Botanical Medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Polygonum cuspidatum, and Alchornea cordifolia Demonstrate Inhibitory Activity Against Babesia duncani

Yumin Zhang¹, Hector Alvarez-Manzo¹, Jacob Leone², Sunjya Schweig³ and Ying Zhang⁴*

¹ Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, ² FOCUS Health Group, Naturopathic, Novato, CA, United States, ³ California Center for Functional Medicine, Kensington, CA, United States, ⁴ State Key Laboratory for the Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Human babesiosis is a CDC reportable disease in the United States and is recognized as an emerging health risk in multiple parts of the world. The current treatment for human babesiosis is suboptimal due to treatment failures and unwanted side effects. Although Babesia duncani was first described almost 30 years ago, further research is needed to elucidate its pathogenesis and clarify optimal treatment regimens. Here, we screened a panel of herbal medicines and identified Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Alchornea cordifolia, and Polygonum cuspidatum to have good in vitro inhibitory activity against B. duncani in the hamster erythrocyte model. Furthermore, we found their potential bioactive compounds, cryptolepine, artemisinin, artesunate, artemether, and baicalein, to have good activity against B. duncani, with IC₅₀ values of 3.4 μM, 14 μM, 7.4 μM, 7.8 μM, and 12 μM, respectively, which are comparable or lower than that of the currently used drugs quinine (10 μM) and clindamycin (37 μM). B. duncani treated with cryptolepine and quinine at their respective 1×, 2×, 4× and 8× IC₅₀ values, and by artemether at 8× IC₅₀ for three days could not regrow in subculture. Additionally, Cryptolepis sanguinolenta 90% ethanol extract also exhibited no regrowth after 6 days of subculture at doses of 2×, 4×, and 8× IC₅₀ values. Our results indicate that some botanical medicines and their active constituents have potent activity against B. duncani in vitro and may be further explored for more effective treatment of babesiosis.

Keywords: Babesia duncani, Cryptolepis sanguinolenta, herbal medicine, cryptolepine, artemisinin, baicalein, phytoneutrient, phytotherapy
INTRODUCTION

Babesiosis is a disease caused by the Babesia parasite that infects red blood cells. Babesia genus belongs to the apicomplexan phylum, and over 100 Babesia species have been identified (Birkenheuer et al., 2006). However, only a few of these have been documented to infect humans. Babesia divergens, Babesia microti, and Babesia duncani cause the most human babesiosis cases worldwide. Most human babesiosis infections in the United States are caused by B. microti and B. duncani, and in Europe the majority of reported cases are due to B. divergens (Vannier and Krause, 2012). All of these Babesia species can be acquired by Ixodes ticks that are also reservoirs of Borrelia spirochetes which cause Lyme disease. Recently, Dermacentor albipictus has been implicated as a competent tick vector for Babesia duncani (Swei et al., 2019). Epidemiologic studies have documented that up to 23% of patients with babesiosis also experienced concurrent Lyme disease (Diuk-Wasser et al., 2016) and coinfected patients can experience a greater number of symptoms for a longer duration than those with Lyme disease alone (Krause et al., 1996; Krause et al., 2002). In addition to transmission by tick bite, human babesiosis transmission has also been reported via blood transfusion, organ transplantation, and vertical transmission during pregnancy (Lux et al., 2003; Leiby, 2006). The clinical manifestations of babesiosis range from asymptomatic to severe and common clinical symptoms in patients include fever, chills, and sweats. Patients with immunological diseases, on immunosuppressive therapies, and patients who have undergone splenectomy are at increased risk of more severe symptoms and even death (Vannier et al., 2015).

Babesiosis caused by Babesia microti is endemic in the Northeast and the upper Midwest of the United States. Babesiosis caused by B. duncani was first described in Washington State as strain WA1 in 1991 and is widespread in North America based on molecular and immunological tests (Vannier and Krause, 2012; Scott and Scott, 2018). Animals such as mice, gerbils, and hamsters are susceptible to infection with B. duncani via intraperitoneal injection. Studies in animal models show that B. duncani has a different pathogenesis than B. microti, in that the parasitemia in hamsters infected with B. microti can initially be very high, but then decrease to undetectable levels within several weeks. In contrast, B. duncani can cause severe acute disease and death in hamsters, within 10 days after inoculation (Kjemtrup and Conrad, 2000). In mouse models, B. duncani can give rise to more severe presentations and higher mortality rates than B. microti (Moro et al., 1998). In terms of morphology, there is no obvious difference in intraerythrocytic stages between B. duncani and B. microti, nevertheless, based on the phylogenetic analysis of 18S RNA gene, ITS2 gene, cytb gene, and coxI gene, B. duncani show a distinct evolutionary lineage compared to B. microti and other Babesia species (Virji et al., 2019).

The current treatment protocols for human babesiosis frequently use medications such as atovaquone, azithromycin, clindamycin, quinine, and their combinations (Smith et al., 2020). However, these regimens are suboptimal and are associated with treatment failures and significant side effects (Kjemtrup and Conrad, 2000), even in immunocompetent patients (Krause et al., 1998; Gonzalez et al., 2014). Some cases of Babesia infection can be persistent, and recrudescence may occur up to two years after therapy (Krause et al., 2008; Raffalli and Wormser, 2016). Furthermore, it has been demonstrated that B. duncani showed unexpectedly high tolerance to atovaquone, azithromycin, clindamycin, and quinine in vitro (Abraham et al., 2018), and B. duncani could regrow in vitro after exposure to high concentrations of atovaquone or azithromycin for 3 days (Zhang et al., 2020). Therefore, alternative more effective antimicrobial agents and treatment regimens with fewer side effects need to be developed.

In the past decade, some promising anti-parasite drug candidates or compounds have been identified against B. microti, B. divergens, B. gibsoni, B. bovis, B. bigemina, and B. caballi in vitro or in vivo including mycophenolic acid, pentamidine, doxorubicin hydrochloride, vorinostat, luteolin, pyronaridine, robenidine, primaquine, and diphenyl furan for potential medical and veterinary treatment (Nehrbass-Stuedli et al., 2011; Rizk et al., 2015; Yao et al., 2015; Rizk et al., 2018; Li et al., 2020; Rizk et al., 2020). However, to date, limited research has focused on drug screening against B. duncani.

Herbal medicines were used by ancient cultures and their safety and efficacy have been documented by various traditional medicine systems such as Ayurveda and Traditional Chinese Medicine (Borchardt, 2002; Jaiswal et al., 2016). The adverse effects of botanical products were determined to be rare according to a recent report (Di Lorenzo et al., 2015). In a previous study, we have screened a panel of essential oils and found that garlic oil, black pepper, and their constituents showed good activity against B. duncani in a hamster erythrocyte model (Zhang et al., 2020). These findings indicated that natural products extracted from plants and herbs may serve as a potential source of promising compounds for anti-parasitic drugs. In this current study, we used the same hamster erythrocyte model to screen for inhibition of B. duncani using a panel of 46 herbal medicine extracts, many of which are useful in the treatment of babesiosis and related conditions according to the Buhner book on herbal medicines (Buhner, 2015) or were shown in our previous in vitro studies to have antimicrobial effects (Feng J. et al., 2020). Our results identified Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Alchornea cordifolia, and Polygonum cuspidatum and their bioactive compounds as having good activity against B. duncani.

MATERIALS AND METHODS

Hamster Donor Blood and Babesia duncani Culture

Hamster whole blood was collected from Golden Syrian hamsters (Charles River) by cardiac puncture using phosphate buffer saline containing 15 mM EDTA as anticoagulant according to protocols approved by the Johns Hopkins Institutional Animal Care and Use Committee. Hamster whole Blood was washed three times by centrifugation at 500 g for
15 min in PBS, 15mM EDTA solution with careful removal of the supernatant and buffy coat at each wash. After the last wash, red blood cells (RBCs) were resuspended at a concentration of 50% hematocrit in Puck’s saline glucose buffer with extra glucose (20 g glucose/L) and stored at 4°C less than 2 weeks before use. Babesia duncani strain WA1 (ATCC PRA-302) was cultured in vitro in HaRGM medium with fresh hamster erythrocytes at 37°C under an atmosphere of 5% CO2 with 95% humidity. Hamster erythrocytes were prepared with HaRGM medium, and continuous B. duncani culture method was performed as described in detail in our previous publication (Zhang et al., 2020).

Natural Products and Compounds
A panel of 46 herbal medicine extracts (Table 1) and relevant solvent controls were used for testing. The herbal medicine extracts of Uncaria tomentosa, Stevia rebaudiana, Juglans nigra, Cryptolepis sanguinolenta, Artemisia annua, Andrographis paniculata, Dipsacus fullonum, Withania somnifera, Scutellaria baicalensis, and Polygonum cuspidatum were sourced from KW Botanicals (San Anselmo, California) and Heron Botanicals (Kingston, Washington). Most of these natural products were provided as ethanol extracts at 30%, 60%, and 90% ethanol, and the ethanol solvent was also tested as a control in the respective concentrations. Other herbal medicine extracts were purchased commercially. Detailed information including source, extraction parts, extraction type, and manufacturer of all the herbal medicine extracts tested in this study is shown in Supplementary Table S1. Cryptopine (cryptopine hydrate; Sigma-Aldrich), artemisinin (Alfa Aesar), artemesunate (TCI Chemicals), artether (Frontier Scientific), baicalein (5,6,7-Trihydroxyflavone; Alfa Aesar), quinine (quinine hydrochloride; Frontier Scientific), and clindamycin (clindamycin hydrochloride; Sigma-Aldrich) were prepared in DMSO stock in high concentrations and then diluted to desired concentrations with DMSO.

In Vitro Evaluation of Herbal Medicines and Compounds on Inhibition of B. duncani
Babesia duncani was cultured in 24-well plate at 2.5% hematocrit of hamster erythrocytes at 37°C under an atmosphere of 5% CO2 with 95% humidity for 3–4 days. When parasitemia reached 2%, the infected erythrocytes were split evenly into 96-well plates at 2.5% hematocrit each well for natural products and compounds test. Natural products were prepared in 10% (v/v) DMSO stock solution. Ethanol at 30%, 60%, and 90% was prepared in parallel in DMSO as controls. Then the natural products and ethanol controls were added to 96-well plates containing infected erythrocytes to obtain final concentrations of 0.01%. Treated cultures were inoculated at 37°C for 3 days without replacing medium in a chamber under an atmosphere of 5% CO2 with 95% humidity. Then 1 µl of erythrocytes laid on the plate bottom were taken out for growth test by SYBR Green I assay as described previously (Zhang et al., 2020). Briefly, the 1 µl of RBCs sediment were placed into 100 µl of lysis buffer consisting of 20 mM Tris, pH 7.4, 5 mM EDTA, 0.008% saponin, 0.08% Triton X-100, and 2× SYBR Green I (10,000× stock, Invitrogen) in 96-well plates. The plates were then inoculated in the dark at 37°C for 60 mins followed by plate reading at excitation wavelength (490 nm) and a fluorescence intensity at 520 nm in a microplate reader (HTS 7000 plus Bio Assay Reader, PerkinElmer Inc., USA). For the IC50 susceptibility assay, B. duncani cultures were exposed to increasing concentrations of Artemisia annua (30% ethanol extract), Cryptolepis sanguinolenta (90% ethanol extract), Scutellaria baicalensis (90% ethanol extract), cryptopine, artemisinin, artemesunate, artether, baicalein, quinine, and clindamycin. Each IC50 was performed in triplicate in 96-well plates with a starting 2% parasitemia and 2.5% hematocrit followed by SYBR Green I stain. Dose-response curves with fitting a nonlinear regression curve were performed in GraphPad Prism (version 7.0) and then calculate the best-fit IC50 values. The morphology of Giemsa stained thin blood smears of B. duncani after treatment with compounds at 1×, 2×, and 4× IC50 concentrations for 3 days was observed under BX-710 All-in-One Fluorescence Microscope (KEYENCE, Inc., Itasca, IL, USA).

Subculture Studies to Evaluate Killing Efficacy of the Top Natural Product Hits and Selected Compounds
B. duncani cultures were exposed to Artemisia annua (30% ethanol extract), Cryptolepis sanguinolenta (90% ethanol extract), Scutellaria baicalensis (90% ethanol extract), cryptopine, artemisinin, artemesunate, artether, baicalein, quinine, and clindamycin at their respective 1×, 2×, 4× and 8× IC50 values for 3 days. After three days of exposure, the treated erythrocytes were washed three times in HaRGM medium to wash away drugs and then inoculated in fresh hamster erythrocytes at ratio of 1:5. Each compound was tested in triplicate, and every well had 4–6 replicates for sampling to avoid obvious hematocrit decrease. Culture conditions of the subcultures were identical to those described above. One microliter of erythrocytes laid on the plate bottom was taken out every one or two days and stored at -80°C. The growth of the subculture was examined by the SYBR Green I assay as described above, and the fluorescence units of day zero were normalized as zero.

RESULTS
Evaluation of Natural Product Extracts for Inhibitory Activity Against B. duncani
We evaluated a panel of 46 herbal medicine extracts and their corresponding controls at a concentration of 0.01% (v/v) for inhibitory activity against B. duncani at an initial parasitemia of 2% in vitro after 3 days of incubation (Table 1). Cryptolepis sanguinolenta (60, 90% ethanol extracts), Artemisia annua (30% ethanol extract), Scutellaria baicalensis (60%, 90% ethanol extracts), Polygonum cuspidatum (60% ethanol extract), and Alchornea cordifolia were the top hits with more than 50%
inhibitory effect at 0.01% against *B. duncani*. As a control, we tested the ethanol carrier at concentrations of 30%, 60%, and 90%, which did not show obvious inhibitory effect at up to 1% concentration.

Cryptolepis sanguinolenta, Artemisia annua, and Scutellaria baicalensis showed activity at 30%, 60%, and 90% EE, therefore, we further tested the half-maximal inhibitory concentration values of these three active natural product extracts at different ethanol concentrations. Dose-response assays confirmed that Cryptolepis sanguinolenta and Scutellaria baicalensis at 60% and 90% EE showed approximately 4–10 times lower IC50 values compared to those at 30% EE. In contrast, *Artemisia annua* at 30% EE exhibited the lowest IC50 value, even lower than it was at 60% and 90% EE (Table 2; Figure 1).

| Product Names | Plants | Inhibition (%) |
|---------------|--------|---------------|
| Chinese Skullcap (90% EE) | Scutellaria baicalensis | 84 |
| Cryptolepis (90% EE) | Cryptolepis sanguinolenta | 80 |
| Cryptolepis (60% EE) | Cryptolepis sanguinolenta | 70 |
| Chinese Skullcap (60% EE) | Scutellaria baicalensis | 68 |
| Japanese knotweed (60% EE) | Polygonum cuspidatum | 59 |
| Sweet wormwood (30% EE) | Artemisia annua | 58 |
| Alchornea | Alchornea cordifolia | 54 |
| Japanese knotweed (90% EE) | Polygonum cuspidatum | 42 |
| Andrographis (90% EE) | Andrographis paniculata | 37 |
| Andrographis (60% EE) | Andrographis paniculata | 36 |
| Sweet wormwood (60% EE) | Artemisia annua | 35 |
| Andrographis (30% EE) | Andrographis paniculata | 34 |
| Cistus | Cistus incanus | 34 |
| Ashwagandha (30% EE) | Withania somnifera | 33 |
| Hemp oil | Cannabis sativa | 26 |
| Barberry | Berberis vulgaris | 25 |
| Chuan xin lan | Andrographis paniculata | 23 |
| Black walnut (90% EE) | Juglans nigra | 23 |
| Ashwagandha (60% EE) | Withania somnifera | 23 |
| Stevia/Tian ju ye | Stevia rebaudiana fol | 22 |
| Ashwagandha (90% EE) | Withania somnifera | 21 |
| Echinacea | Echinacea purpurea & Echinacea angustifolia | 20 |
| Andrographis | Andrographis paniculata | 19 |
| Licorice | Glycyrrhiza spp. | 19 |
| Grapefruit seed extract | Grapefruit seed | 17 |
| Usnea | Usnea spp. | 17 |
| Eleutherococcus | Eleutherococcus senticosus | 17 |
| Black walnut (60% EE) | Juglans nigra | 13 |
| Sweet wormwood (90% EE) | Artemisia annua | 13 |
| Gou teng | Uncaria rhynchophylla | 12 |
| Teasel/Gao liang jiang | Dipsacus fullonum | 11 |
| Osha | Ligusticum porto root | 11 |
| Cryptolepis (30% EE) | Cryptolepis sanguinolenta | 9 |
| Chinese Skullcap (30% EE) | Scutellaria baicalensis | 9 |
| Houttuynia | Houttuynia | 9 |
| Bidos | Bidens ptila | 9 |
| Ban zhi lan | Scutellaria barbata | 8 |
| Black walnut (30% EE) | Juglans nigra | 8 |
| Uncaria | Uncaria tomentosa | 6 |
| Samento | Uncaria tomentosa | 0 |
| Cumanda | Campsiandra angustifolia | 0 |
| Bandero | Otoha sp. | 0 |
| Coptis | Rhizoma coptidis | 0 |
| Japanese knotweed (30% EE) | Polygonum cuspidatum | 0 |
| Reishi | Ganoderma lingzhi | 0 |
| Black Walnut | Juglans nigra fruc | 0 |

The growth of infected erythrocytes at day zero was set as 0%. The growth of *B. duncani* in infected erythrocytes treated with 1% DMSO vehicle at day three was set as 100%. EE, ethanol extract. The bold product names indicate the effective hits studied in this study.

**Artemisinin, Cryptolepine, and Baicalein Are the Active Constituents Extracted From Artemisia annua, Cryptolepis sanguinolenta, and Scutellaria baicalensis Respectively and Have High Potent Antibabesial Activity**

Since Cryptolepis sanguinolenta (90% ethanol extract), *Artemisia annua* (30% ethanol extract), and Scutellaria baicalensis (90% ethanol extract)
ethanol extract) extracts showed better inhibitory activity against B. duncani than Polygonum cuspidatum, and Alchornea cordifolia, we focused on testing cryptolepine, artemisinin, and baicalein, which are known bioactive compounds derived from these three herbal medicines (Klayman, 1993; Cimanga et al., 1996; Nishioka et al., 1998). For comparison, we also tested the commonly used clinical drugs quinine and clindamycin. All these compounds and drugs inhibited the growth of B. duncani in a dose-dependent manner (Figure 1). The IC50 values of cryptolepine, artemisinin, and baicalein were 3.4 µM, 14 µM, and 12 µM, respectively. In contrast, the IC50 values of quinine and clindamycin were 10 µM and 37 µM.

Artemisinin Derivatives Artesunate and Artemether Showed Higher Inhibitory Activity Against B. duncani Than Artemisinin

Artemisinin derivatives, including artemunate and arteether, are potent antimalarial drugs which have been shown to reduce malaria parasitemia more rapidly than any other known antimalarial drugs, and are effective against multidrug-resistant malaria parasites (Olliaro et al., 2001). In our research, we compared artemisinin, artemunate, and arteether for their inhibitory effects against B. duncani in our culture system (Figure 2). The IC50 values of artemunate and arteether were 7.4 µM and 7.8 µM, respectively, indicating that both of the artemisinin derivatives showed higher inhibitory activity than artemisinin.

| Natural product extracts | IC50 values |
|--------------------------|-------------|
| Cryptolepis sanguinolenta (30% EE) | 0.039% (v/v) |
| Cryptolepis sanguinolenta (60% EE) | 0.0041% |
| Cryptolepis sanguinolenta (90% EE) | 0.0046% |
| Artemisia annua (30% EE) | 0.0091% |
| Artemisia annua (60% EE) | 0.0097% |
| Artemisia annua (90% EE) | 0.030% |
| Scutellaria baicalensis (30% EE) | 0.034% |
| Scutellaria baicalensis (60% EE) | 0.038% |
| Scutellaria baicalensis (90% EE) | 0.0097% |
| Cryptolepine | 3.4 µM |
| Artemisinin | 14 µM |
| Baicalein | 12 µM |
| Quinine | 10 µM |
| Clindamycin | 37 µM |

Growth was evaluated by SYBR Green stain at day three after B. duncani exposure to natural product extracts. Each natural product concentration was made in triplicate. IC50 values were calculated in GraphPad Prism (version 7.0). EE, ethanol extract.

FIGURE 1 | Evaluation of in vitro natural products and drug compounds’ susceptibility of B. duncani to Cryptolepis sanguinolenta (30%, 60%, and 90% EE), Artemisia annua (30%, 60%, and 90% EE), Scutellaria baicalensis (30%, 60%, and 90% EE), cryptolepine, artemunate, baicalein, clindamycin, and quinine at different concentrations. Each natural product or drug compound concentration was set in triplicate. SYBR Green I assay was performed at day three after exposure to the natural products or drug compounds. The growth of B. duncani in infected erythrocytes at day zero was set as 0%. The growth of B. duncani in infected erythrocytes treated with 1% DMSO vehicle at day three was set as 100%. GraphPad Prism (version 7.0) was used to generate dose-response curves by fitting a nonlinear regression curve to the data. c, concentration; EE, ethanol extract.
Morphological Changes of Treated *B. duncani*

After three days, the morphological changes of *B. duncani* treated with cryptolepine, artemunate, artemether, baicalein, clindamycin, and quinine at their respective concentrations of 1×, 2×, 4× IC₅₀ values were observed. The parasites treated by cryptolepine (1×, 2×, 4× IC₅₀ values) appeared to have dramatically condensed chromatin in both trophozoite and schizont stages (Figure 3). Parasites exposed to artemunate, artemether, and clindamycin at 4× IC₅₀ values displayed teratogenic form, and those exposed to quinine showed gradually enlarged vacuole at the treated concentration of 1×, 2×, 4× IC₅₀ values (Figure 3). *B. duncani* treated with artemunate (1×, 2× IC₅₀ values), artemether (1×, 2×, IC₅₀ values), baicalein (1×, 2×, 4× IC₅₀ values), and clindamycin (1×, 2× IC₅₀ values) did not show significant morphological changes (Figure 3).

Subculture Studies to Evaluate Viability of *B. duncani* Treated With Herb Extract Compounds Artesunate, Artemether, Cryptolepine, Baicalein, As Well as Quinine, and Clindamycin

In order to further confirm our finding that cryptolepine, baicalein, artemunate, and artemether were the most active compounds for inhibiting *B. duncani* growth in vitro and that their corresponding natural product extracts indeed have a killing effect, we performed subculture studies by re-inoculating three day treated infected erythrocytes into fresh uninfected erythrocytes to monitor regrowth. For comparison, we also evaluated the killing activity of quinine and clindamycin. The results showed that *Artemisia annua* (30% ethanol extract) and *Scutellaria baicalensis* (90% ethanol extract) treated *B. duncani* could regrow at 1×, 2×, 4×, and 8× IC₅₀ values, and growth of most of these treated parasites could be observed at day two (Figures 4A, B). *Cryptolepis sanguinolenta* (90% ethanol extract) treated *B. duncani* regrew two days after treatment at 1× IC₅₀ value, however, regrowth did not occur in six days after treatment at 2×, 4×, and 8× IC₅₀ values (Figure 4C). Similarly, *B. duncani* treated with cryptolepine at 1×, 2×, 4×, and 8× IC₅₀ values did not regrow in six days (Figure 4G). *B. duncani* treated with artemunate and artemether regrew after treatment at 1×, 2×, and 4× IC₅₀ values, but no regrowth occurred after treatment with artemether at 8× IC₅₀ (Figures 4D, E). Treatment with baicalein at an IC₅₀ of 1× and 2× did not prevent *B. duncani* regrowth at day two of incubation, and regrowth also occurred at day four when treated with baicalein at an IC₅₀ of 4× and 8× (Figure 4F). The drug quinine showed good killing effect (Figure 4H) at 1×, 2×, 4×, and 8× IC₅₀ values, as did clindamycin at 2×, 4×, and 8× IC₅₀ values. However, *B. duncani* could regrow after clindamycin treatment at 1× IC₅₀ value (Figure 4I).

DISCUSSION

While recovery from clinical babesiosis can be spontaneous in healthy individuals, there is a much greater risk of severe symptoms and fatality in elderly, asplenic, or otherwise immunocompromised patients (Vannier and Krause, 2012; Vannier et al., 2015). Furthermore, some patients can experience persistent infection and clinical illness despite treatment with current therapeutic regimens (Krause et al., 1998; Raffalli and Wormser, 2016; Bloch et al., 2019). Importantly, illness can be more severe and recalcitrant to available treatment regimens when patients are simultaneously infected with multiple tickborne pathogens (Krause et al., 2002; Horowitz and Freeman, 2019; Sanchez-Vicente et al., 2019). In addition, some physicians opine that patients infected with *B. duncani* can have a more protracted course and typically require a longer duration of treatment than those infected with *B. microti* (Scott and Scott, 2018). The combination of quinine and clindamycin is the treatment regimen recommended for *B. duncani* spp. infections (Vannier and Krause, 2009) and all severe babesiosis infections (Centers for Disease, 1983). However, a clinical trial indicated that 72% of patients who received quinine plus clindamycin for babesiosis suffered side effects including tinnitus, vertigo, and gastrointestinal upset, in some cases severe enough to necessitate dosage decrease or treatment suspension (Krause et al., 2000). Previous studies found that quinine showed 30–100 nM of EC₅₀ against *Plasmodium falciparum* (Skinner et al., 1996), and clindamycin showed around 8.8 nM of IC₅₀ against *Plasmodium falciparum* 3D7 (Dahl and Rosenthal, 2007). However, in this study we found that quinine and clindamycin had much higher IC₅₀ values against *B. duncani* growth in hamster erythrocytes (10 μM and 37 μM, respectively), and these results are comparable with research done in vitro in human erythrocytes (Abraham et al., 2018). Our subculture results showed quinine had good killing effect in vitro in the range of 10–80 μM (Figure 4H), but relapse occurred after the treatment of clindamycin at the concentration of 37 μM (Figure 4I). Importantly, we identified that the bioactive compounds derived from *Cryptolepis sanguinolenta*, *Artemisia annua*, and *Scutellaria baicalensis*, namely cryptolepine, artemisinin, artemunate, artemether, and baicalein (3.4 μM, 14 μM, 7.4 μM, 7.8 μM, and 12 μM, respectively) had comparable or even better activity against...
B. duncani than the commonly used medications quinine (10 μM) and clindamycin (37 μM).

Botanical medicine has a long documented history of use over 5,000 years (Borchardt, 2002) and recent retrospective and systematic reviews have concluded severe adverse events associated with botanical medicine usage are rare (Di Lorenzo et al., 2015; Restani et al., 2016). Research over the past few decades has demonstrated that many botanical medicines have antimicrobial, antiparasitic, and anticancer activity (Tagboto and Townson, 2001; Shoemaker et al., 2005; Feng et al., 2011). In our previous study, we identified seven herbal medicines that have good activity against stationary phase Borrelia burgdorferi compared to the control antibiotics used in clinical treatment (Feng J. et al., 2020). In humans, Babesia spp. frequently exist as co-infections with Borrelia burgdorferi through tick transmission (Magnarelli et al., 1995; Parveen and Bhanot, 2019). The screening results of the present study revealed that Cryptolepis sanguinolenta, Artemisia annua, and Scutellaria baicalensis, which previously showed good activity against Borrelia burgdorferi (Tona et al., 1998), also had impressive inhibitory

FIGURE 3 | Morphology of B. duncani observed after treatment with cryptolepine, artesunate, artemether, baicalein, clindamycin, and quinine at their respective concentrations of 1×, 2×, and 4× IC50 values after three days of drug exposure.
effects against *B. duncani* (at even lower concentrations of 0.01%). Additionally, we showed that herbs extracted by different concentrations of alcohol (30%, 60%, and 90% ethanol extracts) had different inhibitory activity. Extracts of *Cryptolepis sanguinolenta* and *Scutellaria baicalensis* in higher concentrations of alcohol (90% and 60% ethanol extracts) had better inhibitory activity than in low alcohol concentration (30% ethanol extract), while *Artemisia annua* performed better at a lower alcohol concentration. This indicates that the corresponding bioactive ingredients of these herbs have diverse solubility in alcohol solvents, and that appropriate solvents and techniques are critical for the isolation and extraction of active constituents.

*Cryptolepis sanguinolenta* has been shown in preclinical studies to have anti-inflammatory, antimicrobial, anti-amoebic, anti-cancer, and anti-malarial properties (Grellier et al., 1996; Tona et al., 1998; Mills-Robertson et al., 2012; Ansah and Mensah, 2013; Hanprasertpong et al., 2014). Cryptolepine, an indoloquinoline alkaloid isolated from *Cryptolepis sanguinolenta*, has been shown *in vitro* to have significant antiplasmodial activity against drug-sensitive and drug-resistant strains (Osafo et al., 2017; Ameyaw et al., 2018), demonstrating an IC$_{50}$ of 0.2–0.6 µM against *Plasmodium falciparum in vitro* (Grellier et al., 1996). *In vivo*, cryptolepine given orally at 50 mg/kg/day to mice infected with *Plasmodium berghei* was found to have moderate antimalarial activity to suppress parasitemia by 80% (Wright et al., 1996). The bioactivity of cryptolepine is believed to contribute to DNA intercalating activity targeting non-alternating CC sites, cell morphology change, and topoisomerase II inhibiting effects (Sawer et al., 1995; Bonjean et al., 1998; Lisgarten et al., 2002). Preliminary preclinical and clinical data indicate that *Cryptolepis sanguinolenta* and cryptolepine can have systemic effects *in vivo* and therefore are candidates for clinical development. Two open-label trials using different formulations of *Cryptolepis sanguinolenta* showed significant efficacy without observed toxicity in the treatment of patients with uncomplicated malaria (Bugyei et al., 2010; Tempesta, 2010). The pharmacokinetics of cryptolepine revealed that it has low bioavailability, a moderate half-life (4.5h) and extensive distribution (Forkuo et al., 2017a). A nanoformulation of cryptolepine exhibited a more favorable pharmacokinetic profile and a stronger antiplasmodial effect compared to the unaltered form of cryptolepine (Forkuo et al., 2017b). Toxicology data suggests that *Cryptolepis sanguinolenta* is generally safe at doses under 500 mg/kg of body weight (Osafo et al., 2017), however further studies are needed to evaluate concerns regarding potential anti-fertility and embryotoxic effects (Ajayi and Akhigbe, 2012; Mensah et al., 2019). Recently, Batiha et al. demonstrated that cryptolepine exhibited significant activity against multiple *Babesia* spp. *In vitro* and inhibited *B. microti* in mice at a dose of 5 mg/kg (Batiha et al., 2020b). To the best of our knowledge no study has reported the *in vitro* or *in vivo* treatment efficacy of whole herb *Cryptolepis sanguinolenta*, against *Babesia* spp. Among the panel of natural product extracts tested in this study, we found *Cryptolepis sanguinolenta*.
The Chinese herb, *Artemisia annua* (Sweet wormwood or Qing Hao) has been used for the treatment of malaria for centuries (Feng X. et al., 2020) and the Chinese scientist who isolated its most famous active constituent, artemisinin, was awarded the Nobel Prize in 2015 in recognition for artemisinin’s role in reducing malaria-associated morbidity and mortality (Liu and Liu, 2016). Artemisinin-based compounds have become pivotal in malaria treatment as they can reduce malarial parasitemia more rapidly than other known antimalarial drugs and are effective against all stages of *Plasmodium* spp. (Price et al., 1999; Adjuik et al., 2003). In contrast, our subculture study showed the regrowth of *B. duncani* could occur six days after treatment of artesunate at as high as 60 µM (8× IC₅₀) and of artemisinin at 31 µM (4× IC₅₀). Because there is limited data in the research literature on the efficacy of artemisinin-related drugs against other *Babesia* spp., it remains unknown whether the low susceptibility to artemisinin, artesunate, and artemether is specific to *B. duncani*. Preliminary animal data suggest that adding a second anti-parasitic agent, such as cryptolepine from *Cryptolepis sanguinolenta*, may increase the susceptibility of artemisinin-based therapy to *Babesia* spp., similar to the use of two or three agents in artemisinin-based combination therapy (ACT) for malaria (Kumar et al., 2003; Iguchi et al., 2015; Forkuo et al., 2016; Carvalho et al., 2020). The development of malarial resistance to ACT raises important points regarding the potential benefits of using a whole plant form (e.g., *Artemisia annua*) vs a single constituent form (e.g., artemisinin) (Haldar et al., 2018). Although theoretic concerns that *Artemisia annua*’s low artemisinin content may increase the risk of antimicrobial resistance (W.H.O.W.P.S, 2012) subsequent testing has revealed that antimicrobial resistance occurred three times slower when a whole plant form of *Artemisia annua* was used compared to artemisinin monotherapy (Elfawal et al., 2015). Additionally, preliminary clinical studies have revealed that treatment with whole plant *Artemisia annua* was associated with improved clinical outcomes and fewer side effects compared to ACT (Daddy et al., 2017; Munyangi et al., 2019). The improved outcomes when using a whole plant form of *Artemisia annua* can be explained by an evolutionarily guided synergism among its phytochemicals resulting in increased bioavailability of active compounds (pharmacodynamic synergy), complementary mechanisms of action (antimicrobial, immune-modulating, anti-inflammatory, etc.), multidrug resistance inhibition, and multiple antimicrobial compounds (Rasoanaivo et al., 2011; Munyangi et al., 2019; Caesar and Cech, 2019; Desrosiers et al., 2020, Zhao et al., 2020).

*Scutellaria baicalensis* (Chinese skullcap) has been widely used as a medicinal plant in China for thousands of years for...
the treatment of diarrhea, dysentery, hypertension, hemorrhage, insomnia, inflammation and respiratory infections (Zhao et al., 2016). Over forty different compounds have been isolated from Scutellaria baicalensis and the antimicrobial activity is hypothesized to be due to flavonoids, volatile oils, terpenoids, and polysaccharides (Wu et al., 2008; Lu et al., 2011; Leung et al., 2016). Previous studies have demonstrated that Scutellaria baicalensis extract and one of its primary bioactive constituents, baicalein, exhibited in vitro activity against various morphologic forms of Borrelia burgdorferi and Borrelia garinii (Goc et al., 2015; Feng J. et al., 2020). In traditional Korean medicine, Scutellaria baicalensis was combined with Artemisia apiacea for the treatment of malaria and other malaria-like diseases (Trinh et al., 2018). Existing clinical trial data on Scutellaria baicalensis indicates it is non-toxic and has systemic effects showing benefits in diabetes, enterovirus encephalitis, and adjunctive cancer care (Smol’ianinov et al., 1997; Lin et al., 2016; Shin et al., 2020). A medical food combination of concentrated baicalin and catechin (Limbrel™, Move Free Advanced™) was linked to reversible liver damage in at least 35 cases (Chalasani et al., 2012), however a recent study found no hepatotoxicity in patients taking a whole herb formulation of Scutellaria baicalensis dosed at 1,335 mg per day for an average of 444 days (Puri et al., 2019). Human safety studies on baicalin have shown that doses between 200–800 mg per day are safe and well-tolerated with a favorable pharmacokinetic profile (Li et al., 2014; Pang et al., 2016). The anti-babesial activity of Scutellaria baicalensis and its active constituents have not been previously reported. In this study, we found Scutellaria baicalensis and baicalein showed good in vitro activity against B. duncani, with the $IC_{50}$ value of baicalein practically the same as quinine (12 µM vs 10 µM), and three times lower than clindamycin (12 µM vs 37 µM). A study of ethanol extracts from Scutellaria baicalensis showed that it had the strongest inhibitory and bactericidal efficacy against six foodborne pathogens and had the highest content of flavonoids and phenolic acids (Lu et al., 2011). These findings are consistent with the present study which demonstrated Scutellaria baicalensis extract in 90% ethanol had a lower $IC_{50}$ against B. duncani than those in 30% and 60% ethanol. Similar to artemesunate, regrowth of B. duncani treated with baicalein did not occur in four days at 4× and 8× $IC_{50}$ values.

It should be noted that Alchornea cordifolia and Polygonum cuspidatum extracts also showed good inhibitory effect against B. duncani in our study. Both have been documented to have antimicrobial and anti-inflammatory activity (Ebi, 2001; Manga et al., 2004; Shan et al., 2008; Ghanim et al., 2010). Alchornea cordifolia has been used by traditional herbalists in several African countries for the treatment of malaria (Boniface et al., 2016) and pre-clinical studies corroborate significant antimalarial effects (Mustofa et al., 2000; Mesia et al., 2008; Ayisi et al., 2011). The active constituents of Alchornea cordifolia extract are complex, including phenolic acid, gallic acid, protocatechuc acid, ellagic acid, and quercetin (Boniface et al., 2016). Of note, ellagic acid has previously shown in vitro antiplasmodial activity (Lamikanra et al., 1990; Banzouzi et al., 2002) and in vivo antibabesial activity (Beshbishy et al., 2019) and quercetin has been shown to have antiplasmodial and antibabesial effects (Batiha et al., 2020a). Although clinical data is lacking, preclinical studies reveal Alchornea cordifolia has favorable toxicology and bioavailability profiles (Gatting et al., 2010; Ajibade and Olayemi, 2015; Boniface et al., 2016; Djimeli et al., 2017). Polygonum cuspidatum contains over 60 active constituents with wide-ranging mechanisms of action and varying degrees of bioavailability (Pan et al., 2019). Stilbenes (e.g., resveratrol) and hydroxyanthraquinones (e.g., emodin) are among the active constituents of Polygonum cuspidatum that exert antibacterial properties (Shan et al., 2008). The bioavailability of orally administered resveratrol and emodin is suboptimal (Walle, 2011; Dong et al., 2016) and therefore numerous strategies have been developed to improve their pharmacokinetic profiles (Chimento et al., 2019; Dewanjee et al., 2020). Despite its low bioavailability, resveratrol has high bioactivity which is thought to be related to extensive biotransformation in enterocytes, hepatocytes, and the gastrointestinal microbiome (Luca et al., 2020). Although various active constituents from Polygonum cuspidatum have been shown to have antiparasitic effects (Passos et al., 2015; Lin et al., 2017), to our knowledge, this is the first time that the antibabesial effects of Polygonum cuspidatum have been reported. Interestingly, our previous study indicated that Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, and Polygonum cuspidatum extracts have activity against Borrelia burgdorferi (Feng J. et al., 2020), indicating that it may be advantageous to use these herbs to simultaneously target these different pathogens in complex Lyme disease with co-infections.

A potential limitation of the study is the descriptive nature of the study. Future studies are needed to determine the active components of the effective hits, to better investigate their effects on Babesia morphology through microscopy techniques, and to characterize if extracts produce Babesia death or just inhibit its growth or cell cycle, and to confirm their activity in animal models.

**CONCLUSIONS**

In this study we identified herbal medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Alchornea cordifolia, and Polygonum cuspidatum that exhibit good in vitro inhibitory activity against B. duncani at 0.01% (v/v). Furthermore, we tested the activity of the potential bioactive constituents and found that cryptolepine, artemisinin-related compounds, and baicalein may be promising alternatives to treat human babesiosis caused by B. duncani. These botanical medicines can be considered candidates for clinical development based on their low cost, favorable toxicity and pharmacokinetic profiles and history of safety and efficacy from clinical trials and/or use in traditional medicine. Future research will be needed to identify the bioactive components and to test if they also have activity against other Babesia species in vitro and in vivo. In order to identify more effective treatments, combinations of these
active herbs and active compounds need to be tested both in vitro and in animal models. This research needs to be done both in isolation and potentially in combination with current antibabesial drugs. Since the botanical medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Alchornea cordifolia, and Polygonum cuspidatum are already in clinical use, it is also important for future studies to evaluate them directly in patients using specific clinical treatment regimens.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Johns Hopkins Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

Conceptualization, YMZ and YZ. Methodology, YMZ, YZ, HA-M, and JL. Data curation, YMZ and HA-M. Funding acquisition, YZ. Writing—original draft, YMZ. Writing—review and editing, JL, SS, and YZ. All authors contributed to the article and approved the submitted version.

FUNDING

Sources of funding included Bay Area Lyme Foundation, the Steven and Alexandra Cohen Foundation, Global Lyme Alliance, NatCapLyme, LivLyme Foundation, and the Einstein–Sim Family Charitable Fund.

ACKNOWLEDGMENTS

We gratefully acknowledge the generous support of Bay Area Lyme Foundation, the Steven and Alexandra Cohen Foundation, Global Lyme Alliance, NatCapLyme, LivLyme Foundation, and the Einstein–Sim Family Charitable Fund. We thank BEI Resources for providing the Babesia duncani strain WA1 (ATCC® PRA-302™) used in this study. We thank herbalists Eric Yarnell ND and Brian Kie Weissbuch for providing some of the botanical extracts for evaluation in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.624745/full#supplementary-material

REFERENCES

Abraham, A., Brasov, I., Thekkiniath, J., Kilian, N., Lawres, L., Gao, R., et al. (2018). Establishment of a continuous in vitro culture of Babesia duncani in human erythrocytes reveals unusually high tolerance to recommended therapies. J. Biol. Chem. 293, 19974–19981. doi: 10.1074/jbc.A118.005771

Adjou, M., Babiker, A., Garner, P., Olliaro, P., Taylor, W., White, N., et al. (2004). Artesunate combinations for treatment of malaria: meta-analysis. Lancet 363, 9–17. doi: 10.1016/S0140-6736(03)15162-8

Ajayi, A. F., and Akhigbe, R. E. (2012). Antifertility activity of Cryptolepis sanguinolenta leaf ethanolic extract in male rats. J. Hum. Reprod. Sci. 5, 43–47. doi: 10.4103/0974-1208.97799

Ajibade, T. O., and Olayemi, F. O. (2015). Reproductive and toxic effects of methanol extract of Alchornea cordifolia leaf in male rats. Andrologia 47, 1034–1040. doi: 10.1111/and.12374

Ameyaw, E. O., Asmah, K. B., Binney, R. P., Henneh, I. T., Owusu-Agyei, P., Prah, J., et al. (2018). Isobolographic analysis of co-administration of two plant-derived antiplasmodial drug candidates, cryptolepine and xylopic acid, in Plasmodium berghei. Malar. J. 17, 153. doi: 10.1186/s12936-018-2283-8

Ansah, C., and Mensah, K. B. (2013). A review of the anticancer potential of the active herbs and active compounds need to be tested both in vitro and in animal models. This research needs to be done both in isolation and potentially in combination with current antibabesial drugs. Since the botanical medicines Cryptolepis sanguinolenta, Artemisia annua, Scutellaria baicalensis, Alchornea cordifolia, and Polygonum cuspidatum are already in clinical use, it is also important for future studies to evaluate them directly in patients using specific clinical treatment regimens.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Johns Hopkins Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

Conceptualization, YMZ and YZ. Methodology, YMZ, YZ, HA-M, and JL. Data curation, YMZ and HA-M. Funding acquisition, YZ. Writing—original draft, YMZ. Writing—review and editing, JL, SS, and YZ. All authors contributed to the article and approved the submitted version.

FUNDING

Sources of funding included Bay Area Lyme Foundation, the Steven and Alexandra Cohen Foundation, Global Lyme Alliance, NatCapLyme, LivLyme Foundation, and the Einstein–Sim Family Charitable Fund.

ACKNOWLEDGMENTS

We gratefully acknowledge the generous support of Bay Area Lyme Foundation, the Steven and Alexandra Cohen Foundation, Global Lyme Alliance, NatCapLyme, LivLyme Foundation, and the Einstein–Sim Family Charitable Fund. We thank BEI Resources for providing the Babesia duncani strain WA1 (ATCC® PRA-302™) used in this study. We thank herbalists Eric Yarnell ND and Brian Kie Weissbuch for providing some of the botanical extracts for evaluation in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2021.624745/full#supplementary-material

Batiha, G. E., Beshbishy, A. M., Ikram, M., Mulla, Z. S., El-Hack, M. E. A., Taha, A. E., et al. (2020a). The Pharmacological Activity, Biochemical Properties, and Pharmacokinetics of the Major Natural Polyphenolic Flavonoid: Quercetin. Foods 9. doi: 10.3390/foods9030374

Batiha, G. E., Beshbishy, A. M., Alkazmi, L. M., Nadwa, E. H., Rashwan, E. K., Yokoyama, N., et al. (2020b). In vitro and in vivo growth inhibitory activities of cryptolepine hydrate against several Babesia species and Theileria equi. PLoS Negl. Trop. Dis. 14 (8), e0008489. doi: 10.1371/journal.pntd.0008489

Beshbishy, A. M., Batiha, G. E., Yokoyama, N., and Igarashi, I. (2019). Ellagic acid microspheres restrict the growth of Babesia and Theileria in vitro and Babesia microti in vivo. Parasit. Vectors 12, 269. doi: 10.1186/s13071-019-3520-x

Birkenheuer, A. J., Whittington, J., Neel, J., Large, E., Barger, A., Levy, M. G., et al. (2006). Molecular characterization of a Babesia species identified in a North American raccoon. J. Wildl. Dis. 42, 375–380. doi: 10.7589/0090-3558-42.2.375

Bloch, E. M., Kumar, S., and Krause, P. J. (2019). Persistence of Babesia microti Infection in Humans. Pathogens 8 (3), 102. doi: 10.3390/pathogens8030102

Boniface, P. K., Ferreira, S. B., and Kaiser, C. R. (2016). Recent trends in phytochemistry, ethnobotany and pharmacological significance of Alchornea cordifolia (Schumach. & Thonn.) Muell. Arg. J. Ethnopharmacol. 191, 216–244. doi: 10.1016/j.jep.2016.06.021

Bonjean, K., De Pauw-Gillet, M. C., Defresne, M. P., Colson, P., Houssier, C., Dassonneville, L., et al. (1998). The DNA intercalating alkaloid cryptolepine interferes with topoisoamerase II and inhibits primarily DNA synthesis in B16 melanoma cells. Biochemistry 37, 5136–5146. doi: 10.1021/bi972927q

Borchardt, J. K. (2002). The Beginnings of Drug Therapy: Ancient Mesopotamian Medicine. Drug News Perspect. 15, 187–192. doi: 10.1358/dnp.2002.15.3.80018

Bugyei, K. A., Boye, G. L., and Addy, M. E. (2010). Clinical efficacy of a tea-bag formulation of Cryptolepis sanguinolenta root in the treatment of acute uncomplicated falciparum malaria. Ghana Med. J. 44, 3–9. doi: 10.4314/gmj.v44i1.68849
Krause, P. J., Telford, S. R. S., Spielman, A., Sikand, V., Ryan, R., Christianson, D., et al. (1996). Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness. JAMA 275, 1657–1660. doi: 10.1001/ jama.1996.03530450047031

Krause, P. J., Spielman, A., Telford, S. R. S., Sikand, V. K., McKay, K., Christianson, D., et al. (1998). Persistent parasitemia after acute babesiosis. N. Engl. J. Med. 339, 160–165. doi: 10.1056/NEJM199807163390304

Krause, P. J., Lepore, T., Sikand, V. K., Gadhaw, J. Jr., Burke, G., Telford, S. R. S., et al. (2000). Atoquavone and azithromycin for the treatment of babesiosis. N. Engl. J. Med. 343, 1454–1458. doi: 10.1056/NEJM200011143430204

Krause, P. J., McKay, K., Thompson, C. A., Sikand, V. K., Lentz, R., Lepore, T., et al. (2002). Disease-specific diagnosis of coinfected tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease. Clin. Infect. Dis. 34, 1184–1191. doi: 10.1086/339813

Krause, P. J., Daily, J., Telford, S. R., Vannier, E., Lantos, P., and Spielman, A. (2007). Shared features in the pathobiology of babesiosis and malaria. Trends Parasitol. 23, 605–610. doi: 10.1016/j.pt.2007.09.005

Krause, P. J., Gewurz, B. E., Hill, D., Marty, F. M., Vannier, E., Foppa, I. M., et al. (2008). Persistent and relapsing babesiosis in immunocompromised patients. Clin. Infect. Dis. 46, 370–376. doi: 10.1086/525852

Kumar, S., Gupta, A. K., Pal, Y., and Dwivedi, S. K. (2003). In-vivo therapeutic efficacy trial with artemisinin derivative, buparvaquone and imidocarb dipropionate against Babesia equi infection in donkeys. J. Vet. Med. Sci. 65, 1171–1177. doi: 10.1292/jvms.65.1171

Lamikanra, A., Ogundaini, A. O., and Ogungbamila, F. O. (1990). Antibacterial Constituents of Alchornea-Cordifolia Leaves. Phytother. Res. 4, 198–200. doi: 10.1002/ptr.2650040508

Leiby, D. A. (2006). Babesiosis and blood transfusion: flying under the radar. Vox. Sang. 90, 157–165. doi: 10.1111/j.1423-0410.2006.00740.x

Leung, K. C., Seneviratne, C. J., Li, X., Leung, P. C., Lau, C. B., Wong, C. H., et al. (2016). Synergistic Antibacterial Effects of Nanoparticles Encapsulated with Scutellaria baicalensis and Pure Chlorhexidine on Oral Bacterial Biofilms. Nanomater. (Basel) 6 (4), 61. doi: 10.3390/nano6040061

Lin, H., Zhou, J., Lin, K., Wang, H., Xue, W., Li, Y., Cao, G., et al. (2014). Safety, tolerability, and pharmacokinetics of a single ascending dose of baicalein chewable tablets in healthy subjects. J. Ethnopharmacol. 156, 210–215. doi: 10.1016/j.jep.2014.08.031

Li, Y., Liu, M., Rizk, M. A., Momouni, P. F. A., Lee, S. H., Galon, E. M., et al. (2020). Drug screening of food and drug administration-approved compounds against Babesia bovis in vitro. Exp. Parasitol. 210, 107831. doi: 10.1016/j.exparpar.2020.107831

Lin, H., Zhou, J., Lin, K., Wang, H., Liang, Z., Ren, X., et al. (2016). Efficacy of Scutellaria baicalensis for the Treatment of Hand, Foot, and Mouth Disease Associated with Encephalitis in Patients Infected with EV71: A Multicenter, Randomized, Placebo-controlled Trial. Pharmacol. Res. 106, 569751. doi: 10.1016/j.pharmres.2015.09.006

Lin, B. C., Harris, D. R., Kirkman, L. M. D., Perez, A. M., Qian, Y. W., Mustofa, A., Benoit-Vical, F., Pelissier, Y., Kone-Bamba, D., and Mallie, M. (2000). Antiplasmodial activity of plant extracts used in west African traditional medicine. J. Ethnopharmacol. 73, 145–151. doi: 10.1016/S0378-8741(00)00296-8

Nagai, A., Yokoyama, N., Matsu, T., Bork, S., Hirata, H., Xuan, X., et al. (2003). Inhibitory activity of Scutellaria baicalensis on the inflammatory activity of LPS-stimulated RAW264.7 cells. Agents Chemother. 47, 800–805. doi: 10.1128/AAC.47.5.800-805.2003

Nehrbass-Stuedli, A., Boykin, D., Tidwell, R. R., and Brun, R. (2011). Novel diamidines as therapeutic agents against Plasmodium falciparum. J. Med. Chem. 54, 4924–4928. doi: 10.1021/jm2007693

Mustafa, A., Benoit-Vical, F., Pelissier, Y., Kone-Bamba, D., and Mallie, M. (2000). Antiplasmodial activity of plant extracts used in west African traditional medicine. J. Ethnopharmacol. 73, 145–151. doi: 10.1016/S0378-8741(00)00296-8

Ngai, A., Yokoyama, N., Matsu, T., Bork, S., Hirata, H., Xuan, X., et al. (2003). Growth-inhibitory effects of artemisinin fractions of Artemisia annua on schizosporidia in a large clinical trial. Phytomedicine 10, 233–240. doi: 10.1016/j.phymed.2010.01.014

Nakaj, Y., Cornet-Vernet, L., Idumbo, M., Lu, C., Lutgen, P., Perronne, C., et al. (2018). Effect of Artemisia annua and Artemisia afra tea infusions on schistosomiasis in a large clinical trial. Phytomedicine 51, 233–240. doi: 10.1016/j.phymed.2018.10.014

Nakaj, Y., Cornet-Vernet, L., Idumbo, M., Lu, C., Lutgen, P., Perronne, C., et al. (2019). Artemisia annua and Artemisia afra tea infusions vs. artemesia-amoedique (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial. Phytomedicine 57, 49–56. doi: 10.1016/j.phymed.2018.12.002

Nishioha, T., Kawabata, J., and Aoyama, Y. (1998). Baicalein, an alpha-glucosidase inhibitor from Scutellaria baicalensis. J. Agric. Food Chem. 46, 3439–3445. doi: 10.1021/jf9711117

Nishioha, T., Kawabata, J., and Aoyama, Y. (1998). Baicalein, an alpha-glucosidase inhibitor from Scutellaria baicalensis. J. Agric. Food Chem. 46, 3439–3445. doi: 10.1021/jf9711117

Oliveira, P. L., Haynes, R. K., Meunier, B., and Yuthavong, Y. (2001). Possible modes of action of the artemisinin-type compounds. Trends Parasitol. 17, 122–126. doi: 10.1016/S1471-4922(00)01838-9

OsoA, N., Mensah, K. B., and Yeboah, O. K. (2017). Phytochemical and Pharmacological Review of Cryptolepis saundersi (Lindl.) Schlecht. Adv. Pharmacol. Sci. 2017, 3026370. doi: 10.1155/2017/3026370

Pan, B., Shi, X., Ding, T., and Liu, L. (2019). Unraveling the action mechanism of Polygonum cuspidatum by a network pharmacology approach. Am. J. Transl. Res. 11, 6790–6811

Pang, H., Xue, W., Shi, A., Li, M., Li, Y., Cao, G., et al. (2016). Multiple-Ascending-Dose Pharmacokinetics and Safety Evaluation of Baicalein Chewable Tablets in...
patients with tick-borne diseases. JL does receive profits from medical services and botanical preparations he exclusively makes available to patients in these two practices and does not currently sell botanical products commercially.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.