Apr 3rd, 11:30 AM - 1:30 PM

Anti-fungal Compounds from Native Prairie Plants

Sarah McMichael
University of Northern Iowa

Kirk Manfredi
University of Northern Iowa, kirk.manfredi@uni.edu

Anti-Fungal Compounds from Native Prairie Plants

Sarah McMichael[2] , Kirk P. Manfredi[1]
[1]Department of Chemistry and Biochemistry, University of Northern Iowa, Cedar Falls, IA 50614;
[2]Department of Biology, University of Northern Iowa, Cedar Falls, IA 50614

University of Northern Iowa

INTRODUCTION

Four compounds were identified from *Junipers virginiana* that showed anti-microbial activity against C. Albicans (yeast), B. Subtilis (gram +), and S. Aureaus (gram +). This project examines these compound's activity against filamentous fungus (A. Niger). Over the past three years our lab has been interested in identifying compounds from native prairie plants that inhibit bacterial and fungal growth at a level that would allow these compounds to be used for preservatives in personal care products. The goal of this research is to develop a botanical based product that can compete with current commercial preservatives. Current commercial personal care product preservatives include Parabens (glycerol formulations of derivatives of p-hydroxybenzoic acid) and DMDM hydantoin (a compound capable of donating formaldehyde). In addition to synthetic preservatives a number of "green / organic" preservatives are also available. Examples include Lucidal liquid (derived from the lactic acid bacteria *Leuconostoc kimchi*), Biovert (a mixture of glucose peroxidase, lactose peroxidase, and glucose oxidase), and grape seed extract (vitamin E, flavonoids and other natural compounds). In addition to natural preservatives, we are interested in finding natural alternatives to the synthetic antimicrobial, triclosan, which is commonly used in water based antimicrobial soaps. Triclosan has recently been banned and there needs to be alternatives to take triclosan's place. This project is focusing on the anti-fungal capabilities of the 4 isolated compounds.

EXPERIMENTAL

Over 100 different species of native North American Prairie plants have been collected and identified in our lab. These plants have been subjected to a methylene chloride: methanol-methylene chloride: water extraction scheme. Each extract was then subjected to a disk-agar antibacterial assay using *B. subtilis* and *E.coli* as test organisms. Crude extracts were dissolved in DMSO to give a final concentration of 0.100 mg / 5ul. Five uL of solution was then placed on a disk and put on a petri containing a lawn of test organism. After incubation (8 hrs) the plates were analyzed for zones of growth inhibition. Tetracycline (30 ug) and Triclosan (50 ug) were used as positive controls in the assay. Extracts that showed significant inhibition in the disk assay were then further separated to identify the compounds associated with the observed biological activity. The most promising lead(s) came from the Eastern Red Cedar (*Juniperus virginiana*). Further separations and bioassays lead to the isolation and purification of 4 active compounds that have been identified as diterpene carboxylic acids (figure 1).

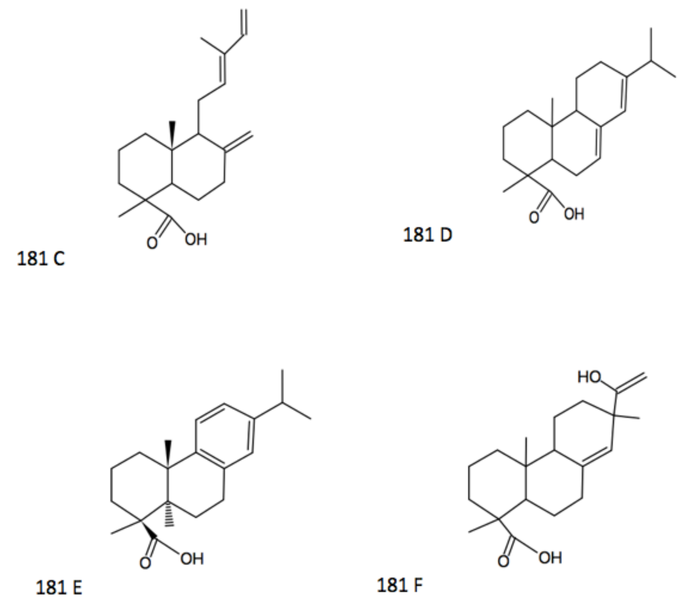

Figure 1. ^{1}H NMR spectra (400 MHz) of the 4 (C,D,E,F) active compounds isolated from *J. virginiana*.

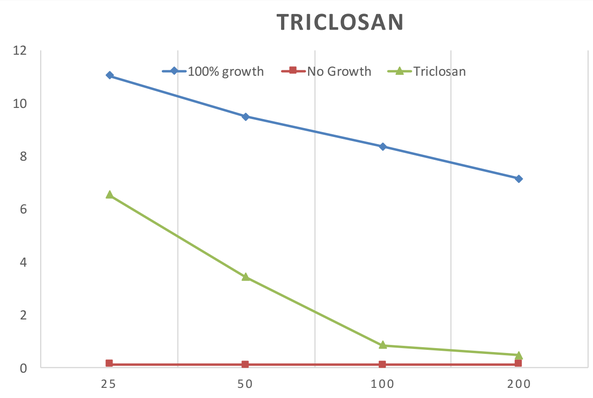

Figure 2.

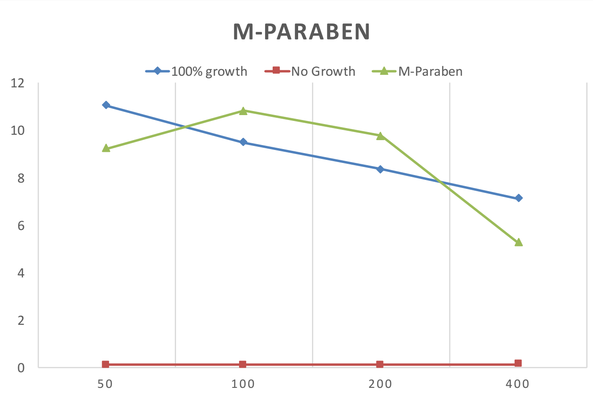

Figure 3.

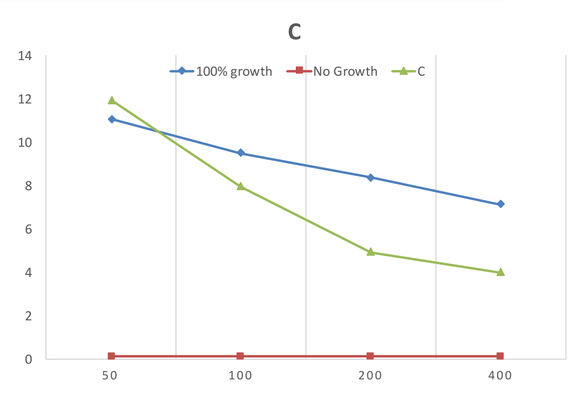

Figure 4.

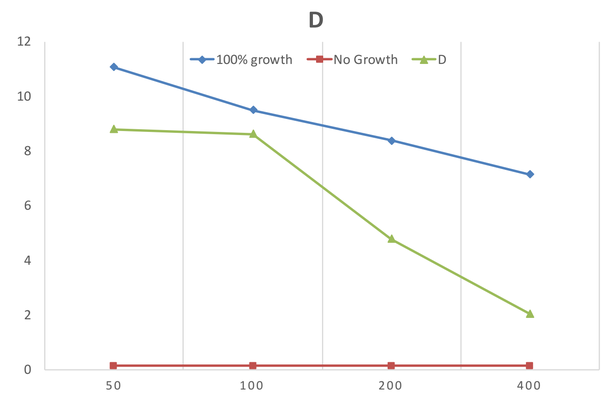

Figure 5.

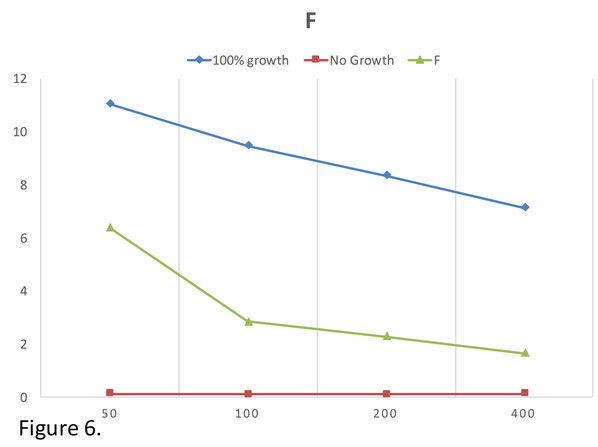

Figure 6.

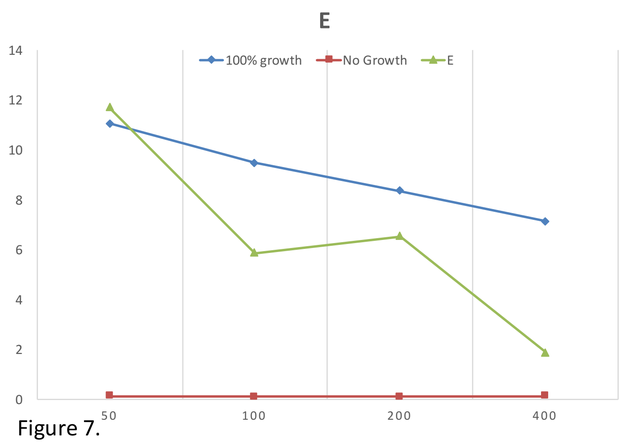

Figure 7.

Figures 2-7: These figures represent the growth vs concentration of each compound. All growth values are 10^5 of the values that are on each graph. Compounds E (fig. 7) and D (fig. 5) show inhibition at the highest concentration of 400 micrograms/mL, but at lesser concentrations the inhibition is not seen. Triclosan (fig. 2), the commercially used anti-microbial compound shows inhibition for the 2 highest concentrations and possibly the 3rd lowest concentration as well. Compound F (fig. 6) has comparable results to triclosan. The 2 highest concentrations shows inhibition towards the fungus. The 3rd lowest concentration shows almost more inhibition than triclosan, granted F is at a higher concentration.

CURRENT TEST

Given that our goal is to produce botanical based preservatives, we attempted to measure the spectrum of biological activity of the compounds from *Junipers virginiana* . The assay was done while performing sterile technique. All assays were ran using 1 mL micro-test tubes. Each tube was inoculated with *A. Niger* cells that were suspended in RPMI broth. Blue Alamar dye was then added to determine the growth of the fungus. The compounds were added at various concentrations and then all of the samples were covered and incubated for 24 hours. The assay was evaluated by a fluorescence measurement. When fungal growth is present the blue dye turns pinks and emits a higher fluorescence value. The samples were all measure at 582 nm. Various samples were then chosen and plated to confirm the growth was from *A. Niger* and if the compound still presented inhibitory effects.

CURRENT RESULTS

(Fig. 8, 9, 10, & 11) These plates were done to ensure that the positive growth that was measured was *A. Niger* as well as to see how well the compound was inhibiting the fungus. Plating the samples allows us to take out some of the guesswork. The samples that we chose to plate were based off of the fluorescence data. 100 ul of the sample was sterilely pipetted onto a plate and then incubated for 24 hours. Figure 9 and 10 show that the compound inhibited the fungal growth, as no colonies were formed. Figure 8, the positive control has many colonies formed, as it should. The negative control of DMSO shows no growth as it should too (Fig. 11). Compounds C and D were also plated, but fungal growth was observed, confirming the fluorescence data. As of now the compound that looks the most promising is F, due to it having 3 active points of inhibition towards *A. Niger*.

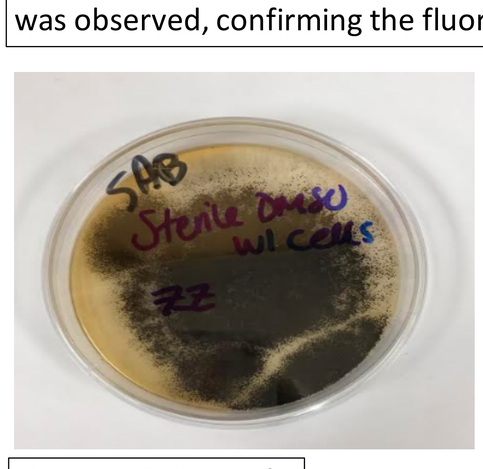

Figure 8: 100% growth

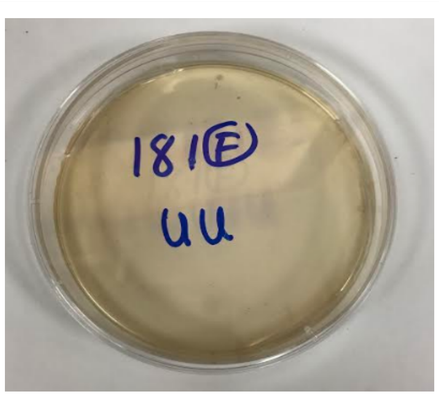

Figure 9: Compound F at 100 ug/mL

Figure 10: Triclosan at 200 ug/mL

Figure 11: DMSO NO CELLS

CONCLUSIONS AND FUTURE WORK

The 4 related carboxylic diterpenes that were tested show promising data. The activity of of compound F is comparable to triclosan when inoculated with the filamentous fungus *A. Niger*. Compound F is being tested at a higher concentration than triclosan. Triclosan is being tested at half the concentration. Both triclosan and compound F have definitive active points of inhibition. The third data point was questionable for both, but with further testing (Fig. 9) we were able to see that even the 3rd lowest concentration for compound F showed active inhibition towards the filamentous fungus. The 100% growth should be a linear relationship with no slope. Our 100% growth has a small slope and in future experiments more data points will be evaluated to ensure a line with no slope. The activity of the mixtures will be further tested to determine if the increased activity is additive or synergistic. Our data shows a promising future for our compound F to potentially be a preservative in personal care products.

Acknowledgements

The authors acknowledge The UNI SOAR Grant for the support of this research project. The authors also would like to thank Kelly Kindscher (University of Kansas) and Gregory Houseal (University of Northern Iowa) for their assistance with plant collection and identification.