

Introduction

Lyme disease or Lyme borreliosis is a tickborne multisystemic disease caused by different species of *Borrelia*.¹ Administering antibiotics is the primary treatment for this disease, however, relapse often occurs when antibiotic treatment is discontinued.¹ One possible reasoning is that *Borrelia* becomes resistant to antibiotic treatment by converting from their spirochete form to several defensive forms such as cysts and biofilms.^{1,2} Recent studies on antibiotic resistant pathogens demonstrated that certain herbal extracts could have significant antimicrobial, antiprotozoal, antiviral, properties and in addition to that they are proven to be nontoxic and can be taken safely for longer period of time.³ The aim is to test the effectiveness of three different medicinal agents (Cumanda, Lakato and Stevia) on the different morphological forms of *Borrelia burgdorferi* using fluorescent, dark field microscopic techniques, special staining techniques including BacLight viability staining methods and by total carbohydrate analysis. As antibiotics are proven to be ineffective in eliminating all the morphological forms, natural antimicrobial agents might provide a more effective therapeutic option for Lyme disease patients.

Methods

The three morphological forms of *Borrelia burgdorferi* B31 (spirochetes, round bodies and biofilm) were analyzed in this study.¹ The antimicrobial sensitivity of spirochetal and cyst forms was tested using dark field microscopy (direct cell counting).^{1,3} Qualitative effects of antimicrobials against biofilms were assessed using fluorescent microscopy and quantitative analysis on biofilm was performed following the Total Carbohydrate Assay using standard published technique.⁴ The three antimicrobial agents tested in this study has shown significant antimicrobial effects in previous studies.^{5,6,7}

Direct counting of *Borrelia* spirochetes and cysts after 96 hours of treatment with different antimicrobial agents using dark field microscopy

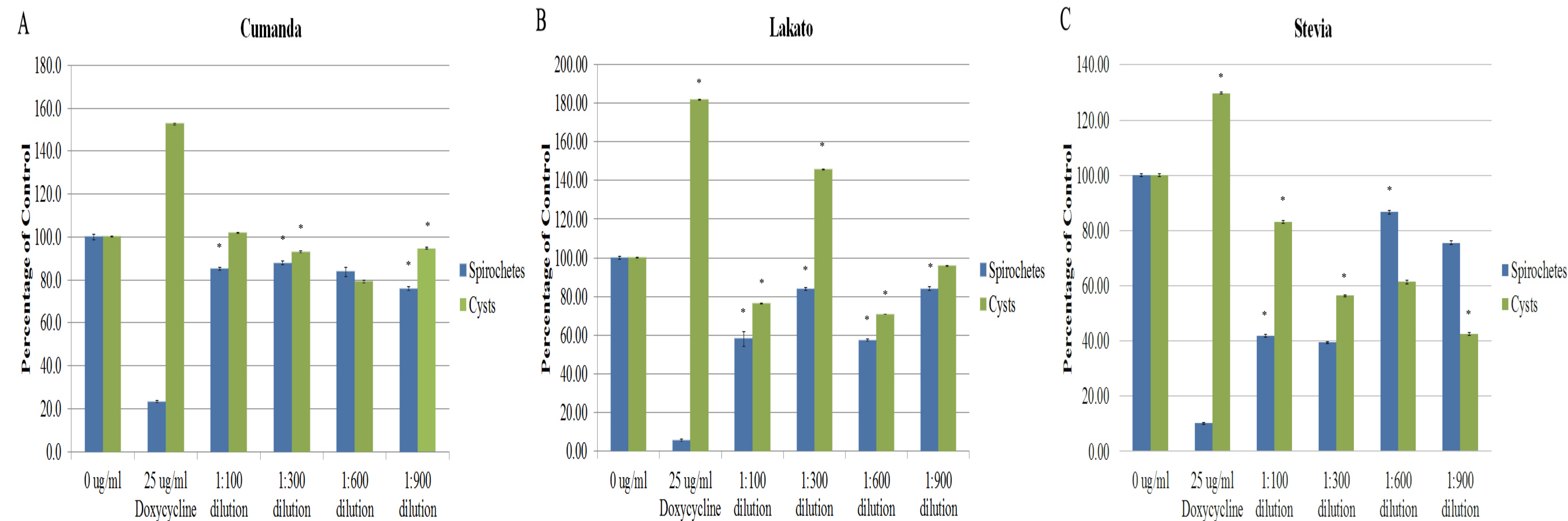


Figure1: The *in vitro* susceptibility of spirochete and cyst forms of B31 *Borrelia burgdorferi* to Cumanda, Lakato and Stevia and to doxycycline (25 µg/ml) for 96 hours' treatment period using direct cell counting and dark field morphological evaluation methods. P- values <0.05 indicates statistical significance (*).

Quantitative analysis of *Borrelia burgdorferi* after 96 hours of treatment with different antimicrobial agents

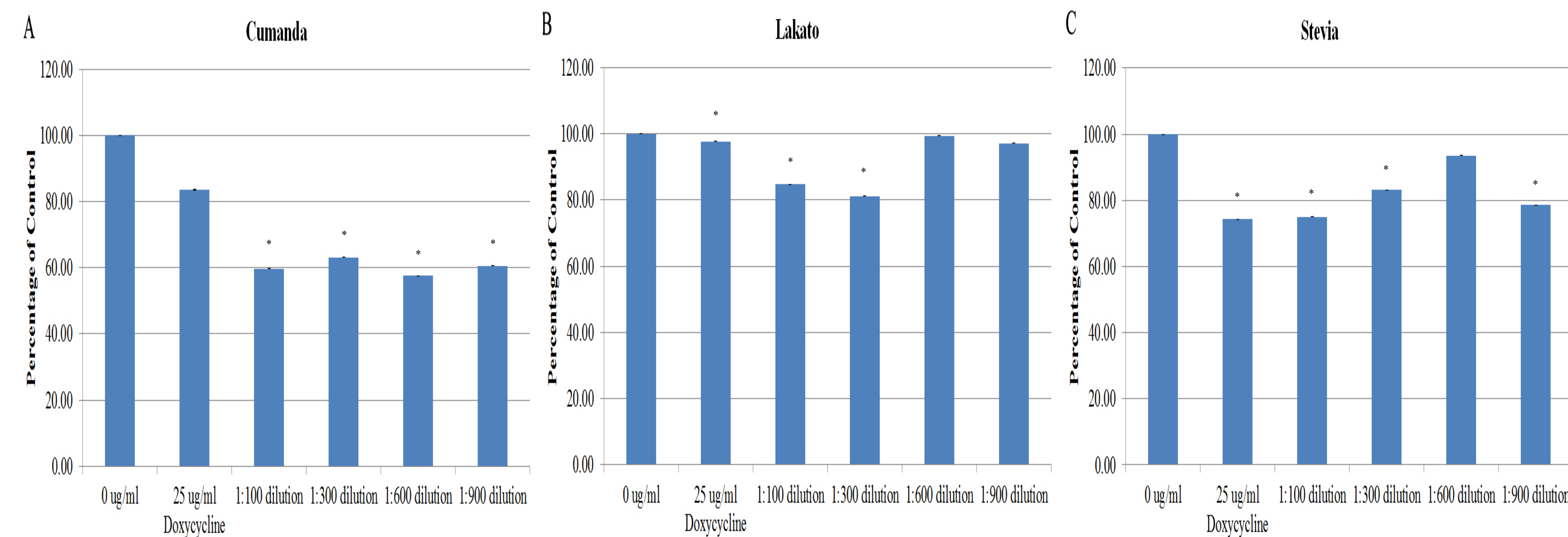


Figure2: Total carbohydrate assay on the biofilm of *Borrelia burgdorferi* treated with different antimicrobials namely Cumanda, Lakato and Stevia in various dilutions. P- values <0.05 indicates statistical significance (*).

Qualitative analysis of *Borrelia burgdorferi* after 96 hours of treatment with different antimicrobial agents

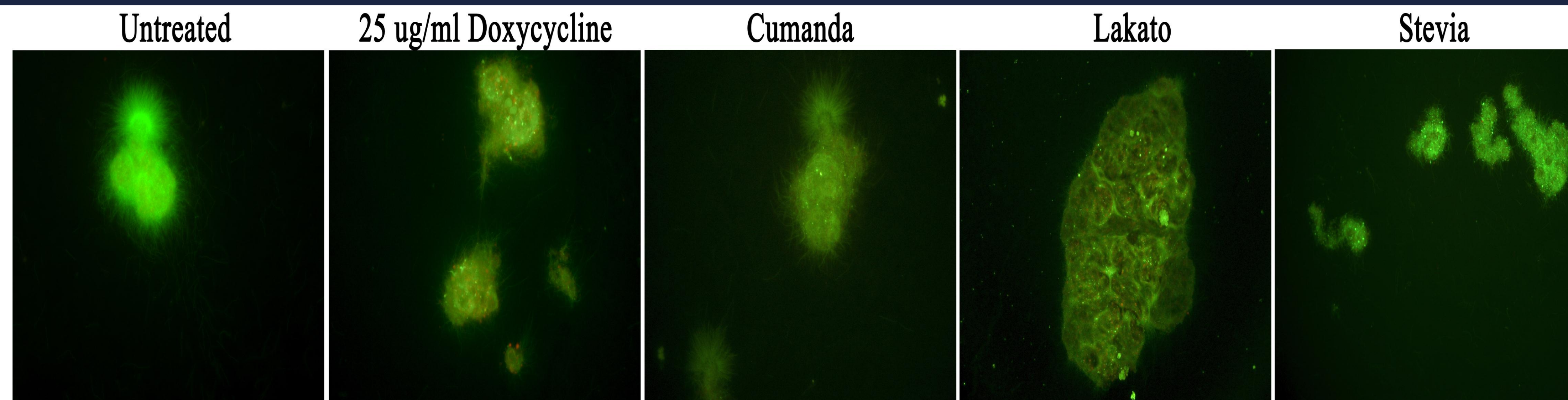


Figure3: BacLight viability staining of *Borrelia burgdorferi* B31 strain after 96-hour treatment using SYTO 9 green-fluorescent nucleic acid stain (live cells) and propidium iodide, a red-fluorescent nucleic acid stain (dead cells). Representative images of untreated cells, cells treated with 25 ug/ml Doxycycline, 1:300 dilutions of Cumanda, Lakato and Stevia.

Results

In summary, the treatment involving Stevia, resulted in a significant reduction in the numbers of spirochetal form by 60% and the cyst forms by 98%. Doxycycline reduced the spirochetal forms by 77% but showed a marked increase in the cyst form at higher concentration. The treatment with Lakato also show similar trends but less effective than Stevia. Furthermore, Cumanda at a higher dilution (1:900 dilution) reduced the spirochete form by 24% and the cyst forms by 5%.When quantitative effects on biofilm were evaluated, Cumanda reduced the formation of colonies by 43% (1:600 dilution).

Conclusions

Therefore in conclusion the treatment involving Stevia show a significant reduction in both the spirochetal and cyst forms, while Cumanda had the most significant effect in reducing the colony formation. Moreover, our results demonstrated that herbal agents had significant effects on all forms of *Borrelia burgdorferi*, implying that these antimicrobial agents could provide an effective therapeutic approach for Lyme disease patients.

References

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Acknowledgements

We are grateful to the University of New Haven, Nutramedix LLC (Jupiter, Florida), TBDA (Turn the Corner Foundation), LDA, Lyme Research Alliance, Lyme disease. org (CALDA) and Schwartz Foundation for all their support.