

Analysis of metabolites present in *Limonium carolinianum* endophytes

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Abstract

Limonium carolinianum is a perennial plant that has been shown to contain medicinal properties and has been used to treat diarrhea in folk medicine, with recent research showing antiviral, antioxidant, and potential anticancer properties.¹ Endophytes found in the plant were studied to determine if they produce compounds that could be the origin of its medicinal properties. The metabolites produced by two of the endophytes were studied and LCMS data shows both endophytes produce chemicals with varying effects. The Bioactivity of the metabolites were also found to inhibit two types of gram-negative bacteria from growing on PDA media.

Introduction

Sea Lavender (*Limonium carolinianum*) is a coastal perennial plant that grows along the northeastern coast of the United States. The plant is often found in salt marshes in the upper elevated regions.² The genus *Limonium* has been shown to contain antiviral properties, antioxidants, and used in folk medicine as a remedy for diarrhea. The genus has also been shown to host a large amount of endophytes.³

Endophytes are microorganisms that have formed a symbiotic relationship with the plant host, typically a species of fungi or bacteria. Endophytes live in the plant tissue, and can be found in the stem, roots, and leaves. Endophytes have been shown to give the plant increased resistance to diseases and predators, while the plant provides a safe location for the endophytes to grow.⁴ Some endophytes produce potent chemicals for the plant and have been shown to be the source of pharmaceutical drugs associated with the parent plant. This prevents the need for overharvesting wild populations of the plant, instead cultivation of the endophyte can be done on its own, and the drug then extracted directly from the endophyte growth.

In this study endophytes contained in *Limonium carolinianum* were grown and analyzed for production of any drugs that are used to treat a variety of illnesses. This data was then compared to previous knowledge of medicinal properties the plant and others in its genus have shown. The results of this study could have applications in biosynthesis of drugs as well as the discovery of new compounds.



Figure 1. Image of *Limonium carolinianum* plant that samples were taken from.



Figure 2. Images of isolated endophytes. Top: endophyte 1, isolated from leaf. Bottom: endophyte 2.1, 2.2, isolated from stem.

Methods and Results

Samples of *Limonium carolinianum* leaves and stems were collected from local salt marshes. The samples were sterilized with bleach and ethanol on the exterior, before being cut into small pieces and then plated on potato dextrose agar. Any growth coming from the cut portions was isolated onto another agar plate. Once established on plate the endophyte was then transferred to potato broth. The metabolites produced by the fungi were extracted from the broth using ethyl acetate and a separatory funnel, then the ethyl acetate was removed using a rotary evaporator. The extracted metabolites were analyzed using LCMS. The bioactivity of the metabolites were also analyzed, by spotting the extracted metabolites along with the crude broth on an agar plate that contained bacteria. The inhibition of bacterial growth was measured as the diameter of the area where no growth was found.



Figure 3. Images of endophytes growing in broth prior to metabolite extraction. From left to right is endophyte 1, endophyte 2.1, and endophyte 2.2.

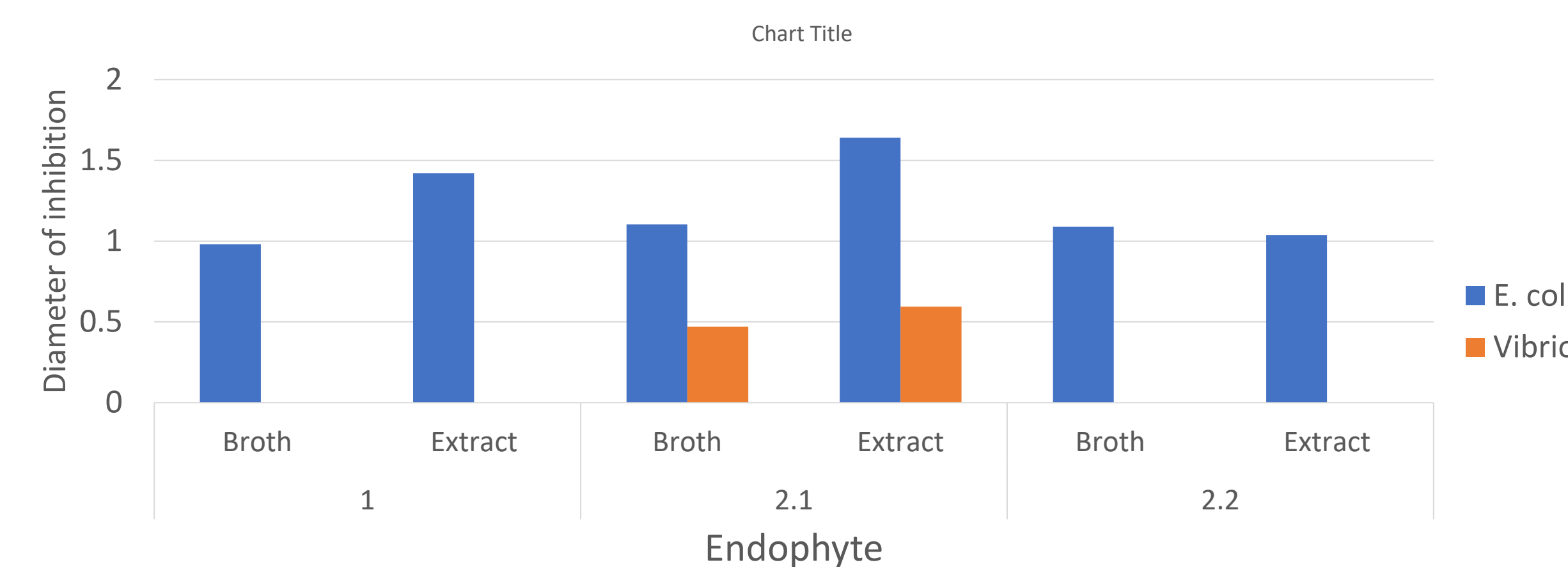
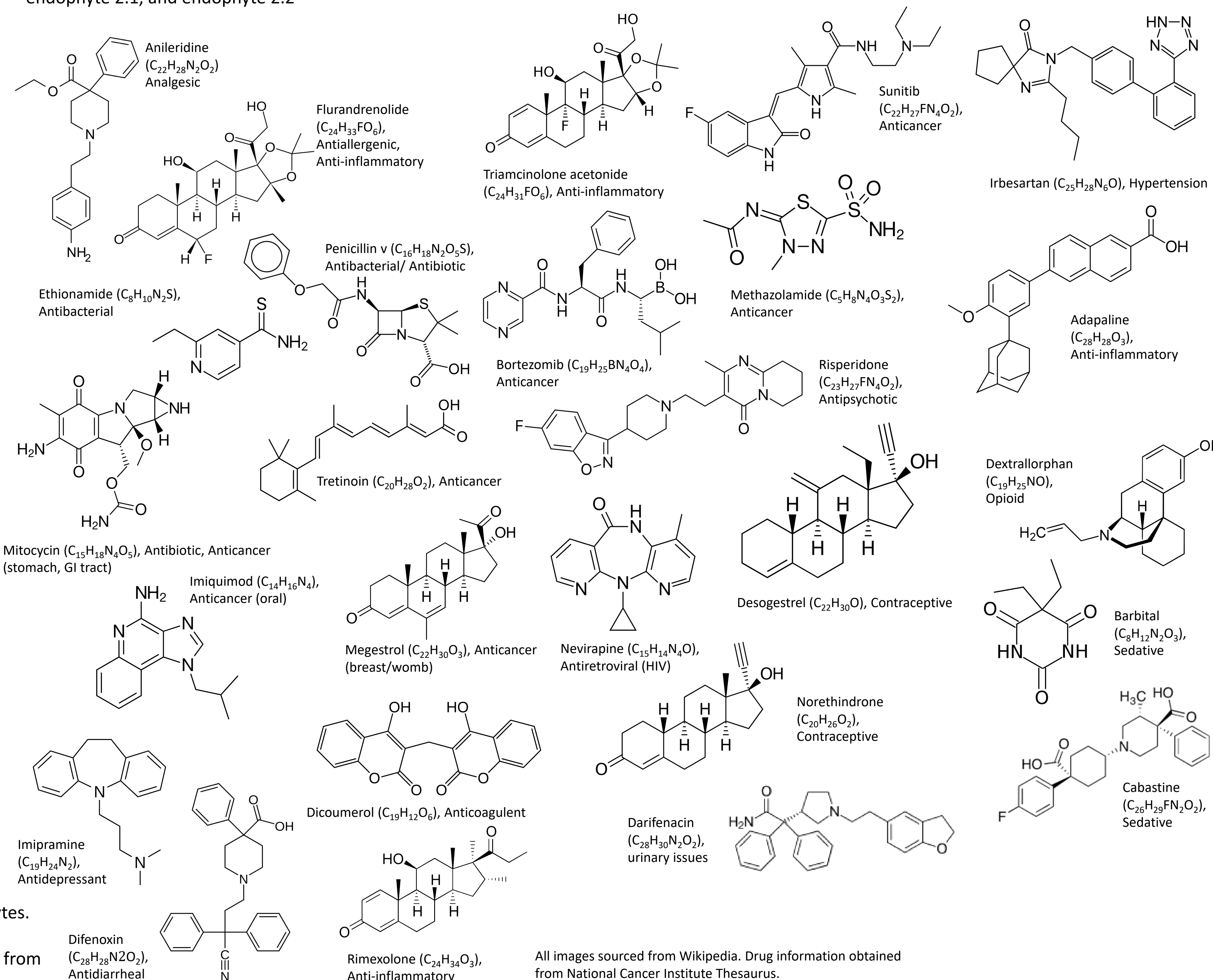


Figure 4. Graph showing inhibition of bacterial growth on plate containing endophyte metabolites. Inhibition measured as the diameter around spot where no bacterial growth was seen. Endophyte 2.1 was potentially contaminated during growth in potato broth.



All images sourced from Wikipedia. Drug information obtained from National Cancer Institute Thesaurus.

Results (cont.)

Table 1. Qualitative descriptions of studied endophytes

Endophyte	Origin	Location	Description
1	<i>Limonium carolinianum</i>	Leaf	Yellowish fluff that matures to deep orange
2.1	<i>Limonium carolinianum</i>	Stem	White fluff, matures to slight yellow
2.2	<i>Limonium carolinianum</i>	Stem	White fluff, matures to slight yellow

Both the leaves and stems of *Limonium carolinianum* contained fungal endophytes that were successfully cultivated. All endophytes showed strong resistance to *E. coli*, along with some inhibition of *Vibrio*. Endophyte 1 showed stronger inhibition of the extracted broth, along with Endophyte 2.1. Nonpolar metabolites were extracted and analyzed using LCMS. The metabolites contained the following properties, and their names can be found to the left. Both endophytes grown contained in the LCMS results the compounds difenoxy, tretinoin, imiquimod, and mitomycin. Difenoxy is used to treat diarrhea which has been reported as one of the medicinal properties of the plant. Tretinoin, imiquimod, and mitomycin are all anticancer drugs, a property that has recently been looked into in the *Limonium* genus. The finding of these compounds in metabolites produced by endophytes could point to the medicinal properties in the plant being produced by endophytes, not the plant directly synthesizing them.

Discussion and conclusion

The endophytes grown were shown to produce drugs that give the parent plant, *Limonium carolinianum*, the medicinal properties that have been previously reported. In addition to the reported properties, LCMS data shows the potential production of more biologically active compounds than thought. Further research into these endophytes and plant extracts should be done to determine just how much *Limonium carolinianum* might offer to the biotech industry. Endophyte 2.1 and 2.2 are from the same isolated fungus however were grown in separate broths as endophyte 2.1 was thought to have been contaminated. The inhibition of *Vibrio* by only endophyte 2.1 might be the additional metabolites produced when in the presence of bacteria during growth. Further research into the medicinal properties of the plant, along with the source of these compounds requires further research.

References

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