg/mL GEN, and 7.8 μg/mL RIF.

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892 **Table S1. Herbal product sources, validation, and extract details**

Herbal Products	Trade Names of Herbal Products	Source	Validation/ID	Extract Details
Juglans nigra (husk/hull)	Black walnut	Pacific Botanicals (Wild harvested)	Organoleptic, KW Botanicals	45% ETOH extract of husk/hulls by KW Botanicals
Juglans nigra (husk)	Black walnut	Heron Botanicals (Wild harvested, New York)	Organoleptic, Heron Botanicals	30, 60, 90% ETOH by Heron Botanicals
Rhizoma coptidis	Coptis	Hawaii Pharm LLC (Certified organic coptis)	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite , Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Polygonum cuspidatum	Japanese knotweed	Heron Botanicals (Organic cultivation, China)	Organoleptic, Heron Botanicals	30% ETOH by Heron Botanicals
Stevia rebaudiana	Tian ju ye	Sonoma County Herb Exchange (Cultivated)	Organoleptic, KW Botanicals	25% ETOH extract by KW Botanicals
Uncaria tomentosa bark	Samento	NutraMedix, LLC (Jupiter, Florida)	Per manufacturer	Samento bark 20-24% ETOH extract
Citrus paradisi	Grapefruit seed extract	Cintamani, Poland (Citrosept ™)	Cintamani, Poland	Organic grapefruit seed extract
Andrographis paniculata	Chuan xin lian	Hawaii Pharm LLC (Wild harvested, China)	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite , Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Artemisia annua	Sweet wormwood	Heron Botanicals (Organic cultivation)	American Herbal Pharmacopoeia (Scotts Valley, CA), Organoleptic, Heron Botanicals Confirmed 0.11% Artemisinin content, The Institute for Food Safety and Defense	30, 60, 90% ETOH by Heron Botanicals
Otoba spp. bark	Banderol	NutraMedix, LLC (Jupiter, Florida)	Per manufacturer	Banderol bark 20-24% ETOH extract
Polygonum cuspidatum	Hu zhang	Hawaii Pharm LLC (Wild harvested,	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite ,

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		China)		Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Cryptolepis sanguinolenta	Cryptolepis	Heron Botanicals (Wild harvested, Ghana)	HPTLC, The Institute for Food Safety and Defense Organoleptic, Heron Botanicals	30, 60, 90% ETOH by Heron Botanicals
Scutellaria baicalensis	Huang qin	Hawaii Pharm LLC (Wild harvested, China)	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite , Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Scutellaria barbata	Ban zhi lian	Hawaii Pharm LLC (Wild harvested, China)	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite , Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Campsiandra angustifolia bark	Cumanda	NutraMedix, LLC (Jupiter, Florida)	Per manufacturer	Cumanda bark 20-24% ETOH extract
Uncaria rhynchophylla	Gou Teng	Hawaii Pharm LLC (Certified organic, China)	Organoleptic, Hawaii Pharm, LLC	Dry Herb Glycerite , Herb/Menstruum ratio 1:3, Glycerin/Water ratio 6:4
Dipsacus fullonum	Gao liang jiang	Friend's of the Trees (wild harvested, Washington State)	DNA Species Identification, NSF International	40% ETOH by KW Botanicals (Inadvertently comingled with D. asper sample prior to testing)
Uncaria tomentosa	Uncaria	Mountain Rose Herbs (Wild harvested)	DNA Species Identification, Christopher Hobbs, Ph.D.	50% ETOH by KW Botanicals
Andrographis paniculata	Andrographis	Heron Botanicals (Organic cultivation, China)	Organoleptic, Heron Botanicals	30% ETOH by Heron Botanicals
Colloidal silver	Argentyn 23®	Argentyn 23®	Per manufacturer	Bio-Active Silver Hydrosol™

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896 **Table S2. Activity of other tested herbal products against stationary phase *B. henselae* [1]**

Herbal Products (HP) and Control Drugs	Trade Names of Herbal Products	Residual Viability (%) after 1% HP or Antibiotic Treatment		Residual Viability (%) after 0.5% HP Treatment	
		Plate Reader[2]	Microscope[3]	Plate Reader[2]	Microscope[3]
Drug free control		70%	75%		
DMSO control		60%	70%		
30% alcohol		70%	78%		
60% alcohol		80%	82%		
90% alcohol		65%	63%		
AZI		70%	65%		
DOX		66%	60%		
GEN		44%	50%		
RIF		31%	45%		
DAP		6%	10%		
Methylene Blue		29%	35%		
Miconazole		40%	50%		
Colloidal silver	Argentyn 23®	18%	37%	45%	76%
Andrographis paniculata	Chuan xin lian GE (Hawaii Pharm)	19%	38%	24%	50%
Uncaria rhynchophylla	Gou teng GE (Hawaii Pharm)	19%	39%	43%	88%
Rhizoma coptidis	Coptis GE (Hawaii Pharm)	21%	41%	22%	50%
Citrus paradisi	Grapefruit seed extract (Citrocept)	21%	38%	48%	89%
Uncaria tomentosa bark	Samento 20-24% AE (NutraMedix)	24%	50%	29%	65%
Stevia rebaudiana fol	Tian ju ye 25% AE (Sonoma County Herb Exchange)	24%	40%	48%	83%
Artemisia annua	Sweet wormwood 60% AE (Heron Botanical)	26%	42%	43%	75%
Dipsacus fullonum	Teasel 40% AE (Friend's of the Trees)	28%	57%	40%	78%
Artemisia annua	Sweet wormwood 90% AE (Heron Botanical)	30%	50%	45%	80%
Uncaria tomentosa cort	Uncaria 50% AE (Mountain Rose Herbs)	32%	60%	45%	82%

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Andrographis paniculata	Andrographis 30% AE (Heron Botanicals)	35%	65%	44%	81%
Artemisia annua	Sweet wormwood 30% AE (Heron Botanical)	40%	70%	58%	90%
Otoba sp. bark	Banderol 20-24% AE (NutraMedix)	41%	83%	50%	87%
Campsiandra angustifolia bark	Cumanda 20-24% AE (NutraMedix)	50%	85%	56%	90%

897 [1] A five-day-old stationary phase *B. henselae* culture was treated with herbal products (1% or 0.5%) (*v/v*)
898 or control drugs for three days. Drug concentrations used in this experiment were based on their Cmax
899 and were as follows: 0.2 μg/mL AZI, 2.4 μg/mL DOX, 10 μg/mL GEN, 7.8 μg/mL RIF, 60 μg/mL DAP,
900 2.9 μg/mL methylene blue, and 6.3 μg/mL miconazole. [2] Residual viability was calculated according to
901 the regression equation and the ratio of Green/Red fluorescence obtained by SYBR Green I/PI assay. [3]
902 Residual viability was assayed by fluorescence microscope counting as described in the Methods.

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