The anti-borreliae efficacy of phytochemicals and micronutrients: an update

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Abstract: Naturally occurring substances have been used for centuries to fight against various pathogens. They serve as a source for new chemical entities or provide options to already existing therapeutics. While there is an increasing interest in studying antimicrobial properties of naturally derived agents, little is known about their effects against Borrelia burgdorferi sensu lato, the causative pathogens of Lyme disease. A better understanding of this aspect could advance knowledge about pathophysiology of these bacteria and help improve the efficacy of current approaches against Lyme disease. Here, we review all naturally occurring substances scientifically evaluated to date, including plant extracts, their metabolites, and micronutrients, against vegetative (spirochetes) and latent (rounded bodies, biofilm) forms of Borrelia sp. This summary reveals the potent anti-borreliae activity of several of these natural compounds indicating their potential in enhancing the efficacy of current treatments for Lyme disease, and offering new options to already existing therapeutic regiments.

Keywords: biofilm, Borrelia sp., cysts, micronutrients, phytochemicals, spirochetes

Borrelia sp. and Lyme disease

Borrelia sp. are the causative pathogens of Lyme disease (LD) that are classified in at least 38 species [Dryden and Hodgkins, 2010; Stanek et al. 2012; Zajkowska et al. 2012]. To date, 14 of them have been recognized to cause this illness. They are jointly named as Borrelia burgdorferi sensu lato and include species such as Borrelia burgdorferi sensu stricto and the recently identified Borrelia mayonii (predominantly causing LD in the United States), as well as Borrelia afzelii and Borrelia garinii (predominantly causing LD in Eurasia) [Calderaro et al. 2014; Lovrich et al. 1994; Rudenko et al. 2011; Welsh et al. 1992]. For over 30 years, since the link between these bacteria and LD was established, scientists have been studying the biology and pathology of Borrelia sp.

Although, it is a phylogenetically distinct group of bacteria, all of them are microaerophilic, host-dependent, and slow-growing organisms transmitted by ticks of genus Ixodes [Burgdorfer et al. 1985; Embers et al. 2012; Hodzic et al. 2008; Hodzic et al. 2014; Miklossy et al. 2008; Sapi et al. 2011; Stanek et al. 2012; Stricker and Johnson, 2013]. Today, it is also known that these bacteria convert from their active spirochete form into latent forms, namely rounded forms (bodies) and biofilm. The latent forms are considered to be responsible for the persistency of LD [Brorson and Brorson, 1998; Embres et al. 2012; Gruntar et al. 2001; Hodzic et al. 2008; Hodzic et al. 2014; Miklossy et al. 2008; Sapi et al. 2011; Sapi et al. 2012; Stricker and Johnson, 2013; Timmaraju et al. 2015]. Spirochetes, which are motile and able to survive in viscous conditions, transform upon threats into latent forms, undergoing at the same time genotypic followed by phenotypic changes [Alban et al. 2000; Brorson and Brorson, 1998; Coleman et al. 1995; Gruntar et al. 2001; Miller et al. 2014; Wu et al. 2011; Zhao et al. 2014]. In such forms, they can survive even decades evading the host immune system [Berndtson, 2013; Hodzic et al. 2008; Miklossy et al. 2008; Murgia and Cinco, 2004]. Latent rounded forms are living forms with a low metabolic rate. Unlike spirochetes, they are not motile; however, like spirochetes they may be transmissible and capable of inducing infection. They are morphologically diverted into granular...
form (dot-like spirochetes), cell wall deficient (CWD) form (spheroplast, L-form, bleb-like spirochete), or cystic form (round body/form) [Al-Robaie et al. 2010; Sapi et al. 2011]. Biofilm, on the other hand, is a thin-layered agglomerate of bacteria covered with self-produced extra-polysaccharide substance (EPS) formed for protection from severe and harsh conditions. Biofilm may harbor a heterogeneous population of spirochetes and rounded forms with distinct genetic and protein profiles. *Borrelia* sp. within the biofilm is much more difficult to eliminate as well and can be even up to 1000 times more resistant to antibiotics because of limitation in their distribution and dissemination [Mah, 2012; Sapi et al. 2012; Timmaraju et al. 2015].

The clinical symptoms of LD manifest as a multisystem inflammation that in its early (localized) stage mainly affects the skin, and in later (disseminated and/or persistent) stages affects the joints, nervous system, and, to a lesser extent, the heart, muscles, or other organs [Massarotti, 2002; Zimering et al. 2014]. Because of a growing number of patients facing ongoing or relapsing symptoms, LD has emerged as the most common vector-borne disease in the United States and Europe [Dryden and Hodgkins, 2010; Hodzic et al. 2014; Johnson et al. 2014; Krause et al. 2006; Rudenko et al. 2011]. With 300,000 cases per year in the United States and 500,000 in Europe, and an unknown number of unreported cases, this disease has been acknowledged as a significant health concern [Krause et al. 2006; Johnson et al. 2014; Meek et al. 1996; Stricker and Johnson, 2014].

The first line treatment of LD is based on antibiotics such as doxycycline used for adults and amoxicillin or cefuroxime axetil for adults and children. These antibiotics have been found to be quite effective when administrated at the early stages of LD, but not at its late stages [Embers et al. 2012; Eppes and Childs, 2002; Donta, 2002; Donta, 2007; Massarotti et al. 1992; Stanek et al. 2012]. Moreover, according to the Centers for Disease Control (CDC), among all patients diagnosed with LD that were treated with antibiotics for a recommended period of time, approximately 20% of them experienced side effects such as symptoms of fatigue, and pain/aches in the joints and/or muscles lasting up to even 6 months [Fallon et al. 2008; Johnson et al. 2014; Klemper et al. 2013; Theophilus et al. 2015]. Although the mechanism associated with this condition in patients, which is referred to as ‘Post-Treatment Lyme Disease Syndrome (PTLDS)’ or ‘chronic Lyme disease’, is not well explained, it is suggested that one of the reasons is the failure of the host immune system to clear infection from either the persistent forms of *Borrelia* sp. or their antigens [Berndtson, 2013; Diterich et al. 2003; Theophilus et al. 2015]. Taking all of these considerations into account, there is an urgent need to develop well tolerated and more effective approaches to LD.

**Plant extracts and micronutrients with anti-borreliae efficacy**

Plants, their extracts, and metabolites have been a valuable source of natural agents for human and animal health for a long time. However, a rather small number of these substances with proven anti-borreliae efficacy have been identified and are reviewed below.

*Dipsacus sylvestris*

*Dipsacus sylvestris* (*Dipsacus fullonum*) is a species of plant native to Eurasia and North Africa. It is known by its common names as wild teasel or fuller’s teasel. *Dipsacus sylvestris* extracts have been studied against *Borrelia afzelii* [Liebold et al. 2011]. Its extracts, 70% ethanol (hydrophilic) as well as ethyl acetate and dichloromethane (both lipophilic), were tested for their activity *in vitro* during an 8-day period. The ethanolic extract showed no growth inhibition against spirochetes, whereas two lipophilic fractions demonstrated significant growth-inhibiting activity, with the strongest inhibition found in the ethyl acetate extract. The >95% growth inhibition by these apolar extracts was achieved at concentration of 2 mg/ml on the first day of treatment with highest inhibition on day IV, and it was kept up to the eighth day when this study was completed. Efficacy of the teasel root extract against rounded forms or biofilm was not evaluated in that study; however, we observed that when used up to 2 mg/ml, it does not have any significant effects against these latent forms.

**Grapefruit seed extract.** *In vitro* evaluation showed that grapefruit seed extract (GSE) can be a powerful agent against spirochetes and the cysts of *Borrelia afzelii ACA-1* when applied in the range of concentration between 0.165% and 0.00032%. Susceptibility testing of mobile spirochetes exposed to GSE for 1 h revealed lack of motile...
bacteria at concentrations of 0.041%, and at concentration of 0.165%, they either completely dissolved or displayed a degenerated shape. When the mobile spirochetes were exposed to GSE for 1 week, the estimated minimal inhibitory concentration (MIC) value was \( \leq 0.00032\% \), and the minimal bactericidal concentration (MBC) value was 0.0052%. Moreover, visible breaking of cysts incubated for 1h with GSE in a concentration range from 0.165% to 0.021% was noticed, and rupturing of cysts was observed after treatment with GSE diluted from 0.01% to 0.00064% with efficacy from 90% to 5%. The MBC value was established to be 0.0013%. Interestingly, the authors observed the abnormal protrusion of membranes and their eventual disruption with the contents leaking at lower applied concentrations of GSE, while the highest GSE concentrations made the bacteria and cysts disappear completely. The MBC was strongly dependent on the length of the incubation, but even short incubation with GSE showed to be very effective against active and latent rounded forms of *Borrelia afzelii* [Brorson and Brorson, 2007]. Effect of GSE on biofilm was not performed in the study. However, we noticed that GSE was ineffective at the MIC and MBC concentrations that were used in testing against spirochetes for 1 week, and with 30–40% efficacy at the highest MBC concentrations tested against spirochetes and cysts for 1h [Goc et al. unpublished]. The study corroborates with results of Heggars et al., who demonstrated that mechanism of GSE’s antibacterial activity manifests by disruption of the bacterial membrane and liberating the cytoplasmic contents within 15 min after its application [Heggars et al. 2002]. However, it has also been shown that GSE is an efflux inhibitor; thus, the anti-borreliae activity of this agent can express itself using this mechanism as well [Abulrob et al. 2004; Fraser et al. 1997]. Interestingly, GSE showed to be effective against many pathogenic strains but not against intestinal microflora [Ionescu et al. 1990; Reagor et al. 2002]. One study indicated that antimicrobial activity of GSE can be attributed to just the synthetic preservative agents contained within [Von Woedtke et al. 1999]. Also contradictory studies are published in regard to its cytotoxicity. One study showed GSE to be gastro-protective and anti-inflammatory [Zayachkivska et al. 2005]. Another study reported about cytotoxicity of GSE on fibroblasts and severely damaging inflammatory effect to the connective tissue just after 24h of incubation, leading to greater toxicity at higher GSE concentrations [Guedes et al. 2013].

**Uncaria tomentosa and Otoba parvifolia**

Two herbal extracts *Uncaria tomentosa* and *Otoba parvifolia* were evaluated for their anti-borreliae efficacy *in vitro*. *Uncaria tomentosa* (commonly known as Cat’s Claw) is a vine, whereas *Otoba parvifolia* is a tree and both can be found in the tropical jungles of South and Central America. Extracts from these two plants were tested for their *in vitro* effectiveness on active and dormant forms of *Borrelia burgdorferi sensu stricto* demonstrating significant effects on all of its morphological forms, especially when used in combination. For both herbal extracts used individually, the dilution of 1:400 was the most efficient in eliminating both the spirochetes and rounded forms. However, they showed to be the most effective, when they were applied in combination at lower (1:300) dilution. In regard to biofilm (biofilm-like colonies), the extract from *Uncaria tomentosa* at 1:300 dilution showed significant reduction of biofilm size and its disruption but without the bactericidal effect. In the presence of extract from *Otoba parvifolia*, the size of biofilm colonies was not reduced; however, bactericidal effect was achieved in >90%. In the presence of both herbal extracts, no sign of any colony formation was observed, with only a few nonmotile live spirochetes and rounded bodies present [Datar et al. 2010]. There is a need for more detailed toxicological evaluation of these extracts, although, the available data show no indication of severe toxicity and a low potential for acute and sub-acute oral toxicity, and there was no evidence of genotoxic or mutagenic activity [Valero and Gonzales, 2005].

**Stevia rebaudiana**

The same research team reported about significant efficacy of extracts from leaves of *Stevia rebaudiana* against all forms of *Borrelia burgdorferi sensu stricto* [Theophilus et al. 2015]. The researchers observed that this extract used at a concentration of 1.2µg/ml significantly affected the viability of spirochetes and rounded forms of *Borrelia burgdorferi sensu stricto*. This observation was confirmed with additional subculture experiments using *Stevia rebaudiana*-treated bacteria which showed either no regrowth after 7 days or 10% regrowth of viable cells after 14 days of incubation. In the same study, *Stevia rebaudiana* extract at a concentration of 1.2µg/ml was evaluated on growth and viability of biofilm. It demonstrated ~40% efficacy in reducing attached biofilm mass on both plastic- and collagen-coated surfaces with affected EPS layer.
Interestingly, Stevia leaf extract is commonly used as a sugar substitute and toxicological studies have shown that it does not have mutagenic, teratogenic, or carcinogenic effects. Various studies demonstrated its safety at high dietary intake doses [Anton et al. 2010; Carakostas et al. 2008].

Other plant extracts. We have documented that some other plant extracts and micronutrients also have anti-borreliae properties. Altogether we have examined 25 different agents, including enzymes, amino acids, fatty acids, chelators, and vitamins. Our study revealed that vitamins like D3 and C, as well as kelp (iodine), exhibit inhibitory effect against spirochetes of Borrelia burgdorferi sensu stricto and Borrelia garinii [Goc et al. 2015]. Additionally, we observed that vitamin B-complex, serrapeptase, and extracts from wild cherry, grape seed, black walnut green hull, apricot seed, and anise have bacteriostatic effect as well (Tables 1 and 2). Yet, only few of these compounds were found to be effective against latent forms of Borrelia sp. This confirms how these forms are difficult to combat, since even antibiotics commonly prescribed for Lyme patients are not effective against latent forms of Borrelia sp. Despite this, a few of them like kelp and extracts from wild cherry, grape seed, black walnut green hull, and apricot seed revealed bactericidal effects toward rounded forms. Biofilm-eradicating effect has

### Table 1. Efficacy of plant extracts against Borrelia sp.

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Spirochetes [µg/ml]</th>
<th>Rounded Forms [µg/ml]</th>
<th>Biofilm [µg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape seed</td>
<td>MIC$_{C90}$ at 100</td>
<td>MBC$_{B90}$ at 100</td>
<td>NS</td>
</tr>
<tr>
<td>Wild cherry</td>
<td>MIC$_{C90}$ at 125</td>
<td>MBC$_{B90}$ at 150</td>
<td>NS</td>
</tr>
<tr>
<td>Black walnut green hull</td>
<td>MIC$_{C90}$ at 100</td>
<td>MBC$_{B90}$ at 125</td>
<td>MBC$_{B50}$ at 250</td>
</tr>
<tr>
<td>White peony</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apricot seed</td>
<td>MIC$_{C75}$ at 125</td>
<td>MBC$_{B70}$ at 125</td>
<td>MBC$_{B45}$ at 250</td>
</tr>
<tr>
<td>Olive leaf</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Oregano</td>
<td>MIC$_{C45}$ at 250</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Anise</td>
<td>MIC$_{C45}$ at 250</td>
<td>MIC$_{C30}$ at 250</td>
<td>NS</td>
</tr>
<tr>
<td>Sage</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*BSH medium, Buffered Schamm and Hestrin’s; BSK; Barbour–Stoenner–Kelly; EC, effective concentration eradicating biofilm; MBC, minimal bactericidal concentration; MIC, minimal inhibitory concentration; NS, not susceptible.*

Testing was performed with two species such as Borrelia burgdorferi and Borrelia garinii. Subscript index at the MIC value indicates the % of inhibition at the provided concentration expressed as µg/ml in 1.5 ml of BSK complete medium at 33°C after 72h; the MBC value indicates the percent of killing at the provided concentration expressed as µg/ml in 1.5 ml of BSH complete medium at 33°C after 72h; subscript index at the EC value indicates the percent of biofilm eradication at the provided concentration expressed as µg/ml in 1.0 ml of BSK complete medium at 33°C after 72h, according to [Goc et al. 2015].

### Table 2. Efficacy of micronutrients and other natural substances against Borrelia sp.

<table>
<thead>
<tr>
<th>Micronutrients and others</th>
<th>Spirochetes [µg/ml]</th>
<th>Rounded forms [µg/ml]</th>
<th>Biofilm [µg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B-complex</td>
<td>MIC$_{C50}$ at 200</td>
<td>MBC$_{B40}$ at 500</td>
<td>NS</td>
</tr>
<tr>
<td>Serrapeptase</td>
<td>MIC$_{C25}$ at 50</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L-lysine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>L-arginine</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Fulvic acid</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

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been observed upon serrapeptase and kelp application. Neither plant extracts nor micronutrients tested in our study severely affected viability of HepG2 cells showing either no or mild cytotoxicity at their MIC and MBC values [Goc et al. unpublished]. Moreover, with respect to grape seed extract, protective and anti-inflammatory properties have been shown [Chu et al. 2016; Strathearn et al. 2014; Wang et al. 2015].

**Phytochemicals with anti-borreliae efficacy**

Phytochemicals represent a vast group of agents with antimicrobial activities which have been investigated by our research group for their anti-borreliae efficacy. Evaluation of 23 naturally derived compounds tested against spirochetes, rounded forms, and biofilm, demonstrated that several of them are effective against spirochetes of *Borrelia burgdorferi sensu stricto* and *Borrelia garinii*. They included polyphenols and fatty acids that showed bacteriostatic and bactericidal effects against spirochetes with the MIC values ranging from 50 to 250 µg/ml, and the MBC values between 100 and 250 µg/ml. We also observed that, although they could not eliminate rounded forms in 90–99%, a few of them were potent enough to induce their death in 50%. These compounds included hydroxytyrosol, 10-HAD (cis-2-decenoic acid), baicalein and monolaurin at their MBC50 values ranging from 300 to 500 µg/ml. In addition, flavones such as baicalein and luteolin, as well as fatty acids such as monolaurin and 10-HAD, used at concentrations 200–500 µg/ml could reduce biofilm-like colonies formed by *Borrelia burgdorferi sensu stricto* by 30–60%. However, only baicalein and monolaurin applied at the same concentrations were effective in reducing biofilm formed by *Borrelia garinii* by approximately 40–60% [Goc et al. 2015]. Moreover, our additional study has shown that polyphenols like apigenin, malvidin, quercetin, resveratrol and its dimer viniferin, ellagic acid, and nordihydroguaralretic acid as well as other compounds expressed bacteriostatic and/or bactericidal anti-spirochetal effects. However, their efficacy against anti-rounded forms could reach only 30–50% at concentrations between 150 and 500 µg/ml, and they did not demonstrate any significant anti-biofilm effects (Table 3). Evaluation of cytotoxicity of these compounds warrants more study; however, we did not notice statistically significant cytotoxic effects toward human HepG2 cells up to 125 µg/ml, although moderate cytotoxicity was seen when these compounds were applied at higher concentrations up to 250 µg/ml [Goc et al. 2015; Goc et al. unpublished].

**Conclusion**

Antimicrobial agents derived from natural sources such as plants, herbs, spices, fruits, and essential oils, have shown activity against an abundance of bacterial species. There is a vast potential in exploring further antibacterial properties of these natural substances since most of them have been recognized

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**Table 3. Efficacy of phytochemicals against *Borrelia sp.***

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Spirochetes (µg/ml)</th>
<th>Rounded forms (µg/ml)</th>
<th>Biofilm (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MIC</strong></td>
<td><strong>MBC</strong></td>
<td><strong>MIC</strong></td>
<td><strong>MBC</strong></td>
</tr>
<tr>
<td>Apigenin</td>
<td>MIC40 at 125</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Malvidin</td>
<td>MIC45 at 75</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Quercetin</td>
<td>MIC45 at 75</td>
<td>MBC50 at 125</td>
<td>MBC45 at 250</td>
</tr>
<tr>
<td>E-viniferin</td>
<td>MIC40 at 75</td>
<td>MBC40 at 125</td>
<td>MBC45 at 250</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>MIC40 at 125</td>
<td>MBC40 at 250</td>
<td>MBC45 at 300</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>MIC50 at 100</td>
<td>MIC45 at 250</td>
<td>NS</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>MIC40 at 250</td>
<td>NS</td>
<td>MBC50 at 500</td>
</tr>
<tr>
<td>Nordihydroguaralretic</td>
<td>MIC40 at 125</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>MIC40 at 75</td>
<td>MBC40 at 100</td>
<td>MBC50 at 150</td>
</tr>
<tr>
<td>Fucoidan</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Berberine sulfate</td>
<td>MIC40 at 100</td>
<td>MBC40 at 150</td>
<td>MBC50 at 250</td>
</tr>
</tbody>
</table>

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as generally well tolerated and effective. This is especially important in the aspect of infections caused by *Borrelia sp.* since side effects of conventional antibiotic treatment as well as bacterial persistence are well known and have become a main health concern in LD patients. This summary documents *in vitro* efficacy of naturally occurring substances against active and latent forms of *Borrelia burgdorferi sensu lato*. The results indicate that several of these agents present a promising alternative for combating these bacteria. In addition, the potential of applying these compounds as adjuvants in current antimicrobial therapy should be explored. Finally, clinical trials and animal studies involving natural substances alone or in conjunction with conventional antibiotics are highly desirable, since their lack hinder the application of such agents in public health practice.

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**Conflict of interest statement**
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