Review and Research on Cancer Treatment
Volume 8, Issue 1 (2022)

The effect of plant extracts from *Scutellaria baicalensis, Stevia rebaudiana, Eleutherococcus senticosus, Schisandra chinensis* and *Boswellia serrata* on human fibroblasts and *Borrelia burgdorferi spirocheates* – in vitro study

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ABSTRACT

Lyme disease caused by *Borrelia burgdorferi* is a multisystemic disease affecting numerous tissues and organs of the human body. Plant extracts with antimicrobial properties can be used to support the treatment of *Borrelia burgdorferi* infections. It was decided to evaluate the toxic effects of extracts from *Scutellaria baicalensis*, *Stevia rebaudiana*, *Eleutherococcus senticosus*, *Schisandra chinensis* and *Boswellia serrata* on human cells and to evaluate the MIC (Minimal Inhibitory Concentration) on *Borrelia burgorferi* spirochetes. The extracts from these plants present scientifically proven antimicrobial activity. The plant extracts were obtained with 80% methanol and then the content of flavonoids, phenolic acids, tannins, flavonoids were determined. The antioxidant potential of plant extracts was also evaluated. Cytotoxicity tests were performed on human dermal fibroblasts exposed to the concentration of 0.05 to 1 mg/ml of the plant extracts because fibroblasts have been widely used in cell culture as an *in vitro* model to tests of cell viability and toxicity of many compounds. There was also determined MIC for *Borrelia burgdorferi*. In the case of human cells, *Boswellia serrata* extract shows no cytotoxicity. The remaining ones are characterized by toxicity above 50/100 micrograms/ml. The highest ability to inhibit the growth of *Borrelia burgdorferi* was demonstrated by *Stevia rebaudiana, Scutellaria baicalensis* and *Eleutherococcus senticosus* (1.0 mg/ml). The weakest antibacterial properties were demonstrated by extract of *Schisandra chinensis* and *Boswellia serrata* (2.0 mg/ml). *Boswelia serrata* extract may be of interest in the context of application in Lyme disease therapy due to the lack of cytotoxic activity against human cells.

Keywords: *Borrelia burgdorferi*, MIC, NHDF, *Scutellaria baicalensis, Stevia rebaudiana, Eleutherococcus senticosus, Schisandra chinensis, Boswellia serrata*

INTRODUCTION

The bacteria *Borrelia burgdorferi sensu lato* belongs to the family of spirochetes (*spirochetae*). It is a Gram-negative bacterium with a highly twisted elongated cell. Borreliosis (Lyme disease) caused by *Borrelia burgdorferi* is a multisystemic disease affecting numerous tissues and organs of the human body. The main problems are the life strategy of *Borrelia burgdorferi*, the complex immune response and the inadequacies of diagnostic methods. Among the problems complicating the treatment of Lyme borreliosis are the high efficiency of the spirochete in the initial colonization of tissues, its rapid dissemination and rapid penetration of the central nervous system. This is compounded by the ability to infect almost all tissues, to evade immune response by penetrating cells (e.g. fibroblasts) (Fikrig, 2006). Bacterial insensitivity to commonly used antibiotics is becoming increasingly common (Lantos, 2015).

Plant extracts with antimicrobial properties can be used to support the treatment of *Borrelia burgdorferi* infections. Plant extracts containing active substances with proven antibacterial, anti-inflammatory and immune system stimulating properties can significantly contribute to the cure of borreliosis (Feng, 2015; Zhao, 2019).

Baikal skullcap (*Scutellaria baicalensis*) is a plant that comes from the family *Lamiaceae*. The homeland of Baikal skullcap is eastern Siberia, the Zabaikal Mountains, the Maritime Country, northern China, Mongolia and Japan. The pharmaceutical raw material, which is used for medicinal purposes, is the root of the *Scutellaria baicalensis*. It is a small (25-60 cm high) perennial plant. It develops simple stems with not large lanceolate leaves, at the top of which not too large blue or blue-violet labial flowers grow (Zhao, 2019). The main chemicals that can be found in *Scutellaria baicalensis* root are mainly flavonoids: baicalin, wogonoside, baicalein, oroxylin A, norwogonin, wogonin, chrysin as well as terpenes and tannins (Wang 2018). Due to its rich composition, Baikal skullcap has multidirectional therapeutic effects. One of its properties is anti-inflammatory

action. Products made from the *Scutellaria baicalensis* can be used not only in acute inflammatory conditions, but they are also highly effective against chronic inflammation (Kim, 2009). Moreover, after their use there is also a reduction of pain and swelling in arthritis, among others (Yang, 2013). *Scutellaria baicalensis* exhibits also antiviral activity (e.g. against HIV, HCV, HBV, EBV, and influenza viruses (Błach-Olszewska, 2008), anti-bacterial (e.g. against *Staphylococcus aureus*) (Qian, 2015) and antifungal (e.g., against *Candida albicans*) (Wong, 2010).

Stevia rebaudiana is a perennial plant belonging to the *Asteraceae* family, native to the northeastern region of Paraguay and the border region of Brazil. It contains compounds known as steviol glycosides (e.g. Stevioside, Rubusoside A-F, Dulcoside A). Although they are 300 to 400 times sweeter than sugar, they do not contribute any calories to our diet because they are not absorbed in the human digestive tract (Leszczyńska, 2011). Apart from glycosides, *Stevia* also contains polyphenols e.g. Caffeic acid, Cinnamic acid, Syringic acid due to stevia extracts may have the following effects: antioxidant, antidiabetic, anti-inflammatory, and anticancer (Myint, 2020).

Siberian ginseng (*Eleutherococcus senticosus*, syn. *Acanthopanax*) belongs to *the Araliaceae* family. The area of occurrence of this species covers mainly north-eastern Asia: China, Korea, Japan, Manchuria, Siberia. *Eleutherococcus senticosus* is a shrub growing up to two meters tall, occasionally reaching four meters, very strongly branched. The stems are covered with light gray bark, from which numerous thin spines grow (Goulet, 2020). Seven compounds were isolated and classified into a new group of glycosides called eleuterosides. Each compound was assigned a classification symbol in the form of a letter and possibly a number indicating the subgroup. The isolated compounds were named eleuterosides: A, B (syringin), C, D, E (acanthoside D), F and G. Besides them, the composition of the root includes, among others: caffeic acid, chlorogenic acid, ferulic acid, vanillin, flavones, resistin, polysaccharide complexes, thymidine, campesterol, stigmasterol, β-carotene, pectins, macro- and microelements, vitamins, glycoproteins, sessiloside and tauroside H1 (Załuski, 2008). *Eleutherococcus senticosus* is a medicinal plant known for 4000 years, used in Chinese medicine to increase human vitality. Studies on species of the *Araliaceae* family indicate that rhizome extracts of *Eleutherococcus senticosus* have biological activity similar to that of root extracts. The broad spectrum of activities of *Eleutherococcus senticosus* extracts include anticancer, antioxidant, immunostimulatory, immunomodulatory and antidepressant properties. Immunostimulatory properties are revealed by increasing the proliferation and differentiation of T lymphocytes and by increasing cytokine production by macrophages. The similar mechanism of activation of these cells is based on the interaction between the toll-like receptor (TLR) located on their surface and the polysaccharide complex in the extract (Załuski, 2008; Lee, 2015). Antimicrobial activity was studied on *Pseudomonas aeruginosa*, *Streptococcus aureus*, *Salmonella*, *Escherichia coli*, *Bacillus cereus*, *Bacillus subtilis* and *Micrococcus luteus* (Chen, 2021). Anti-inflammatory activity associated with modulation of pro-inflammatory cytokines (TNF-α and IL-6) has been demonstrated in animal model experiments using mice (Takahashi, 2014). Studies using oligonucleotide microarrays in animal models have shown that the extracts may have neuroprotective effects (Li, 2016).

Schisandra chinensis is a dioecious climber belonging to the family *Schisandraceae*, growing up to 8-15 meters long. Its natural habitat is in northeastern China, Korea, Japan, the eastern part of Russia, the Kuril Islands and Sakhalin. The most important part of the plant from a medicinal point of view is its fruit – small red berries with a lemony taste grouped in clusters (Szopa, 2012). The most important components of the fruit are lignans, of which more than 40 have been identified to date: e.g. schisandrin, deoxyschisandrin, schisandrin B, schisandrin C, gomisin A, schisanthenol, and schisantherin A (Sowndhararajan, 2018). There are also triterpene compounds (schintrilactone A i B, wuweizidilactone C–F), fhytosterols (stigmasterol and β-sitosterol), vitamins (C and E), organic acids (citric, fumaric, malic, malonic and tartaric), poly- and monosaccharides (glucose, fructose, arabinose and galactose) and numerous bioelements (Ca, Mg, Fe, Mn, B, Zn, Cr, Ni, Cu and Co). The plant is characterized by neuroprotective, immunomodulatory, antioxidant, antitumor, hepatoprotective, antimicrobial (mainly on *Staphylococcus aureus* and *Bacillus subtilis*) (Sowndhararajan, 2018; Zhang, 2020; Li, 2018; Yuan, 2018; Mocan, 2014).

Boswellia serrata (*Burseraceae*) is a deciduous tree reaching four-five meters in height. It grows throughout India. It is a species of tree that grows mainly in dry and mountainous areas of North Africa, India and the Middle East. The resin extract from the frankincense tree has been used for thousands of years in Hindu folk medicine to treat various ailments. The oleoresin is harvested by incising the bark of at least five-year-old plants. A viscous substance slowly oozes from the wounded areas and hardens in the air. Fresh guggul is golden in color, semi-solid in consistency and highly viscous. It darkens to a yellow-brown color after hardening (Sharma 2009). *Boswellia serrata* extract contains many active ingredients such as mono-, di-, tri, tetracyclic triterpene acids and pentacyclic triterpene acids such as β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic-acid oraz 3-acetyl-11-keto-β-boswellic-acid (AKBA) (Roy, 2019). *In vitro* and *in vivo* studies using animal models have shown that boswellic acids exhibit anti-inflammatory and anti-arthritic effects. They have been shown to inhibit TNF-α, IL-1β, IL-6, and MMPs, decrease nitric oxide (NO) levels, and as a result, reduce swelling in rats with induced arthritis (Yu, 2020, Umar. 2014). AKBA has also shown promise as an antimicrobial agent against all Gram-positive and Gram-negative bacteria tested, e.g. *Staphylococcus aureus*, *Enterobacter aerogenes* and antifungal for *Candida albicans* and *Malassezia furfur* (Ismail, 2014; Raja, 2011; Stefano, 2020).

In light of above information, it was decided to evaluate the toxic effects of these extracts with scientifically proven antimicrobial properties on human cells and to evaluate the MIC (Minimal Inhibitory Concentration) on spirochetes in order to assess the potential possibility of their supplementary use in Lyme disease therapy.

MATERIALS AND METHODS

PLANT MATERIALS

The plant materials used to prepare the extracts were purchased from producers of herbal products. The samples of dried plants were extracted using methanol/water (80:20). After extraction, the extracts were separated from the solid plant material by filtering process. Plant extracts were evaporated in rotary evaporator under reduced pressure, and then were freeze-dried to completely remove the solvents. The lyophilized powder was weighed and next, serial dilution of extracts were prepared. Tween 80 was added to dissolve lipophilic compounds (Liebold, 2011).

CELL CULTURES

Normal human dermal fibroblasts (NHDF cell line) were obtained from the Clonetics (CC-2511; San Diego, CA, USA). The reference strain of *Borrelia afzelii* (VS 461, ATCC 51567) was obtained from the National Institute of Public Health – National Institute of Hygiene in Warsaw (Poland).

The bacterial culture was carried out in BSK-H medium (Barbour'a, Stoenner'a, Kelly'ego; Sigma-Aldrich, St. Louis, MO, USA) at 35˚C in microaerophilic conditions. The microscopic analysis of culture was performed upon seven days of growth. Cell number was monitored by cell counting in the Bürker chamber.

Normal human dermal fibroblasts were routinely maintained in the FBM medium (Fibroblast Basal Medium; Lonza, Basel, Switzerland), supplemented with a human fibroblast growth factor-basic (hFGF-B), insulin and gentamicin (FGM™ SingleQuots™; Lonza, Basel, Switzerland) at 37˚C in a 5% CO_2 incubator (Direct Heat CO_2; Thermo Scientific, Waltham, MA, USA). Both, cell number and viability were monitored by cell counting in the Bürker chamber, after staining them with 0.2% trypan blue (Biological Industries, Beit HaEmek, Israel). The experiment was performed on cells in the logarithmic phase of growth under condition of ≥98% viability assessed by trypan blue exclusion. For the experiments, NHDF cells will be used at four – six passages.

ANALYSIS OF BIOACTIVE COMPOUNDS IN PLANT EXTRACTS

The content of total polar phenolic compounds in extracts was determined colorimetrically using Folin-Ciocalteu reagent. The reaction mixture contained of an extract, Folin-Ciocalteu reagent and a sodium carbonate solution. The final mixture was diluted with deionized water. The mixture was kept in the dark at ambient conditions for 60 min in order to complete the reaction (Dewanto, 2002). Then, the absorbance at 760 nm was measured using an Infinite 200

PRO NanoQuant (Tecan, Männedorf, Switzerland). The phenol content (mg/ml) was read from the calibration curve and was expressed in terms of gallic acid. All samples were analyzed in three replicates.

The content of flavonoids in extracts was determined colorimetrically using aluminum chloride solution (Dewanto, 2002). Then the absorbance of the mixture was measured at 415 nm by using an Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). The flavonoid content (mg/ml) was read from the calibration curve and was expressed in terms of quercetin. All samples were analyzed in three replicates.

The total content of phenolic acids was determined spectrophotometrically using Arnova reagent and was read from the calibration curve and expressed in terms of caffeic acid. The absorbance at 490 nm was measured using an Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). All samples were analyzed in three replicates.

The total content of tannins was determined spectrophotometrically (Sun 1998). The absorbance of the mixture was measured at 500 nm by using an Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). The tannins content (mg/ml) was read from the calibration curve and was expressed in terms of catechin. All samples were analyzed in three replicates.

Determination of the antioxidant potential was determined spectrophotometrically using the ABTS solution. The absorbance at 734 nm was measured using an Infinite 200 PRO NanoQuant (Tecan, Männedorf, Switzerland). All samples were analyzed in three replicates. The value of the antioxidant potential was calculated from the standard curve prepared for the Trolox solution in the concentration range of 10-1000 μmol/l.

MIC (MINIMAL INHIBITORY CONCENTRATION)

Growth of *Borrelia burgdorferi* could be detected reliably by software-assisted kinetic measurement of the decrease of absorbance. MIC for *Borrelia burgdorferi* was determined by serial micro-dilution in BSK-H liquid medium (with the 25 μg/ml of phenol red) using 96-well titration plates (Hunfeld, 2000). A series of dilutions of the plants extract were made to concentrations ranging from 0.064 to 4 mg/ml. Final concentrations of the lyophilized plants extracts were reconstituted by adding of 200 μl of the final inoculum suspension in BSK containing phenol red as growth indicator. Microtitre plates with *Borrelia* samples and growth controls were sealed with sterile adhesive plastic and cultured at 35°C with 5% CO_2. The presence or absence of growth was examined after 0, 24, 48, 72, 96, 120, 144 and 168 h by kinetic measurement of indicator colour shift at 450:630 nm applying a commercially available ELISA-reader (Tecan Infinite 200 PRO; Tecan Austria, Grödig, Austria). Amoxycyline at a concentration of 0.5 μg/ml was used as a negative control (Sigma-Aldrich, St Louis, MO, USA) (Sicklinger, 2003).

CYTOTOXICITY

The MTT conversion method was used to determine whether plant extracts at concentrations between 0.05 and 1.0 mg/ml was toxic to the normal fibroblast cell cultures. The MTT assay is often used to measure metabolic activity of cells as an indicator of cell viablity and cytotoxicity of various compounds. It is based on the reduction of a yellow tetrazolium salt to purple formazan crystals by metabolically active cells. The absorbance of the formazan product is measured at a wawelength of 570 nm.

A stock solution of the plant extracts was prepared and then, diluted in cell culture medium. The viability of the cells was assessed after 24 h of plant extracts treatment. Fibroblasts were selected for cytotoxicity research because these cells have been widely used in cell culture as an *in vitro* model to tests of cell viability and toxicity of many compounds.

STATISTICAL ANALYSES

Statistical analyses were performed using Statistica 10.0 software (StatSoft, Tulsa, OK, USA), and the level of significance was set at $p<0.05$. Values were expressed as means and standard deviation (SD) of two independent experiments. A one-way ANOVA test, which was followed by Tukey's post hoc test or Dunnett's test, were used to assess any significant differences among the groups.

RESULTS

In this study, the content of active ingredients was determined, which are presented below (Tab. I). *Scutellaria baicalensis* extract is characterized by the greatest amount of active ingredients and antioxidant potential.

Table I. Concentration of active compounds (phenols, phenolic acids and flavonoids, tannins) and antioxidant potential

	Phenols [µg/ml]	**Phenolic acids [µg/ml]**	**Flavonoids [µg/ml]**	**Tannins [µg/ml]**	**AOP [µM/l]**
Scutellaria baicalensis	963	321	609	0.85	1431
Stevia rebaudiana	687	229	435	0.74	879
Eleutherococcus senticosus	421	194	246	0.38	781
Schisandra chinensis	889	211	544	0.63	1043
Boswellia serrata	721	183	317	0.42	970

AOP – antioxidant potential.

MIC

Growth of *Borrelia* spirochaetes was evaluated based on the decrease of absorbance after seven days (D7) in comparison to the initial absorbance values (first day, D1). The lowest concentration of the tested extracts that did not reduce the absorbance relative to the initial time was considered the MIC.

Compared to the control – amoxycilin (0.5 µg/ml), *Stevia rebaudiana, Scutellaria baicalensis* and *Eleutherococcus senticosus* (1.0 mg/ml) showed the greatest ability to inhibit the development of *Borrelia burgdorferi* spirochetes. Extracts of *Schisandra chinensis* and also *Boswelia serrata* were characterized by lower MIC index – 2.0 mg/ml that suggests their weaker antibacterial properties. The results are shown in Table II and Figs. 1-5.

Table II. Change of absorbance value after seven days (D7) in comparison to the initial absorbance values (D1)

Extract	***Stevia rebaudiana***	***Eleutherococcus senticosus***	***Scutellaria baicalensis***	***Schisandra chinensis***	***Boswelia serrata***	***p**
	Absorbance $_{D7-D1}$ ± SD					
4 mg/ml	0.79 ±0.07^{a}	0.66 ±0.08^{ab}	0.43 ±0.09^{b}	0.70 ±0.16^{ab}	0.54 ±0.20^{ab}	p = 0.012
2 mg/ml	0.33 ±0.27^{a}	0.71 ±0.17^{b}	0.15 ±0.05^{acd}	**0.48 ±0.12^{abd}**	**0.10 ±0.18^{c}**	p < 0.001
1 mg/ml	0.11 ±0.16	0.11 ±0.09	0.01 ±0.05	-0.12 ±0.18	-0.18 ±0.18	NS
0.5 mg/ml	**-0.12 ±0.18**	**-0.08 ±0.04**	**-0.10 ±0.03**	-0.09 ±0.15	-0.22 ±0.15	-
0.25 mg/ml	-0.14 ±0.11	-0.24 ±0.17	-0.17 ±0.05	-0.17 ±0.18	-0.18 ±0.08	-
0.125 mg/ml	-0.21 ±0.14	-0.26 ±0.17	-0.19 ±0.07	-0.33 ±0.29	-0.33 ±0.15	-
0.065 mg/ml	-0.25 ±0.14	-0.32 ±0.13	-0.28 ±0.08	-0.39 ±0.06	-0.24 ±0.10	-
K (+)	-0.43 ±0.24	-0.43 ±0.18	-0.64 ±0.27	-0.47 ±0.12	-0.36 ±0.23	-
K (-)	1.21 ±0.21	1.12 ±0.37	1.33 ±0.07	1.20 ±0.21	1.19 ±0.27	-

the results are presented as the mean ± standard deviation;
MIC values are bolded;
one-way ANOVA test – *p < 0.05; NS – not significance
post hoc Tukey test – statistically different data groups are indicated using different letters.

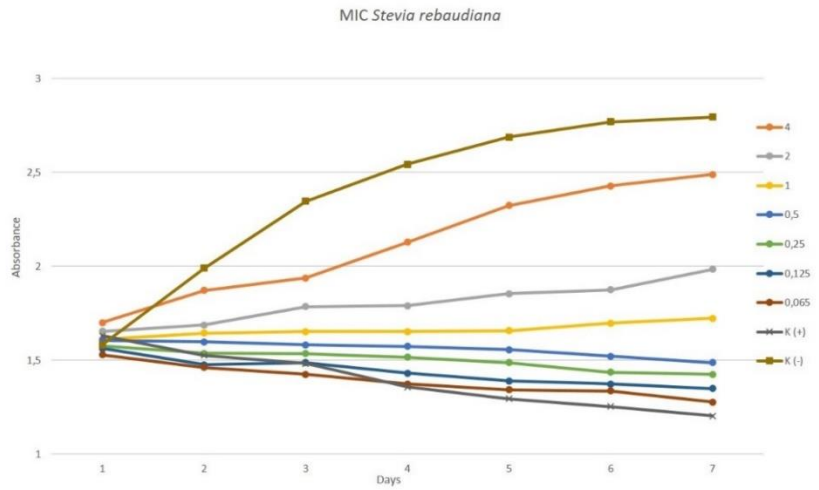

Figure 1. The Minimal Inhibitory Concentration of *Stevia rebaudiana* inhibiting the growth of *Borrelia burgdorferi* spirochetes after 7 days

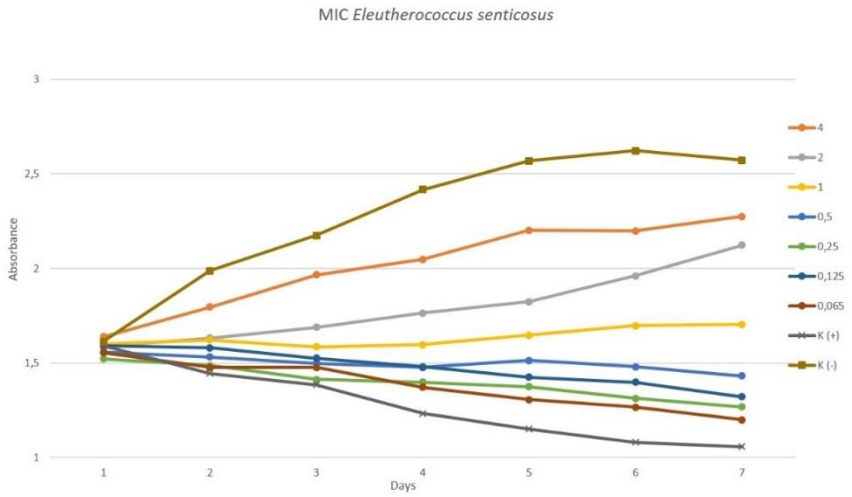

Figure 2. The Minimal Inhibitory Concentration of *Eleutherococcus senticosus* inhibiting the growth of *Borrelia burgdorferi* spirochetes after 7 days

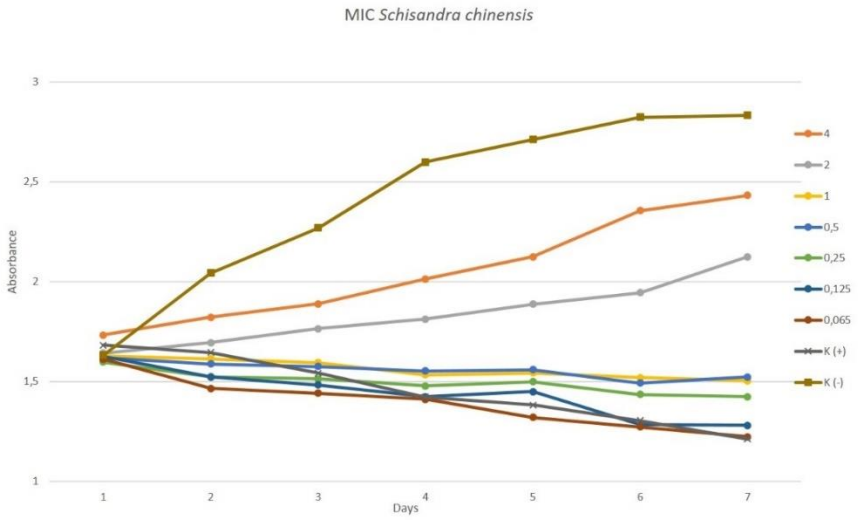

Figure 3. The Minimal Inhibitory Concentration of *Schisandra chinensis* inhibiting the growth of *Borrelia burgdorferi* spirochetes after 7 days

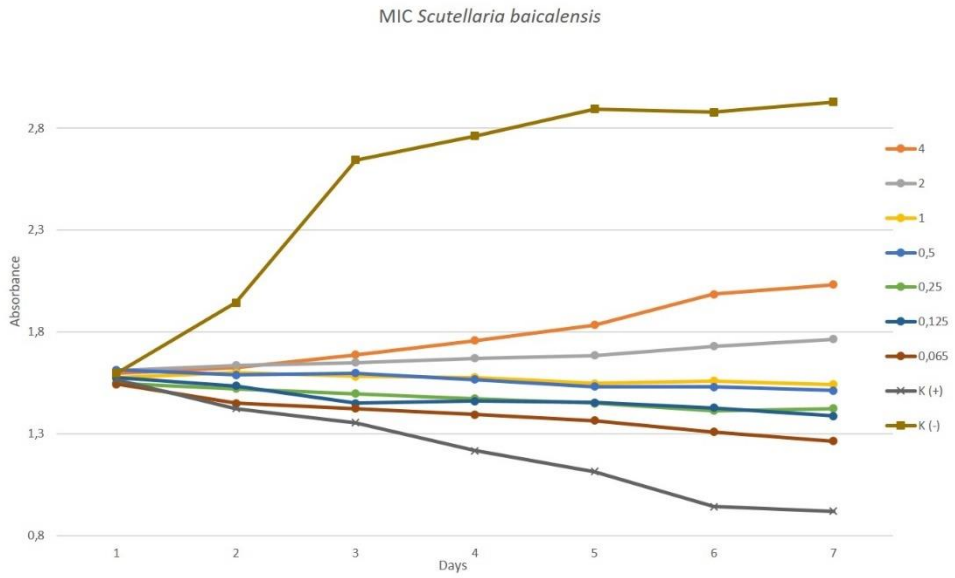

Figure 4. The Minimal Inhibitory Concentration of *Scutellaria baicalensis* inhibiting the growth of *Borrelia burgdorferi* spirochetes after 7 days

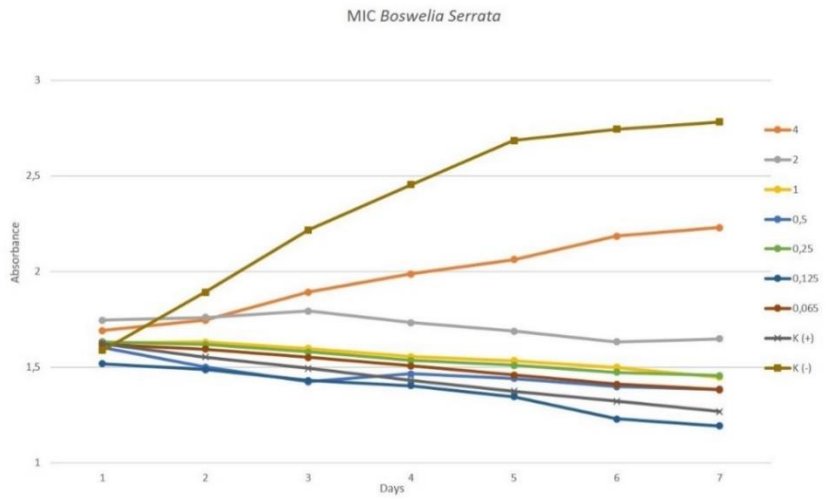

Figure 5. The Minimal Inhibitory Concentration of *Boswelia serrata* inhibiting the growth of *Borrelia burgdorferi* spirochetes after 7 days

EFFECT OF PLANT EXTRACTS ON NHDF VIABILITY

According to the results of a cell viability test. plant extracts was slightly cytotoxic to the normal human dermal fibroblasts at lower concentrations. Significant growth inhibition was observed in cultures incubated with higher concentrations of plant extracts except for *Boswelia serrata* (Tab. III).

Table III. Cell viability in normal human dermal fibroblast cultures in the presence of plant extracts for 24 h

NHDF cytotoxicity – *Scutellaria baicalensis*								
	Control	50 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml	750 ug/ml	1000 ug/ml	Control -
% viability	100.00	104.64	103.36	60.01*	34.44*	33.53*	37.11*	88.56
% SD	10.76	12.39	7.56	5.65	1.75	1.63	2.17	9.73

NHDF cytotoxicity – *Stevia rebaudiana*

	Control	50 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml	750 ug/ml	1000 ug/ml	Control -
% viability	100.00	77.76*	38.76*	46.51*	42.73*	36.08*	26.86*	84.96
% SD	9.96	14.53	2.60	5.32	7.18	8.51	2.17	11.90

NHDF cytotoxicity – *Eleutherococcus senticosus*

	Control	50 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml	750 ug/ml	1000 ug/ml	Control -
% viability	100.00	104.69	39.49*	38.54*	40.43*	42.77*	49.92*	93.28
% SD	8.27	5.99	2.88	2.65	1.84	4.03	5.52	12.81

NHDF cytotoxicity – *Schisandra chinensis*

	Control	50 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml	750 ug/ml	1000 ug/ml	Control -
% viability	100.00	90.30	82.80*	95.65	61.22*	24.34*	23.37*	99.12
% SD	14.65	11.35	15.34	7.52	6.97	1.06	0.42	11.03

NHDF cytotoxicity – *Boswelia Serrata*

	Control	50 ug/ml	100 ug/ml	250 ug/ml	500 ug/ml	750 ug/ml	1000 ug/ml	Control -
% viability	100	103.5	109.19	106.43	106.85	112.82	99.53	100.1
% SD	14.49	11.83	11.43	12.93	9.80	7.71	7.07	10.39

* Statistical significance: $p<0.05$ vs. Control.

DISCUSSION

Pharmacognostic methods may be effective in treating *Borrelia burgdorferi* infection. Standard antibiotic therapy is often supplemented by patients with plants that have antibacterial and strengthening effects on the body. Many plants. or pure substances extracted from them. are commercially available today and used to treat Lyme disease. However. the effect of plant extracts on *Borrelia burgdorferi* is still insufficiently studied especially since *in vitro* experiments predominate. The results of such experiments do not give a clear answer as to how the living organism will react and whether this will be reproducible in results obtained on cell lines. Nonetheless. they are essential for selecting plants with antimicrobial potential. An additional aspect to consider is the bioavailability of plant extracts due to instability of plant-derived compounds and their low bioavailability that results from the large size of compounds and their poor solubility. Nowadays. in order to improve the bioavailability of natural compounds and to achieve better therapeutic response. many methods are tested. such as nanoparticles or phytosome technology (Lu 2018; Rahman 2020; Myint 2021). This is very important for potential biomedical application of natural plant extracts. It should also be noted that the *in vivo* effect can be modulated by various factors for example biotransformation. pH or tissue properties hence *in vivo* studies with the use of an animal model are needed (Izah, 2018 Bubonja-Šonje 2020; Vaou 2021).

In the case of *Stevia rebaudiana*. Theophilus et al. confirmed that alcoholic extracts of stevia leaves exhibit chiropractic properties (Theophilus, 2015). Another research team also counts *Stevia rebaudiana* leaf extract as a natural agent that can kill spirochetes *in vitro*. However. in their compilation. *Stevia rebaudiana* extract is not as potent as the Theophilus et al. study (Feng. 2020). In this study. *Stevia rebaudiana* leaf extract showed the antibacterial efficacy with an MIC of 1.0 mg/ml. The safety of sugar-saccharose substitutes used in the food market has been confirmed (Bender. 2015; Sharma. 2016). Thus. it seems that steviosides isolated from *Stevia rebaudiana* or whole leaf extracts. in addition to their health-promoting properties. can be used as adjunctive preparations in the treatment of Lyme disease.

In the present study. extracts of *Scutellaria baicalensis* and *Eleutherococcus senticosus* were

shown to have similar ability to kill *Borrelia burgdorferi*. The relatively low toxicity in concentration up to 100 ug/ml to human cells in the case *of Scutellaria baicalensis*. allows us to suggest that this plant could also be used as an adjuvant therapy against *Borrelia burgdorferi*. This is also supported by the study of Feng et al. where the MIC for Baikal skullcap was determined to be >2% (Feng 2020). In addition to Lyme disease. flavonoids extracted from *Scutellaria baicalensis* could also potentially be used to treat Tick-Borne Encephalitis Virus (Leonova 2020).

However. there is little data on the antimicrobial activity of the other plants that were tested in this experiment. Of course. all of them showed antimicrobial activity. but examples were given mainly in relation to *Staphylococcus aureus* (Qian 2015; Mocan 2014; Ismail 2014). In the case of *Eleutherococcus senticosus*. which is often studied as an enhancing and adaptogenic plant. Extracts from the root stimulate bacterial migration and phagocytosis by macrophages. released increased amounts of TNF-α and IL-4. IL-6. IL-10 (Jin. 2020). In this study, the MIC with Siberian Ginseng was 1.0 mg/ml and the cytotoxicity against human cells was relatively high. However. it is important to note that *in vitro* studies are not equivalent to effects on the human body. The cytotoxicity of *Eleutherococcus senticosus* extracts was also tested against cancer cells (Chen 2021). Using Siberian Ginseng to treat Lyme disease may be a good choice especially because of its immunomodulatory potential.

Schisandra chinensis has the higher MIC than *Eleutherococcus senticosus* but lower cytotoxicity to human cells. In scientific reports there is no information whether the plant can be used as a raw material in the therapy of Lyme disease. However, there is quite a lot of information about its antibacterial potential against e.g. *Salmonella* (Kwon 2008). *Escherichia coli* (Cui 2020). *Chlamydia pneumoniae* and *Chlamydia trachomatis* (Hakala 2015) and in an animal model of sepsis (Lee 2012).

Boswellia Serrata has the same MIC as *Schisandra chinensis*. but has virtually no cytotoxicity to human cells. The antimicrobial activity of this plant is limited to the few bacterial strains tested. while the mainstream research seems to oscillate around the anticancerogenic properties of this plant (Feng 2021; Ahmed 2015).

SHORT CONCLUSION

In conclusion. our study showed that *Stevia rebaudiana. Scutellaria baicalensis* and *Eleutherococcus senticosus* have the greatest ability to inhibit the growth of *Borrelia burgdorferi.* but *Boswelia serrata* extract may be of interest in the context of application in Lyme disease therapy due to the lack of cytotoxic activity against human cells. Further studies would be recommended due to the relatively high toxic effects of higher concentrations of other plant extracts on human fibroblasts.

Acknowledgments

This study was supported by grant No KNW-2-058/N/0/I from the Medical University of Silesia, Katowice, Poland.

Conflicts of Interest

The authors declare no conflict of interest.

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